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, cyclovalone, 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 instability and bioavailability (Prasad et al. 2014; Anand et al., 2008; Wang et al., 1997). The monocarbonyl 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-inflammatory (Zhang et al., 2014a; Zhang et al., 2014b; Aluwi et al., 2016). Introduction of morpholine Mannich base moiety into asymmetrical MACs bearing a cyclohexane 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 Mannich 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-dimethylaminomethyl-4'-methoxy ACA (2a) and 5-(N-methyl)piperazino)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-methoxybezyldiene)cyclohexanone (= 4'-methoxy ACA) (1), 5-diethylaminomethyl-4'-methoxy ACA (2c), 5-morpholinomethyl-4'-methoxy ACA (2d), and cyclovalone, were donated 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 (UNIF1, 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 2b.

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 0.5 mL of standard or test compounds solution in methanol (15.72 uM) or solvent (for control) and 4.5 mL Bovine Saline Albumin (BSA) 0.5% in tris-buffer saline pH 6.3 was incubated at 37°C for 15 min, then heated at 70°C in a water bath for 5 min, and cooled to reach room temperature. The turbidity was measured at 660 nm. The inhibition (%) of the denaturation was calculated with the formula:

\[
\text{% inhibition} = \left( \frac{A_c - A_s}{A_c} \right) \times 100\%
\]

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).

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

Infrared, 1H proton, and 13C 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 1,246-1,023 cm\(^{-1}\); C-H aliphatic at 2,935-2,829, while the \(\alpha,\beta\)-unsaturated carbonyl groups, the C=C aromatic or ethylenic and alkyl were observed as strong band peaks at 1,658-1,507 and 1,459 cm\(^{-1}\), respectively. The 1H proton NMR (Figures 4), the protons of methylene, or ethylene of N=methylpiperazine 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 pipеразин group in 2b appeared as multiplet peak at 2.99 ppm, and three protons of N-methylpiperazine group in 2b appeared as a singlet at 2.31 ppm. While, the two protons from the ethenyl chain appeared as two triplets at 7.61-7.67 ppm (1H, respectively) confirming the structures were asymmetric (Silverstein et al., 2005). The structures elucidation was supported further by 13C 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 | Infra red (cm\(^{-1}\)) | \(^1\)Proton NMR, \(\delta\) (ppm) | \(^13\)Carbon NMR, \(\delta\) (ppm) | Massa (M+H\(^+\)) (m/z) |
|--------|----------|-------------------------|----------------------------------|----------------------------------|--------------------------|
| 2a     | Red crystal, mp. 100-102°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 (2H,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, 149 (2C), 137-115 (13C), 63 (1C), 55 & 56 | Found: 408.21680; Calc for neutral mass of C25H29NO4 = 407.20966; Mass Error = -0.3 mDa |
| 2b     | Yellow crystal, mp. 123-126°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), 7.46 (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, 148 (2C), 138-114 (13C), 59 (1C), 55 & 56, 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
Mannich base derivatives of Asymmetrical Cyclovalone Analogs

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.

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