HARIYANTI-Synthesis and Preliminary In Vitro Anti-inflammatory Evaluation of Mannich Bases Derivatives of 4’-Methoxy-substituted of Asymmetrical Cyclovalone Analogs

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Synthesis and Preliminary *In Vitro* Anti-inflammatory Evaluation of Mannich Bases Derivatives of 4'-Methoxy-substituted of Asymmetrical Cyclovalone Analogs

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ABSTRACT

Two of Mannich bases derivatives of 4'-methoxy-substituted of asymmetrical cyclovalone analog (ACA) (**2a** and **2b**) were synthesized. The synthesized compounds and the other two Mannich bases derivatives of 4'-methoxy-substituted ACA (**2c** and **2d**) were evaluated for their *in-vitro* anti-inflammatory activity preliminary by protein denaturation inhibition method using a final concentration of 1.57 µM. The study found that all the Mannich bases exhibited anti-inflammatory potential with inhibition ranging from 33.17- 42.47%. The activity of **2b** (42,47%) and **2d** (41.90%) was higher than that of diclofenac sodium (35.27%) and the parent compound **1** (38.16%). As a conclusion, **2b** and **2d** have a prospect as a potential candidate for an anti-inflammatory agent. Further study should be done using more specific methods.

Keywords: Mannich bases derivatives, asymmetrical, ayclovalone, synthesis, *in-vitro* anti-inflammatory, protein denaturation.

INTRODUCTION

Curcumin is well-documented to have anti-inflammatory activity with low toxicity. However, clinical usage of the compound is limited due to its stability and bioavailability (Prasad *et al.*, 2014; Anand *et al.*, 2008; Wang *et al.*, 1997). The mono-carbonyl analogs of curcumin (MACs), such as cyclovalone, showed a more stable chemical structure and better pharmacokinetic profile. Several of them were more active as anti-inflammatory agents than curcumin. (Liang *et al.*, 2008; Zhao *et al.*, 2013; Lamperti *et al.*, 2014; Zhao *et al.*, 2015)..

In the past few years, some asymmetrical MACs were prepared, and among them indicated potent biological activity as anti-inflammation (Zhang *et al.*, 2014a; Zhang *et al.*, 2014b; Aluwi *et al.*, 2016). Introduction of morpholine Mannich base moiety into asymmetrical MACs bearing a cyclohexanone linker (= asymmetrical cyclovalone analogs, ACA) (Figure 1), increased the anti-inflammatory activity of the parent compound. 5-Morpholinomethyl-4'-methoxy ACA showed the highest activity, which almost comparable to diclofenac sodium (Putri *et al.*, 2018). Further investigation found that 5-dimethylaminomethyl of ACA (Figure 1, 4'-R = H) exhibited anti-inflammatory about four-fold than curcumin and equal to diclofenac sodium (Hayun *et al.*, 2019). However, the anti-inflammatory activity of Manncih bases derivatives of 4'-methoxy-substituted of ACA has not elucidated yet.

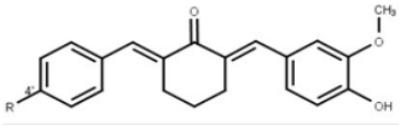


Figure 1. Asymmetrical Cyclovalone Analogs (ACA) (Putri *et al.*, 2018; Hayun *et al.*, 2019)

Hence, as a continuation of the study in exploring the anti-inflammatory activity of Mannich bases derivatives of ACA, in the present study, we synthesized 5-dimethylamino-methyl-4'-methoxy ACA (**2a**) and 5-(N-methylpiperazino)methyl-4'-methoxy ACA (**2b**) (Figure 2). The synthesized compounds and the other two Mannich bases derivatives of 4'-methoxy-substituted ACA (**2c** and **2d**) then were evaluated for their preliminary *in-vitro* anti-inflammatory activity.

MATERIAL AND METHODS

2,6-(4'-Methoxybenzylidene-4-hydroxy-3-methoxybezylidene)cyclohexanone (= 4'-methoxy ACA) (**1**), 5-diethylaminomethyl-4'-methoxy ACA (**2c**), 5-morpholinomethy-4'-methoxy ACA (**2d**), and cyclovalone, were dedicated by earlier researchers (Hayun *et al.*, 2017; Prasetyaningrum *et al.*, 2018; and Putri *et al.*, 2018). Diclofenac sodium was obtained from PT Kimia Farma, Indonesia, while other chemicals and solvent were supplied from chemical suppliers of Sigma-Aldrich or Merck. The compounds' melting point was measured by the melting point apparatus (Stuart Scientific, UK), infrared spectra and NMR spectra were recorded by FT-IR Spectrometer (Agilent Technologies, USA) and NMR spectrometer (Agilent Technologies, USA) respectively. While the mass spectra were recorded by LC-MS with ESI (+) mode (UNIFI, Waters, USA).

Synthesis of Mannich Bases derivatives of 4'-methoxy ACA (2a and 2b)

The compounds **2a** and **2b** were synthesized using the method for preparation of **2c** and **2d** reported previously (Prasetyaningrum *et al.*, 2018, Putri *et al.*, 2018). A cooled solution in ethanol of **1** (2 mmol) was stirred and added a solution in ethanol of dimethylamine (for **2a**) or 1-methylpiperazine (for **2b**) (6 mmol) and formaldehyde solution (6 mmol). The mixture was further stirred for 30 minutes at r.t., then refluxed for 3 h (for **2a**) and 6 h (for **2b**) until the reaction was completed (TLC monitoring). After that, the products were isolated and then purified using a column chromatography technique to get **2a** and **b**.

Preliminary *in-vitro* anti-inflammatory Evaluation.

The evaluation was done using inhibition of albumin denaturation technique, as reported previously with minor modification (Putri *et al.*, 2018). Mixture of 0.5mL of standard or test compounds solution in methanol (15.72 uM) or solvent (for control) and 4.5mL Bovine Saline Albumin (BSA) 0,5% in tris-buffer saline pH 6.3 was incubated at 37°C for 15min, then heated at 70°C in a water bath for 5min, and cooled to reach room temperature. The turbidity was measured at 660nm. The inhibition (%) of the denaturation was calculated with the formula:

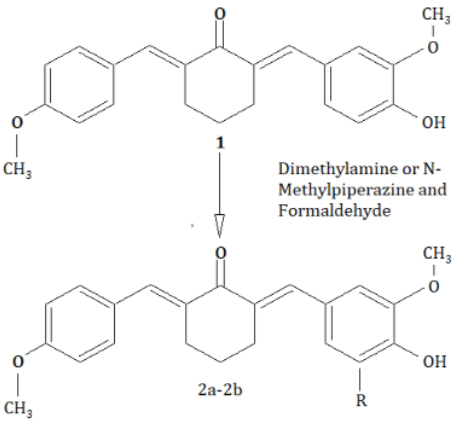
$$\% \text{ inhibition} = [Ac - \frac{As}{Ac}] \times 100\%$$

where Ac was absorbance of control; As was absorbance with sample addition.

RESULT AND DISCUSSION

Chemistry

Scheme of the synthesis of compound **2a-b** (Figure 2).



2a : R = Dimethylaminomethyl
2b : R = (1. Methylpiperazin-4-y)methyl

Figure 2. Scheme of the synthesis of Mannich bases derivatives of 4'-methoxy-substituted ACA

Infrared, ^{1}proton, and ^{13}carbon NMR and mass spectral data obtained were presented in Table II. The infrared spectra **2a** and **2b** (Figures 3) showed the bands of C-O-C and C-N at 1,260-1.023cm^{-1}; C-H aliphatic at 2.935-2.829, while the a,ß-unsaturated carbonyl groups, the C=C aromatic or ethylenic and alkyl were observed as strong band peaks at 1.658-1.507 and 1.459cm^{-1}, respectively. The ^{1}proton NMR (Figures 4), the protons of methylene connecting N of dimethylamine or N-methylpiperazine to aromatic ring appeared as a singlet at 3.71 ppm. Six protons from dimethylamine group in **2a** appeared as singlet peak at 2.34 ppm, eight protons from piperazine group in **2b** appeared as multiplet peak at 2.99ppm, and three protons of N-methylpinerazine group in **2b** appeared as a singlet at 2.31ppm. While, the two protons from the ethenyl chain appeared as two singlets at 7.61-7.67ppm (1H, respectively) confirming the structures were asymmetric (Silverstein *et al.*, 2005). The structures elucidation was supported further by ^{13}carbon NMR (Figures 5 and mass spectra (Figures 6). All data confirmed full agreement with the structures expected.

Table I. Physical and spectral data of compound **2a and 2b**

Compd	Physical	Spectra			
		Infra red (cm^{-1})	^{1}Proton NMR, δ (ppm)	^{13}Carbon NMR, δ (ppm)	Massa (M+H^{+}) (m/z)
2a	Red crystal, mp. 100-102^{o}C	2,931-2,829, 1,658, 1,554, 1,507, 1,459, 1,246, 1,138, and 1,023.	7.67 (1H,s), 7.62 (1H,s), 7.50 (2H,d), 7.07 (1H,s), 7.03 (2 H,d), 6.91(1H,s), 3.86 (3H,s), 3.83 (3H,s), 3.72 (2H,s),2.94 (4H,m), 2.34 (6H,s), 1.78 (2H,m)	189 (1C), 162 (1C), 150 & 149 (2C), 137-115 (13C), 63 (1C), 55 & 56 (2C), 45 (2C), 28-23 (3C)	Found: 408.21680 Calc for neutral mass of C25H29NO4 = 407.20966; Mass Error = -0.3 mDa
2b	Yellow crystal, mp. 123-126^{o}C	2,935-2,864, 1,655, 1,589, 1,508, 1,459, 1,246, 1,142, and 1,023.	7.67 (1H,s), 7.62 (1H,s), 7.56 (2H,d), 7.01 (1H,s), 6.99 (1H,s), 6.97 (2H,d), 3.84 (3H,s), 3.83 (3H,s), 3.75 (2H,s), 2.92(4H,m), 2.59 (8H,m),2.30 (3H,s), 1.80 (2H, m)	192 (1C), 161 (1C), 149 & 148 (2C), 138-114 (13C), 59 (1C), 55 & 56 (2C),53 & 56 (4C), 46 (1C), 30-24 (3C)	Found: 463.25921 Calc for neutral mass of C28H34N2O4 = 462.25186; Mass Error = -0.3 mDa

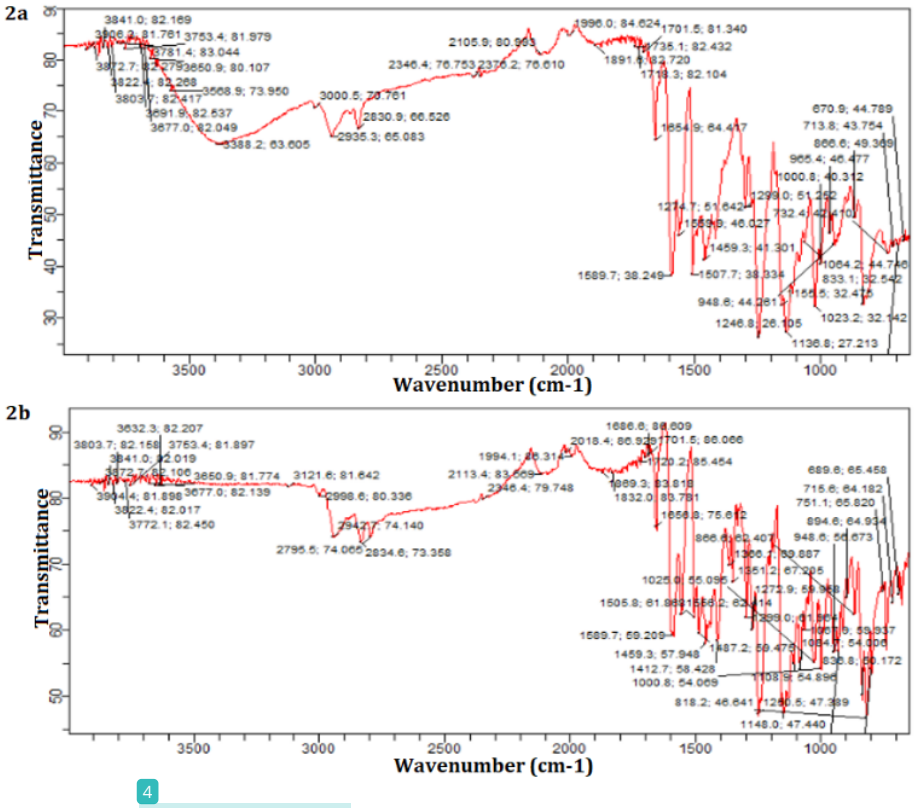


Figure 3. Infra red spectrum of **2a** and **2b**

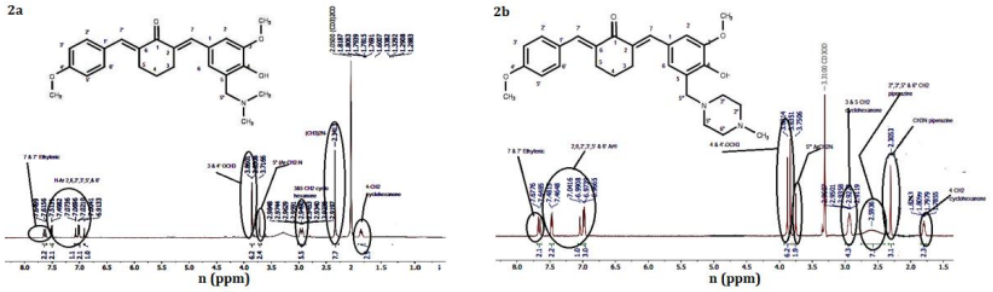


Figure 4. [1]Proton NMR spectrum of **2a** and **2b**

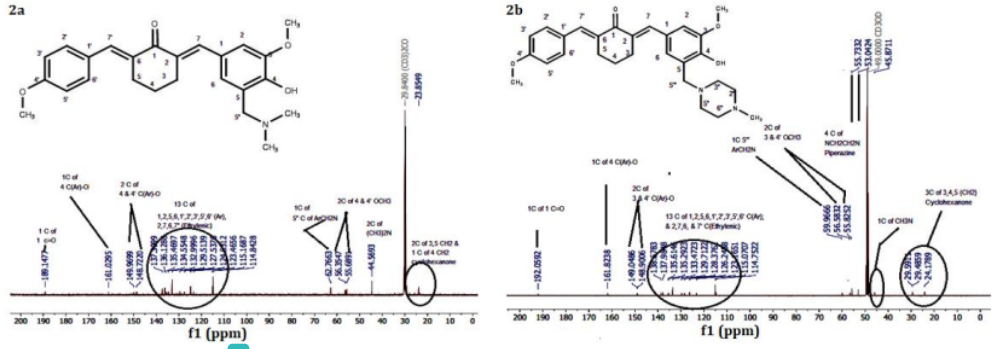


Figure 5. [13]Carbon NMR spectrum of **2a** and **2b**

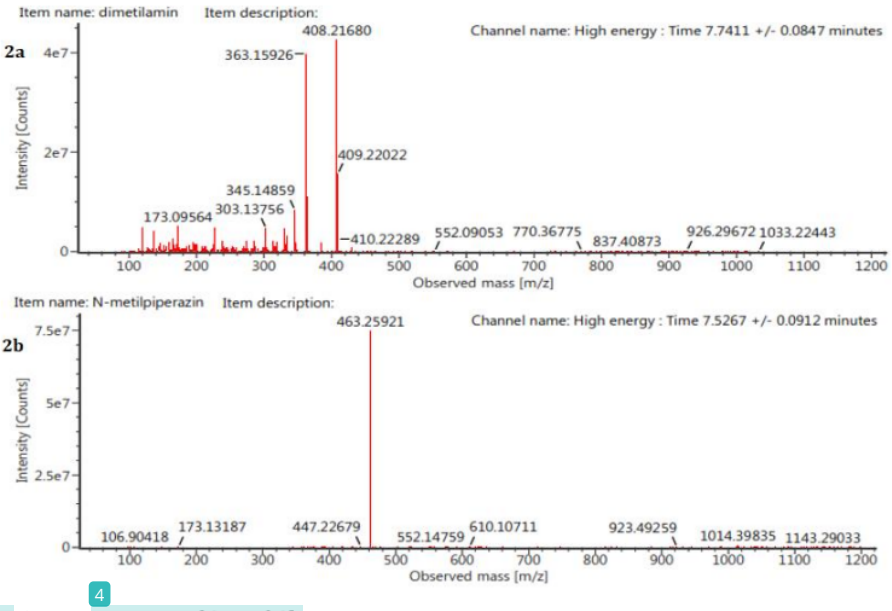


Figure 6. Mass spectrum of **2a** and **2b**

In-vitro Anti-inflammatory activity

Protein denaturation *in vivo* is one of the causes of inflammation. One of the mechanisms of antirheumatic activity of NSAIDs is by inhibiting this denaturation (Umapathy *et al.* 2010; Gunathilake *et al.*, 2018). Denaturation of protein can be induced by UV or heat. The denaturation is making protein aggregation, which can lead to the production of superoxide and nitric oxide by macrophages, which stimulates inflammation (Guzik *et al.*, 2003). Compounds inhibiting the heat-induced protein denaturation are considered to have potential anti-inflammatory activity (Chandra *et al.* 2012; Jagtap *et al.*, 2011).

Table II. Inhibition (%) of heat-induced albumin denaturation by 1.57 µM of the test compounds.

Compound	Inhibition (%) ± SD	
1	38.16	0.14
2a	33.17	0.06
2b	42.47	0.02
2c	38.86	0.04
2d	41.90	1.63
Cyclovalone	19.64	0.09
Diclofenac Sodium	35.27	0.02

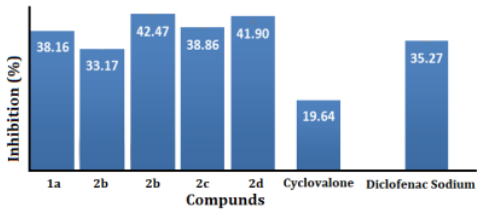


Figure 7. Inhibition ± SD (%) (n=3) of heat-induced albumin denaturation the Mannich base derivatives of 4'-methoxy-substituted of asymmetrical cyclovalone analogs (ACA) (2a-d) compared to the parent compound (1), cyclovalone and sodium diclofenac at a final concentration of 1.57µM.

The preliminary activity of the synthesized compounds (Table II and Figure 7). At a final concentration of 1.57µM indicated that all the Mannich bases inhibited the heat-induced protein denaturation ranging from 33.17–42.47%. This study found that the activity of **2b** [5-(N-methylpiperazino)methyl-4'-methoxy ACA] (42.47%) and **2d** (5-morpholinomethyl-4'-methoxy ACA) (41.90%) was higher than that of diclofenac sodium (35.27%), the parent compound **1** (38.16%), and cyclovalone (19.64%). It was in line with the result of the introduction of the Mannich bases to dehydrozingerone reported earlier (Hayun *et al.*, 2018). Therefore, the compounds have a prospect as a potential candidate for an anti-inflammatory agent. However, to ensure their biological activities, further study should be done using more specific methods.

CONCLUSION

Two Mannich base derivatives of asymmetrical cyclovalone analogs (ACA) were synthesized successfully. Their preliminary *in-vitro* anti-inflammatory evaluation indicated that the compound **2b** [5-(N-methylpiperazino)methyl-4'-methoxy ACA] and **2d** (5-morpholinomethyl-4'-methoxy ACA) exhibited the highest activity. Their biological activities were higher than diclofenac sodium. However, further study should be done using more specific methods.

ACKNOWLEDGMENT

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