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CONTENT

SYNTHESIS AND ACTIVITY TEST AS DEOXYRIBOSE DEGRADATION INHIBITOR OF TWO ASYMMETRIC DIBENZALACETONES

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ABSTRACT

Synthesis and activity test as deoxyribose degradation inhibitor of two asymmetric dibenzalacetones have been conducted. The first compound, 1(E),4(E)-1-(4'-methoxyphenyl)-5-phenyl-1,4-pentadiene-3-one was synthesized from benzaldehyde, acetone and 4-methoxybenzaldehyde. The second, 1(E),4(E)-1-(3',4'-dimethoxyphenyl)-5-phenyl-1,4-pentadiene-3-one was from benzaldehyde, acetone and 3,4-dimethoxybenzaldehyde. Those compounds were synthesized by crossed aldol condensation in base condition with water-ethanol solvent. The synthesize of both compounds using ice bath throughout the stirring. The precipitate was purified by coloumn chromatography. Each product was characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR, HMQC and HMBC. Activity test as deoxyribose degradation inhibitor showed IC_{50} of both compounds are 1,7μM and 1,02 μM respectively.

Keywords: *asymmetric dibenzalacetone, deoxyribose degradation inhibitor*

INTRODUCTION

Aldol Condensation has been occurred by a nucleophilic addition of enolate ion to a carbonyl group. Acetone also undergoes aldol condensation, but the equilibrium concentration of the product was generally small. Cross aldol condensation between p-annisaldehyde from fennel oil with acetophenone produced 2-hydroxy-4-methoxychalcone compound [1]. The influence of base concentration and reaction time on the cross aldol condensation reaction has also been reported [2]. Alnustone or 4(E),6(E)-1,7-diphenyl-4,6-heptadiene-3-one is an asymmetric compound that isolated from Curcuma xanthorrhiza (*Zingiberaceae*). This compound was synthesized by Goksu, et al. using crossed aldol condensation between benzaldehyde and acetone, which then followed by reaction with cynnamaldehyde [3].

Handayani and Arty have synthesized 1(E),4(E)-1.5-diphenyl-1,4-pentadiene-3-one and its derivatives were known as symmetrical dibenzalacetone that have been made by crossed aldol condensation between acetone:benzaldehyde (1:2). It also tested as a radical hydroxyl scavenger [4]. Asymmetric crossed aldol condensation have been done with various catalyst [5,6,7]. Tutik D has synthesized of symmetrical dibenzalacetone which have similar structure with the cinnamic acid derivative [8]. From its structure, it could be estimated that benzalacetone and dibenzalacetone, which have similar structure with cinnamic acid or its derivative, have ultraviolet absorption in the same range. Thus, asymmetric dibenzalacetone was estimated as a radical scavenger and also a sun screen.

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In this research two of asymmetric dibenzalacetones, compounds **5 and 6** namely **1(E),4(E)-1-(4'-methoxyphenyl)-5-phenyl-1,4-pentadiene-3-one and 1(E),4(E)-1-(3',4'-dimethoxyphenyl)-5-phenyl-1,4-pentadiene-3-one** will be synthesized.

EXPERIMENTAL SECTION

Materials

All materials used from Merck, among other acetone, 4-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, chloroform ethanol, benzaldehyde, hexane, and ethyl acetate. TLC was carried out using 0.25-mm plate Silica gel Merck 60 F254, column chromatography were performed by Silica gel 60 (230-400 mesh).

Instrumentation

The ^{1}H, ^{13}C-NMR, HMQC and HMBC Spectra were recorded on 500 MHz Jeol spectrophotometer. IR spectra were conducted using a Shimadzu 8300 FTIR spectrometer.

Procedure

Synthesis of compounds 5.

Into a solution of NaOH (0.025 mol, 1g) in aqueous ethanol (1:1) that was prepared at ambient temperature, **1** benzaldehyde (0.01 mol, 1,06 g), **2** acetone (0,01 mol, 0,58 g) and **3** 4-methoxybenzaldehyde (0,01 mol, 1,66 g) were added drop wise alternately. After additional stirring for 60 minutes, water (20 ml) was added to the reaction mixture which then filtered. The extract was washed with water (20 ml x 3) and separated by column chromatography (d 2.5 cm, h 50 cm), with silica gel 60 (230-400 mesh) as the stationary phase and ethylacetate-hexane 1 : 9 as the eluent. The target compound (**5**) was identified using thin layer chromatography with ethylacetate-hexane 5:1 as the mobile phase.

Synthesis of compound 6.

The similar procedure was repeated for 3,4-dimethoxybenzaldehyde to replace 4-methoxybenzaldehyde in order to synthesize compound **6** (Figure 1). Four fractions obtained from the column chromatography, and the target compound was identified using thin layer chromatography with chloroform-hexane 4: 6.

Deoxyribose assay.

The assay was performed follow the method as described by Halliwell [9]. All solutions were freshly prepared. Into a solution of 0.2 ml 6 mM 2-deoxyribose was added 0.2 ml ascorbic acid 0.01 mM; 0.2 ml buffer phosphate (pH 7.4); 0.2 ml 0.01 mM H_2O_2; 0.02 ml of various concentration sample (0,24; 0,48; 0,95; 1,89; 3,78 µM) and 0.2 ml ferrosulphate 0.1 mM. After an incubation period of 30 minutes at 310^0K, the extent of deoxyribose degradation was measured by the TBA reaction. 3 ml of TBA and 3 ml of TCA were added to the reaction mixture and heated for 15 minutes at 353^0K. After the mixture being cooled, it is read at absorbance 532 nm against a blank (the same solution but without sample). The percentage inhibition was calculated by the formula:

$$I(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} x100\%$$

The IC_{50} value represented the concentration of the compounds that caused 50% inhibition. BHT was used as a positive control.

RESULTS AND DISCUSSION

Improved Synthesis of Compound 5

Separation of the product of crossed aldol condensation between benzaldehyde, acetone and 4-methoxybenzaldehyde was done by coloumn chromatography. The product from coloumn chromatography separation yielded 3 fractions, which were identified by TLC with hexane-ethylacetate 5:1 as the eluent. Retardation factor datas from TLC scanner showed that fraction II was the target of compound **5** (10,6%) determined as yellow residue. Fraction I

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and fraction II supposed to be benzalacetone and dianisalacetone as the side products of crossed aldol condensation reaction.

Characterization of compound **5** by FTIR (KBr) resulted peaks on 3035; 2922; 2842; 1668; 1423; 1446; and 1175 cm^{-1}. A series of one and two dimensional NMR spectroscopic experiment using HMQC and Heteronuclear Multitiple Bond Coherence (HMBC) patterns were performed to assign the proton and carbon resonance correlation of the compounds. The signal pattern of the aromatic ring showed the influence of methoxy (OMe) in δ 3,8 (3H, s) ppm (Figure 1; Table 1).

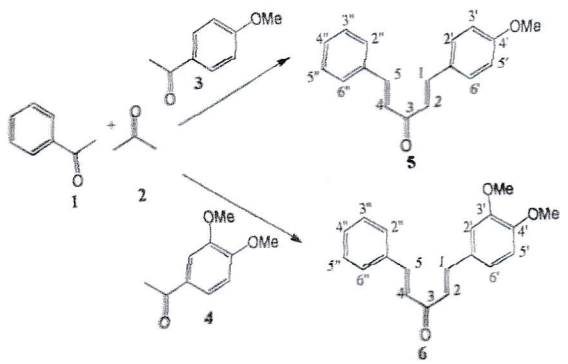

Fig. 1 Scheme of cross aldol condensation to synthesized of compound **5** and **6**.

Table 1. ^{1}H, ^{13}C-NMR and HMBC datas of compound **5** ($CDCl_3$)

C no.	δH (∑H; m) ppm	δC ppm	HMBC (500 MHz)
1	7,70 (1H; d)	143	C6", C2,C3
2	6,97 (1H; d)	123,4	C3, C2'
3	-	189	-
4	7,07 (1H; d)	125,7	C3, C1"
5	**7,74 (1H; d)**	**143,3**	**C3**
2',6'	7,58 (2H; d)	129	C1, C3', C4'
3',5'	6,93 (2H; d)	114,6	C4'
4'-OMe	3,8; (3H, s)	56	C4'
4'	-	**162**	-
2"	7,61 (1H; d)	130,3	C5, C6"
3",5"	7,4 (2H; d)	127,6	C4", C2"
4"	7,41 (1H; d)	128,5	C1", C2"
6"	**7,62 (2H; d)**	**130,5**	**C5, C2"**

Improved Synthesis of compound 6.

The preparation of compound **6** was initiated by the mixing of **1**, **2** and **4** to give **6** (Figure 1). The yield of crossed aldol condensation between acetone, 3,4-dimethoxybenzaldehyde and benzaldehyde was a mixture consist of 4 compounds. There was separated by Column Chromatography (AcOEt-hexane, 1:9) to provide the asymmetric dibenzalacetone **6** (15,53%) determined as pale yellow oil.

The multiple bond correlation of HMBC supported the structure (Figure 1; Table 2). In the ^{1}H-NMR spectrum (500 MHz, $CDCl_3$), two patterns singlet, nine doublet and three double dublet were observed. The double dublet at δ = 7,2; 7,61; and 7,41 was assignable to H2", H3" and H6" respectively. Two equivalence methoxy signals at δ 3,91 and 3,9 were assigned to C3' and C4'. Support spectra data provided by the IR (KBr), which indicates the existence of C=O (1645cm^{-1}), aromatic C=C (1514-1417 cm^{-1}) and CO ether (1255-1139 cm^{-1}). Therefore, the structure of **6** was 1(E),4(E)-1-(3',4'-dimethoxyphenyl)-5-phenyl-1,4-pentadiene-3-one.

Table 2. ^{1}H, ^{13}C-NMR and HMBC datas of compound **6** ($CDCl_3$)

C no.	δH (∑H; m) ppm	δC ppm	HMBC (500 MHz)
1	7,69 (1H; d)	143,6	C2,C3
2	6,94 (1H; d)	124	C3
3	-	188	-
4	7,10 (1H; d)	125,6	C3, C1"
5	7,74 (1H; d)	143	C3
2'	7,06 (1H; d)	110	C3'
3'	-	151	-
3'-OMe	3,9 (3H; s)	56	C4'
4'	-	150	-
4'-OMe	3,91 (3H; s)	56	C3'
5'	6,83 (1H; d)	111	C4', C6', C1'
6'	6,89 (1H; d)	120	C1', C5', C4'
2"	7,61 (1H; dd)	130	C5
3"	7,2 (1H; dd)	123,4	C1", C4"
4"	7,14 (1H; d)	110	C3"
5"	7,33 (1H; d)	129	C1"
6"	7,41 (1H; dd)	128	C1"

Deoxyribosa assay

Activity test as deoxyribose degradation inhibitor was done by fenton reaction [9]. The IC_{50} value represented the consentration of the compounds, that caused 50% inhibition. All experiment were carried out in triplicated.

Determination of IC_{50} values were showed in Table 3.

Table 3. *IC_{50} datas for compounds **5** dan **6***

No	Compound	IC50 (μM)
1.	5	1,7
2.	6	1,02

CONCLUSION

In conclusion, two asymmetric dibenzalacetones exhibited significant deoxyribose degradation inhibitor activity. The IC50 of compund **5** and **6** are 1,7 and 1,02 μM respectively. Compound **6** is more potent than **5** to inhibit deoxyribose degradation.

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