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An Efficient Directly Conversion of the Ethyl *p*-Methoxycinnamate into *N,N*-dimethyl-*p*-Methoxycinnamamide and study the structure-activity relationship on anti-inflammatory activity

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ABSTRACT

Ethyl *p*-methoxycinnamate (EPMC) (**1**) is a major natural ester found in the rhizome of *Kaempferia galanga* and has been reported to have anti-inflammatory activity. Some of the structural modification of this compound has been carried out in order to study the structure-activity relationship on its anti-inflammatory activity. In the present study, we report a new, simple and efficient procedure in the conversion of the ethyl *p*-methoxycinnamate into *N,N*-dimethyl-*p*-methoxycinnamamide (**5**) and then study the structure-activity relationship on its anti-inflammatory activity. The reaction was carried out through a microwave-assisted direct amidation between (EPMC) (**1**) with dimethylformamide (DMF) in the basic condition. The mixture was irradiated by using unmodified microwave-oven at 300 W for 1 minute to obtain compound **(5)** in 88.8% yields. The extensive analysis of the GCMS and NMR data supported that the product of synthesis is *N,N*-dimethyl-*p*-methoxycinnamamide (**5**). Evaluation of the anti-inflammatory activity of compound **5** by using anti-denaturation of heat bovine serum albumin (BSA) assay indicated that *N,N*-dimethyl-*p*-methoxycinnamamide (**5**) still have anti-denaturation activity. Compound **5** has an amide functional group which is more slowly hydrolyzed if compared to **1**. Hence, the reaction has successfully produced a more stable compound which still has anti-inflammatory activity

Keywords: Anti-inflammatory, direct amidation, *N,N*-dimethyl-*p*-methoxycinnamamide, ethyl *p*-methoxycinnamate, *Kaempferia galanga,* microwave-promoted synthesis,

INTRODUCTION

Ethyl *p*-methoxycinnamate (EPMC) (1) is an ester anti-inflammatory compound that is easily isolated from the rhizome of *Kaempferia galanga* (Komala, *et al.*, 2018; Komala *et al.*, 2017). Our previous research suggested that ethyl ester of the EPMC (**1**) is responsible for its anti-inflammatory properties. Conversion of EPMC (**1**) into other ester cause decreasing anti-inflammatory activity (Komala *et al.*, 2018). In the other investigation, we have also successfully converted EPMC (**1**) into cinnamamide derivatives, *N*-(2-hydroxyethyl)-*p*-methoxycinnamamide (**2**), *N,N*-bis-(2-hydroxyethyl)-*p*-smethoxycinnamamide (**3**) and *p*-methoxycinnammamide (**4**) in which these compounds showed higher anti-inflammatory activity than **1** (Figure 1) (Komala *et al.*, 2017).

Cinnamamides are compounds that have an amide bond and have been reported to have various pharmaceutical activities. In the simple method, the amide bond was prepared through direct condensation of carboxylic acids and amines. However, this reaction does not occur spontaneously at ambient temperature. In order to circumvent carboxylate-ammonium salts formation in this reaction, the reaction needs to be conducted at very harsh conditions (>100°C).Therefore, it is necessary to initiate the activate carboxylic acids by using coupling reagent prior to treatment with amines (Figueiredo, *et al.*, 2016; Pattabiraman and Bode, 2011). In recent research, it has been developed the catalytic protocol in performing direct amidation of carboxylic acids with amines, in which boronic acid

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derivatives are found as a prominent catalyst used (Pattabiraman and Bode, 2011; Sabatini, *et al.*, 2017). Another method that is currently being considered in order to form amide bonds is a direct amidation of the ester by using a variation of amines. The aminolysis of the ester by using amines becomes an attraction in the organic synthesis due to its simplicity, economical, and large availability of the starting material (Vrijdag *et al.*, 2014).

In continuing our concern on the study structure-activity relationship of the cinnamamide derivative of the EPMC (**1**), here we are reporting a new, simple and efficient procedure for the synthesis of *N,N*-dimethyl-*p*-methoxycinnamamide (**5**) and then study its structure-activity relationship. The reaction was conducted through a microwave-promoted direct amidation of EPMC (**1**) and DMF in the presence of NaOH (Figure 1). The product of the reaction was evaluated for its anti-inflammatory activity and then further analyzed for its structure-activity relationship on the anti-inflammatory activity.

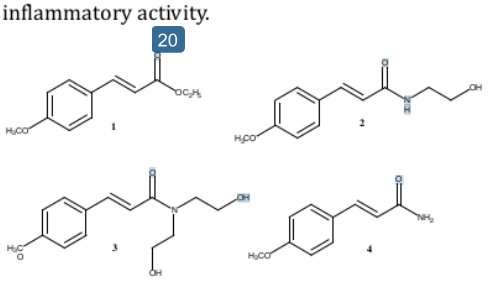

Figure 1. Ethyl *p*-methoxycinnamate (**1**) and its cinnamamide derivatives

MATERIAL AND METHODS

General

The melting point of the synthesized product was measured by using the melting point apparatus Stuart SMP10 without correction. The ^{1}H-NMR was measured on Jeol-500 MHz (^{1}H; 500 MHz) instruments. The reaction was carried out using a microwave oven assisted (Samsung). Product of reaction was analyzed by using GCMS: GC/MS-MSD 7890A/5975C (Agilent Technologies) with the condition was in accordance with our previous reported (Komala, *et al.*, 2018; Komala *et al.*, 2017).

Chemicals

Methanol (Merck), DMF (Merck), ethanol (Merck), Na diclofenac (Sigma-Aldrich), HCl (smart lab), Bovine serum albumin fraction V (Sigma-Aldrich), NaOH (Merck), Trizma Base (Sigma-Aldrich), NaCl (Merck), TLC plate 60 F254 (Merck).

Plant material

The rhizome of *K. galanga* was collected from BALITRO (Balai Penelitian Obat dan Rempah) Bogor, West Java, Indonesia in May 2015. This specimen was identified in Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia.

Extraction and Isolation of EPMC (1)

Extraction and isolation of EPMC (**1**) were conducted in accordance with our previously reported (Komala *et al.*, 2017).

Conversion of EPMC (1) into *N,N*-dimethyl *p*-methoxycinnamamide (5).

In a test tube with the cup, 206mg (1mmol) of EPMC (**1**) was added into the mixture of 10 mmol NaOH in 2mL ethanol and 2mL of dimethylformamide (DMF). The mixture was then irradiated by using an unmodified microwave oven at 300W for 1 minute. The product of the reaction was extracted by using ethyl acetate and H_2O (1:1), and then the ethyl acetate fraction was collected and evaporated to give 182.5mg of white amorphous of *N,N*-dimethyl-*p*-methoxycinnamamide (**5**) (yield 88.6%). mp:132-135°C. GCMS : 205 $[M]^+$, 161 (base peak), 133, 118, 103, 89, 77, 63, 44. ^{1}H-NMR: 3.05 (s, 3H), 3.16 (s, 3H), 3.83 (s,3H), 6.77(d,1H, J=15 Hz), 7.64 (d, 1H, J = 15 Hz), 6.90 (d, 2H, J= 9.1 Hz), 7.48 (d, 2H, J = 8.5 Hz) ^{1}H-NMR data is in accordance to that of the previously reported (Pathan and Patil, 2008).

Anti-Denaturation of Heat BSA Assay

Anti-inflammatory activity was evaluated by using anti denaturation of Heat BSA Assay. The sample was dissolved in methanol at various concentrations and then measured its anti denaturation activity in the procedure is in accordance with our previously reported (Komala, *et al.*, 2018).

RESULTS AND DISCUSSION

In this research, we synthesized the *N,N*-dimethyl-*p*-methoxycinnamate (**5**) through the direct amidation of EPMC (**1**) by using DMF in the presence of NaOH (dissolved in ethanol). In order to find an optimal condition of reaction, initially, EPMC (1 mmol) and DMF (2mL) were reacted in a variety amount of NaOH in 2mL ethanol. The reaction was conducted by using an unmodified microwave oven at 300W for 1min and the result of the reaction was monitored by using TLC (Table I).

Table I. Optimization of the amount of NaOH for the synthesis of *N,N*-dimethyl-*p*-methoxycinnamamide (5)

Entry	EPMC (1) (mol)	DMF (mL)	Ethanol (mL)	NaOH (mmol)	Result
1	1	2	2	1	-
2	1	2	2	5	-
3	1	2	2	10	+

Note: (-): did not give a product of synthesis, (+): successfully gave the product of synthesis

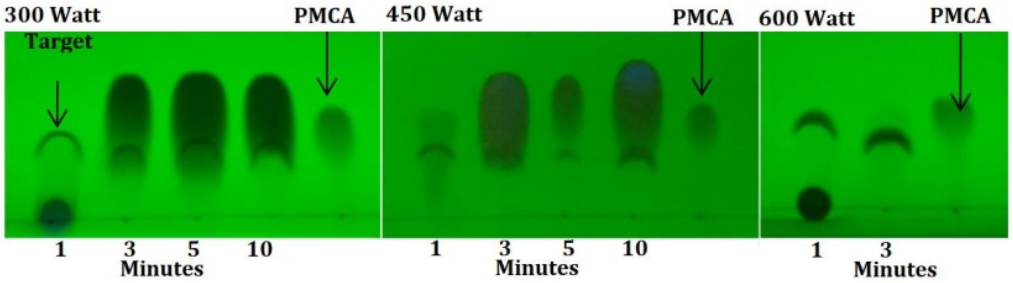

Figure 2. TLC monitoring of the reactions at 300, 450 and 600 Watt in a variation of times (eluent n-hexane-EtOAc 3:2)

Figure 3. TLC monitoring of Product of reaction (eluent n-hexane-EtOAc 3:2). **1**: Amide product (**5**), **2**: Hydrolysis product (**6**), **3**: EPMC (**1**)

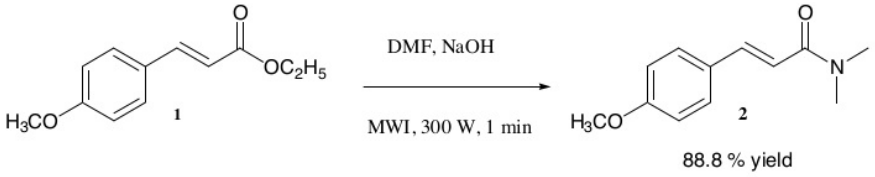

Figure 4. Direct amidation of EPMC (**1**) to synthesize *N,N*-dimethyl-*p*-methoxycinnamamide (**5**)

Unfortunately, there was no indication of product formation in the presence of 1 and 5mmol NaOH in 2mL ethanol. Eventually, the product of synthesis was successfully formed in the presence of 10mmol NaOH in 2mLethanol.

The next optimization was conducted in order to find the optimum power of microwave-oven and time of reaction for the synthesis. The mixture containing NaOH (10mmol) in ethanol (2mL) and DMF (2mL) was then added EPMC (1mmol), subsequently, the mixture was irradiated by using the variations in power microwave-oven and reaction times. The result of the reaction was monitored by using TLC and compared to the product of hydrolysis of EPMC, *p*-methoxycinnamic acid (PMCA) (**6**). The reaction which was carried out by using microwave-oven at 300 Watt in 1min gave a better result compared to the other

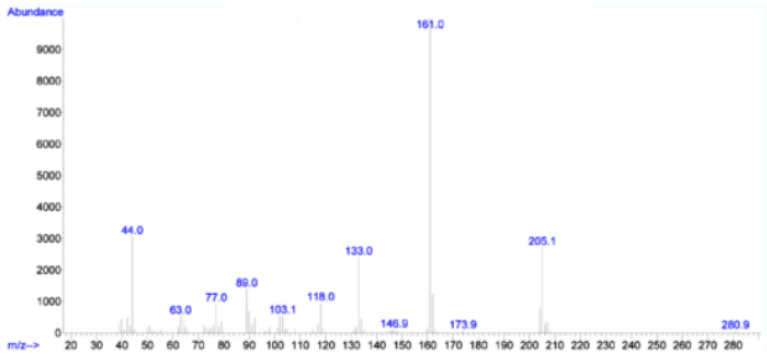

Figure 5. MS data of *N,N*-dimethyl-*p*-methoxycinnamamide (**5**)

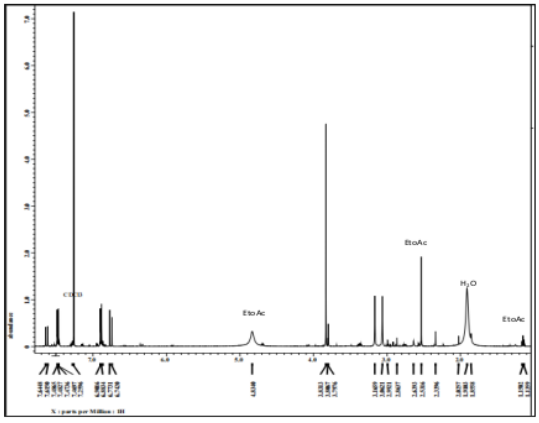

Figure 6. The NMR spectrum of **5**

conditions (Figure 2). The fact in this optimization showed that the longer time used for the reaction, the more product of PCMA (**6**) was formed. It also found that the increasing power of microwave used causes increasing PCMA (**6**) formed. A comparison of the amide product (**5**), PCMA (**6**) and EPMC (**1**) (Figure 3 and 4).

Successful conversion of the EPMC **(1)** to form *N,N*-dimethyl-*p*-methoxycinnamamide (**5**) was occurred through a substitution acyl nucleophilic reaction of EPMC (**1**) with dimethylamine, in which dimethylamine is formed as product degradation of DMF in the solution of NaOH (Buncel and Symons, 1970). The product of the reaction was successfully formed when the reaction was carried out in the mild condition (300W, 1min) and turns to produce a hydrolysis product (*p*-methoxycinnamic acid when the high-power of microwave-oven and a long time of reaction was used more than 300W, 1min. In our prediction, under mild conditions, dimethylamine was rapidly formed and then directly reacted with EPMC. On the other hand, the reaction produced a hydrolysis product when the power of microwave-oven and time of reaction was increased.

Compound **5** was obtained as a white amorphous with the melting point of 132-133. Analysis of the MS data of GCMS indicated the presence of a molecular ion peak $[M]^+$ at 205 m/z, which is correlated to the molecular formula of $C_{12}H_{15}NO_2$. The base peak is found at m/z 161, which is typical of the presence of a *p*-methoxycinnamate moiety (Komala, Supandi, & Hardiansyah, 2018). This peak occurs due to the loss of the $-N(CH_3)_2$ from the whole structure of compound **5** (Figure 5).

The ^{1}H-NMR spectrum (Figure 6) indicated the presence of *trans*-configuration of the *p*-methoxycinnamate moiety which is suggested by the presence of δ.77(d, 1H, J=15 Hz), 7.64 (d, 1H, J = 15 Hz), 6.90 (d, 2H, J= 9.1 Hz), 7.48 (d, 2H, J = 8.5 Hz), 3.83 (s, 3H). The pattern of these signals was in accordance with our previous reported (Komala, *et al.*, 2018). The rest signals at δ 3.05 (s, 3H), 3.16 (s, 3H) were indicated the presence of $-N(CH_3)_2$. Hence it suggested that this compound is *N,N*-dimethyl-*p*-methoxycinnamamide (**5**). ^{1}H-NMR data is in accordance with that of the previously reported which was confirmed that compound 5 is *N,N*-dimethyl-*p*-methoxycinnamamide (Pathan & Patil, 2008).

BSA anti-denaturation assay indicated that compound **5** has anti-inflammatory activity (Figure 7). Williams *et al* have suggested that any compounds that have inhibition denaturation value greater than 20% were considered as having anti-inflammatory properties and have the potency to be developed as anti-inflammatory drugs (Williams *et al*., 2008). Over range concentration of compound **5** at 6.25, 12.5, 25 and 50 ppm, concentration at 25 ppm was found as the minimum concentration that still inhibits denaturation of BSA greater than 20 % (the percentage of inhibition is 23.4%), meanwhile the higher inhibiting denaturation was shown by concentration of 50 ppm (percentage of inhibition is 34.6 %). In our previous reported, cinnamamides that were synthesized from EPMC (**1**), *N*-(2-hydroxyethyl)-*p*-methoxycinnamamide (**2**) and *N,N*-bis-(2-hydroxyethyl)-*p*-methoxycinnamamide (**3**) showed higher anti-inflammatory activity than EPMC (**1**).

However, in this investigation, compound **5** showed lower anti-inflammatory activity compared to EPMC (**1**), in which compound **1** still have inhibition of denaturation activity at a concentration of 0.1 (percentage of inhibition is 32.9%). Compounds **2** and **3** are cinnamamides that have *N*-ethyl hydroxy substituent. Both compounds showed higher anti-inflammatory activity compared to EPMC (**1**). Compound **4** is a cinnammamide that does not have substituent attached to its Nitrogen atom, and anti-inflammatory activity tends to decrease if compared to EPMC (**1**) (Komala, *et al*., 2018; Komala *et al*., 2017). When the EPMC (**1**) was converted to a cinnamamide that has dimethyl substituent at the nitrogen atom, the anti-inflammatory of this derivative is lower compared to EPMC (**1**). Hence, it suggested that the more hydrophilic substituent attaches to the nitrogen atom of cinnamamide derivatives cause increasing its anti-inflammatory activity. Meanwhile, the presence of the lipophilic substituents such as N,N-dimethyl causes a decrease in anti-inflammatory activity. As already known, drugs that contain ester group tend to susceptible to be hydrolyzed, therefore in the further pharmaceutically development of this drug, we must concern about its stability. Even though a drug that has amide drugs are also susceptible to hydrolysis, but these compounds have a lower rate if compare to the ester drugs (Waterman *et al*., 2002). Hence, in the view of the stability, compound **5** is more stable than **1**. Hence, the conversion of the EPMC (**1**) into *N,N*-dimethyl-*p*-methoxycinnamamide (**5**) has produced more stable derivatives that still have anti-inflammatory activity.

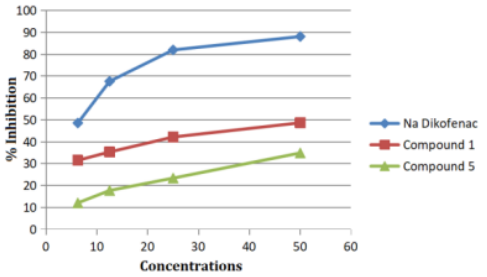

Figure 7. The antidenaturation activity of 1 and 5

CONCLUSION

Direct amidation of EPMC (**1**) by using DMF and NaOH has successful produced N,N-dimethyl-p-methoxycinnamamide (**5**). The reaction was carried out by using the assistance of the irradiation of the unmodified microwaved oven at 300Watt for 1min. The product of synthesis was found to still have anti-inflammatory. Structure-activity relationship study suggested that the presence of the N,N-dimethyl at the nitrogen atom of the *p*-methoxycinnamamide derivative causes a decrease in anti-inflammatory activity. Compound **5** that has an amide functional group is more slowly hydrolyzed if compared to **1**. Hence, it can be concluded that product of synthesis is a more stable compound that still has anti-inflammatory activity.

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