SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF DIBENZYLIDENE-CYCLOHEXANONE

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ABSTRACT

Dibenzylidene-cyclohexanone was synthesized by reacting aromatic aldehydes with cyclohexanone in the acidic condition through the carbonyl condensation reaction. The antibacterial activity was tested by using microdilution method against *K. pneumonia*, *E. coli*, *S. aureus*, *B. subtilis* and *E. faecalis*. The isolated products were obtained as pure curcumin analogs with moderate to high yield. The antibacterial assay showed that 2,6-bis-(3'-hydroxybenzylidene)-cyclohexanone gave MIC and MBC at 50 µg/mL in *E. coli*, *S. aureus*, and *E. faecalis*. Moreover, it showed that the percentage inhibition of *B. subtilis* was around 54 % at a concentration of 25 µg/mL. Among all the tested compounds, **A146** showed better activity against all those bacteria while none showed activity against *K. pneumonia*. **A111** was obtained as the compound with the highest yield and **A146** was the most potent compound. While **A102** was the most potent compound according to the docking study.

Keywords: Antibacterial Agent, Dibenzylidene-cyclohexanone, Docking Study, Synthesis

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INTRODUCTION

The rush in finding new potent antimicrobials is a global issue at this time and the search for the new novel antimicrobial is urgently needed.¹ Some studies showed that curcumin is effective against *Staphylococcus aureus* (*S. aureus*).² Curcumin is also known as a golden compound and this compound has been studied for the treatment of diseases such as nervous system disorders³, antidepressant effect⁴ and epileptic rats⁵. Due to its lack of properties⁶, many curcumin analogs were synthesized⁷⁻⁹ and some have been developed as antibacterial agent¹⁰. In addition, an analog of curcumin has been also reported to have many biological activities such as anticancer, antimicrobial, antidiabetic, antioxidant, antiinflammatory, antituberculosis, and antileishmanial agents.¹¹⁻¹⁷ Not only analog of curcumin that has been successfully synthesized, but derivative of curcumin also synthesized such as curcumin diclofenac which has been studied for its bioavailability.¹⁸⁻¹⁹ A series of curcumin derivatives containing heterocyclic moiety have been successfully synthesized and evaluated their antibacterial activities.²⁰⁻²¹ From the previous study QSAR of antibacterial activity, against *K. pneumoniae*, *B. subtilis*, and *S. aureus* has also been reported.²² In continuation, some analogs from this series were also tested as antibacterial.²³ Some heterocyclic and monocarbonyl of curcumin analog have been studied for their in silico and in vitro potency as antibacterial agents and other potential biological activities.²⁴⁻²⁸ This study is aimed to synthesize and study the antibacterial potency of curcumin analog, dibenzylidene-cyclohexanone.

EXPERIMENTAL

Material and Method

The main chemicals were aromatic aldehyde and cyclohexanone (Table-1). Other chemicals were organic solvents, HCl, and KMnO₄. The condensation reaction was performed in the synthesis process. The

isolation and purification were carried out by the recrystallization technique. The structure of the pure compounds was determined by using the spectroscopic methods (IR, HNMR, CNMR and MS).

General Procedure

To a round bottom flask, aldehyde (2 mol. eq) and cyclohexanone (1 mol. eq) were added subsequently continued by THF. The reaction was catalyzed by HCl. The reaction mixture was mixed at room temperature for 2 hours and then heated up at 20–50 °C. The reaction was monitored by TLC with ethyl acetate: hexane (1:2 v/v) as eluent and TLC was then stained with KMnO₄. The product was isolated by washing the reaction mixture with ethanol: water (1:1 v/v), followed by water. Recrystallization was carried out by using methanol/water.

Table-1: List of Compounds

No	Compound	R ₁	R ₂	R ₃	R ₄	R ₅
1	A102	-OH	-OEt	-H	-H	-H
2	A103	-H	-OEt	-OH	-H	-H
3	A104	-H	-NO ₂	-H	-H	-H
4	A108	-H	-COH	-H	-H	-H
5	A111	-OMe	-H	-H	-OMe	-H
6	A129	-OMe	-H	-H	-H	-H
7	A146	-H	-OH	-H	-H	-H

Synthesis of 2,6-bis-(3'-ethoxy-2'-hydroxybenzylidene)-cyclohexanone, A102

Brownish-yellow crystals; yield 40 %; m.p. 133.7–134.5 °C (Toluene : Hexane); IR (cm⁻¹, KBr): 1580, 2839-3474, 728, 1214, 1467, 1645, 3037, 2977, 1113, 944; ¹H-NMR (500 MHz, ppm, Acetone): δ 2.90 (4H, m, H_{3,5}), 1.80 (2H, m, H₄), 1.46 (6H, m, H_{22,24}), 4.13 (4 H, m, H_{21,23}), 7.08 (2H, dd, *J*₁ = 7.5 Hz, *J*₂ = 1.5 Hz, H_{13,20}), 7.03 (2H, dd, *J*₁ = 7.5 Hz, *J*₂ = 7.5 Hz, H_{12,19}), 6.93 (dd, *J*₁ = 7.5 Hz, *J*₂ = 1.5 Hz, H_{11,18}), 5.85 (2H, s, H_{7,14}), 7.70 (2H, s, 2 x OH); ¹³C-NMR (125 MHz, ppm, Acetone): δ 23 (s), 28 (s), 53 (CH₃), 64 (s), 114 (t), 124 (t), 128 (t), 134 (t), 137 (t), 145 (q), 146 (q), 190 (C=O); MS (EI-MS, m/z, %): 394([M⁺], 5), 377(100), 347(23), 319(15), 228(65), 197(18), 123(23), 77(15), 55(24). Obtained as C₂₄H₂₆O₅

Synthesis of 2,6-bis-(3'-ethoxy-4'-hydroxybenzylidene)-cyclohexanone, A103

Yellow crystals; yield 73 %; m.p. 156.8 – 157.6 °C (DCM : Hexane); IR (cm⁻¹, KBr) 3530, 3345, 3139, 2873, 1654, 1591, 1506, 1475, 1252, 1036, 1214; ¹H-NMR (500 MHz, ppm, CDCl₃): δ 1.45 (2H, t, *J* = 6 Hz, H_{22,24}), 1.79 (2H, q, *J* = 5 Hz, H₄), 2.90 (2H, t, *J* = 5 Hz, H_{3,5}), 4.11, (2H, q, *J* = 6 Hz, H_{21,23}), 5.85 (2H, s, H_{7,14}), 6.94 (2H, d, *J* = 8 Hz, H_{12,19}), 6.96 (2H, d, *J* = 2 Hz, H_{13,18}), 7.04 (2H, dd, *J*₁ = 2 Hz, *J*₂ = 8 Hz, H_{9,16}), 7.70 (2H, s, 2 x OH); ¹³C-NMR (500 MHz, ppm, CDCl₃): δ 15 (CH₃), 23 (s), 28 (s), 64 (s), 114 (t), 124 (t), 128 (t), 134 (t), 137 (t), 145 (q), 146 (q), 190 (C=O); MS (EI-MS, m/z, %): 394 (M⁺, 100), 366(40), 337(30), 291(25), 115(45), 102(38), 91(35), 77(40), 65(30), 55(60), 43(25) Obtained as C₂₄H₂₆O₅.

Synthesis of 2,6-bis-(3'-nitrobenzylidene)-cyclohexanone, A104

Yellow crystals; yield 54 %; m.p. 194.3 – 194.9 °C (CHCl₃ : Hexane); IR (cm⁻¹, KBr) 3152, 3013, 1663, 1604, 1573, 1518, 1350, 707; ¹H-NMR (500 MHz, ppm, CDCl₃): δ 1.85 (2H, q, *J* = 6.5 Hz, H₄), 2.95 (4H, t, *J* = 6.5 Hz, H_{3,5}), 7.59 (2H, t, *J* = 8 Hz, H_{10,17}), 7.73 (2H, d, *J* = 8 Hz, H_{9,16}), 7.79 (2H, s, H_{7,14}), 8.19 (2H, d, *J* = 8 Hz, H_{11,18}), 8.30 (2H, s, H_{13,20}); ¹³C-NMR (125 MHz, ppm, CDCl₃): δ 22 (s); 28 (s); 123 (t); 129 (t); 134 (t); 136 (t); 137 (t); 138 (t); 148 (q); 189 (C=O); MS (EI-MS, m/z) 364 (M⁺, 19); 347 (100); 336(13), 317(38), 289(25), 215(30); 161(19), 115(70), 89(38), 63(40), 51(34), 39(31). Obtained as C₂₀H₁₆N₂O₅.

Synthesis of 2,6-bis-(3'-formylbenzylidene)-cyclohexanone, A108

Yellow crystals; yield 27 %; m.p. 220.6 – 221.4 °C (DCM); IR (cm⁻¹, KBr) 3043, 2943, 2872, 2726, 2837, 1695, 1658, 1597, 1560, 1505, 1413, 826, 790; ¹H-NMR (500 MHz, ppm, CDCl₃): δ 1.84 (2H, q, *J* = 5.5 Hz, H₄), 2.95 (2H, t, *J* = 5.5 Hz, H₃), 2.99 (2H, t, *J* = 5.5 Hz, H₅), 7.54 (2H, s, H_{9,16}), 7.61 (2H, d, *J* = 8 Hz, H_{13,20}), 7.81 (2H, m, H_{12,19}), 7.93 (2H, d, *J* = 8 Hz, H_{11,18}), 10.04 (2H, s, H_{21,22}); ¹³C-NMR (125 MHz, ppm, CDCl₃): δ 22 (s); 28 (s); 129 (t); 130 (t); 130 (t); 135 (t); 135 (t); 136 (t); 136 (t); 136 (t); 138 (t); 141 (q); 189 (C=O); 191 (C=O); MS (EI-MS, m/z) 330 (M⁺, 100); 301(70); 217(30); 202(8); 128 (45); 115 (74); 77 (30); 57 (28); 43 (18). Obtained as C₂₂H₁₈O₃.

Synthesis of 2,6-bis-(2',5'-dimethoxybenzylidene)-cyclohexanone, A111

Yellow crystals; yield 81 %; m.p. 143.0-143.1 °C (EtOH); IR (cm⁻¹, KBr): 2947, 2831, 1662, 1600, 1583, 1488, 1461, 1369, 1442, 1249, 1033, 933, 864, 810; ¹H-NMR (500.MHz, ppm, CDCl₃): δ 1.73 (2H, *m*, H₁₀), 2.82 (4H, *m*, H_{9,10}), 3.76 (6H, *s*, 2 x -OCH₃), 3.77 (6H, *s*, 2x-OCH₃), 6.81 (2H, *m*, H_{10,17}), 6.83 (2H, *s*, H_{13,20}), 6.85 (2H, *m*, H_{11,18}), 7.91 (2H, *s*, H_{7,14}), ¹³C-NMR(500MHz, ppm, CDCl₃): δ 23 (s), 28 (s), 56 (-OCH₃), 111 (t), 114 (t), 116 (t), 126 (t), 132 (t), 137 (t), 152 (q), 190 (C=O); MS (EI-MS, m/z, %): 394(M⁺, 8), 364(30), 363(100). Obtained as C₂₄H₂₆O₅

Synthesis of 2,6-bis-(2'-methoxybenzylidene)-cyclohexanone, A129

Yellow crystals; yield 74 %; m.p. 143.7 – 143.8°C (EtOH); IR (cm⁻¹, KBr): 3060, 2930, 1664, 1612, 1597, 1484, 1241, 1022, 943, 749; ¹H-HNMR (500 MHz, ppm, CDCl₃): δ 1.72 (2H, *m*; H₄), 2.82 (4H, *m*; H_{3,5}), 3.84 (6H, *s*, 2 x OCH₃), 6.90 (2H, *d*, *J* = 8.5, H_{10,17}), 6.94 (2H, *dd*, *J*₁ = 7 Hz; *J*₂ = 7.5 Hz; H_{12,19}), 7.30 (4H, *dd*, *J*₁ = *J*₂ = 7.5 Hz, H_{11,18}, H_{13,20}), 7.95 (2H, *s*, H_{7,14}); ¹³C-NMR (500 MHz, ppm, CDCl₃): δ 23 (s), 28 (s),

55 (s), 110 (t), 120 (t), 125 (t), 130 (t), 130 (t), 132 (t), 136 (t), 158 (q), 190 (q); MS (EI-MS, m/z, %): 334(M^+ , 3), 303(100), 271(10), 197(22), 131(25), 115(35), 103(10), 91(30), 77(24), 51(10), 39(11); Obtained as $C_{22}H_{22}O_3$

Synthesis of 2,6-bis-(3'-hydroxybenzylidene)-cyclohexanone, A146

Yellow crystals; yield 58 %; m.p. 214.1–215.2 °C ($CH_3OH : H_2O$); IR (ν_{max} , cm^{-1} , KBr): 3331, 3182, 2939, 1650, 1599, 1445, 1349, 1333, 1219, 688, 794, 878; 1H -NMR (500 MHz, ppm, $DMSO-d_6$): δ 1.71 (2H, m, H_4), 2.87 (4H, m, $H_{3,5}$), 6.79 (2H, dd, $J_1 = 8$ Hz; $J_2 = 2$ Hz, $H_{13,20}$), 6.92 (2H, s, $H_{9,19}$), 6.96 (2H, d, $J = 8$ Hz, $H_{11,18}$), 7.25 (2H, dd, $J_1 = J_2 = 8$ Hz, $H_{12,19}$); 7.52 (2H, s, $H_{7,14}$), 9.60 (2H, s, 2 x -OH); ^{13}C -NMR (125 MHz, ppm, $DMSO-d_6$): δ 22 (s), 27 (s), 116 (t), 121 (t), 129 (t), 135 (t), 136 (t), 136 (t), 157 (q), 189 (C=O); MS (EI-MS, m/z, %): 308 ($[M^+ + 2H]$, 35), 306(100), 289(100), 277(12), 157(23), 145(20), 131(55), 115(64), 103(40), 91(32), 77(75), 51(20). Obtained as $C_{20}H_{18}O_3$

Antibacterial Assay

The assay was performed by using the microdilution method, against Gram-negative and Gram-positive bacteria. This process is carried out in four steps, sterilization, preparation of bacteria inoculum, preparation of the compound solution and microdilution test. Sterilization is done with an autoclave at 121 °C for 20 minutes. Five Bacteria were prepared by using standard method²⁹⁻³⁰ and those bacteria are *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Escherichia coli* (*E. coli*) ATCC 25922, *Bacillus subtilis* (*B. subtilis*) ATCC 6633, *Klebsiella pneumoniae* (*K. pneumoniae*), and *Enterococcus faecalis* (*E. faecalis*) ATCC 29212. Solution of tested compounds were prepared in DMSO in concentration series as 100 $\mu g/mL$; 50 $\mu g/mL$; 25 $\mu g/mL$; 12.5 $\mu g/mL$ and 6.25 $\mu g/mL$. It was also prepared DMSO without the tested compound in it as solvent control, and amoxicillin at 25 $\mu g/mL$ of concentration as a positive control. The microdilution tested was carried out according to CLSI.³⁰

RESULTS AND DISCUSSION

The dibenzylidene-cyclohexanones were obtained in moderate to high yields. The synthesis was carried out according to carbonyl condensation between aromatic aldehyde and cyclohexanone like Scheme-1 below. The reaction used HCl as a catalyst and THF as a solvent. The reaction was easy to perform and able to scale up.

Scheme-1: Synthesis of Dibenzylidenecyclohexanone

A111 has a high yield of around 81 % where this compound has two methoxy (-OMe) groups in para position each other. While **A108** gave yield only around 27% where this compound has a formyl (-COH) group at meta position. The best yield was found when two methoxy (-OMe) groups were on the aromatic ring. Those compounds with ethoxy (-OEt), methoxy (-OMe), or hydroxy (-OH) on their aromatic rings have better yield compared to those that have nitro or formyl group on their aromatic ring.

A102 was obtained in 58% yield and did not show any significant activity as an antibacterial agent against *K. pneumoniae*, *E. coli* ATCC 25292, *S. aureus* ATCC 25923, *B. subtilis* and *E. faecalis* ATCC 29212 at 100 – 6.25 $\mu g/mL$ of concentration. **A103** was successfully synthesized in a 72.45% yield. The value of its MIC and MBC of the compound can not be determined (N/A), but it showed maximum growth of inhibition

towards *B. subtilis* around 42,06% at concentration 100 µg/mL. **A104** was obtained in 53% yield. The antibacterial test at a concentration of 6,25-100 µg/mL showed that **A104** inhibited the growth of *E. coli*, *K. pneumoniae*, *B. subtilis*, *S. aureus*, and *E. faecalis*, not significantly. **A108** was synthesized in 27 % and did not show any significant activity against *E. coli*, *S. aureus*, *B. subtilis*, *K. pneumoniae*, and *B. subtilis* at concentrations 100 – 6.25 µg/mL. The highest inhibition number was observed at concentration 25 µg/mL against *B. subtilis* around 30 %. It means that the MIC and MBC cannot be determined. **A111** was successfully synthesized with a yield of 81.02%. The results of its antibacterial activity test on *E. coli*, *K. pneumoniae*, *S. aureus*, *B. subtilis*, and *E. faecalis* did not show any good antibacterial activities. **A129** compound was synthesized in 80 % yield. The result of its antibacterial activity test showed that the concentration of up to 100 µg/mL gave the activity of maximum inhibition growth around 20% against *K. pneumoniae*, *E. coli*, 30% against *S. aureus*, *E. faecalis*, and 50% against *B. subtilis* bacteria. The moderate yield was obtained from the synthesis of **A146**. Its antibacterial assay showed that compound **A146** has MIC and MBC values at 50 µg/mL in *E. coli*, *S. aureus*, and *E. faecalis*. Furthermore, it showed the antibacterial activity by observing the percentage of inhibition in *E. coli* and *S. aureus* 100 %, *E. faecalis* 88 %, *B. subtilis* 50 %, and *K. pneumoniae* 9.45% at 50 µg/mL.

Among all the tested compounds, none is active as an antibacterial against *K. pneumoniae* bacteria in terms of giving inhibition around 50 % and more. **A146** successfully inhibited the growth of bacteria against *E. coli* at 25 µg/mL, *S. aureus* at 50 µg/mL, *E. faecalis* at 12.5 µg/mL and *B. subtilis* at 25 µg/mL. It seems that the microdilution method as the chosen assay for the antibacterial activity is not suitable for these kinds of curcumin analogs. According to the structure-activity relationship, it is found that compound **A146** has hydroxy (-OH) group on the aromatic ring, has a better antibacterial activity compared to others. Then this activity was explained by using its docking study which was carried out against 5OYO protein target. The results were shown below (Fig.-1).

Among the tested compounds, compound **A102** has the smallest score docking value = -13.6521. While compound **A146** only has a score docking value = -13.0232 lower than the score docking value of **A102** but both **A102** and **A146** have better score docking values compared to the score docking value of amoxicillin, score docking value = -12.9697. According to the docking study result, compound **A102** is more potent compare to **A146**. From the experimental result, compound **A146** is the most potent antibacterial agent among the tested compounds but it is still not good enough compared to amoxicillin.

Fig.-1: Docking Study of the tested Compounds

CONCLUSION

Compound **A111**, which has two methoxy groups on its aromatic ring, has the highest yield and compound **A146**, which has hydroxy group on its aromatic ring, is the most potent one according to the experiment but compound **A102**, which has ethoxy and hydroxy groups on its aromatic ring, has the smallest score docking which means that compound **A102** should be the most potent one according to the molecular docking study but this was not approved by experiment.

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