Transformation reaction of prenylated chalcone of pinostrobin derivative and their antibacterial activity

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Abstract. The transformation reaction of chalcone prenylation of pinostrobin derivatives has been done. The pinostrobin was isolated from Kaempferia pandurata rhizome which is the main component. The transformation reaction was approved by reacting monooxyprenylated chalcone (1) through zinc chloride (ZnCl₂) and toluene. The purification of the product was performed with chromatographic methods (radial chromatography and thin layer chromatography). Four compounds have been achieved from the transformation reaction, i.e. monocyclicprenylated chalcone (2), monocyclicprenylated pinostrobin (3), monoprenylated chalcone (4), and monoprenylated pinostrobin (5). The elucidation structures of the compounds were confirmed on the spectra of 1H NMR, 13C NMR, and mass spectrometry data. The antibacterial activity was valued using the minimum inhibitory concentration (MIC) against five bacteria, namely, Staphylococcus aureus ATCC 29737, Proteus mirabilis ATCC 21100, Bacillus subtilis ATCC 6633, Klebsiella pneumoniae ATCC 13733, and Escherichia coli O157:H7. The compound 4 showed a potential compound as antibacterial activity against Bacillus subtilis ATCC 6633 (MIC 7.8 µg/mL) and Proteus mirabilis ATCC 21100 (15.6 µg/mL) bacteria.

1. Introduction
Bacterial infections can cause some of the deadliest diseases and epidemics that are widespread in the world, especially in tropical countries. With increased bacterial resistance to antibiotic, it is necessary to develop new approaches and new antibacterial agents as an alternative to antimicrobial therapy [1].

Chalcones and their derivatives have been a major area of interest in recent years. Many research papers have been published, and chalcones continue to show promise for the investigation of new drugs. A new approach to the synthesis of chalcone derivatives has explored, which have revealed a series of pharmacological and biological effects [2].

Chalcones are derivatives of the 1,3-diaryl-2-propene-1-one parent compound consisting of two phenolic rings, which are related to as rings A and B. Most of them are natural and synthetic products having important pharmacological properties, such as antibacterial [3-5], anti-inflammatory [6], anticancer [7-9], and antioxidant agents [10] etc. Among these bioactivities, antibacterial activity is important because bacterial resistance to antibiotics is increasing.

To develop new antibacterial agents, the synthesis of chalcone derivatives can be done through transformation reactions. We have previously reported the transformation of pinostrobin that has isolated from Kaempferia pandurata rhizome. One of the products was prenylated chalcone. Therefore,
in this research, we will report the reaction of chalcone prenylation transformation from pinostrobin derivatives and investigate their antibacterial activity.

2. Experimental

2.1. General procedures
All of the chemicals used were bought from E. Merck and Sigma Aldrich. Solvents for purification of compounds were distilled before use. Purification of compounds was performed by radial chromatography (silica gel 60 PF254, E. Merck) and thin-layer chromatography (TLC) aluminum plates (silica gel 60 F254 E. Merck) and thin-layer chromatography (TLC) aluminum plates (silica gel 60 F254 E. Merck). Chemicals for transformation were prenyl bromide (Sigma Aldrich), K$_2$CO$_3$ (E. Merck), ZnCl$_2$ (E. Merck), acetone (E. Merck), and toluene (E. Merck). Pinostrobin was isolated from Kaempferia pandurata rhizome. NMR analysis (1H and 13C NMR) used CDCl$_3$ as a solvent at room temperature in an Agilent spectrometer (500 MHz, 125 MHz). The chemical shifts were expressed in δ (ppm) and the TMS was used as an internal standard. LCT Premier XE Micromass MS Technologies (HRESIMS) was used for mass spectra analysis. The melting points were measured in the Fischer tool and uncorrected. The following materials will be synthesized and purified according to the procedure described.

2.2. Transformation reaction of chalcone prenylated
We have previously obtained monooxyprenylated chalcone (1) from the pinostrobin derivative that it was isolated from the K. pandurata rhizome [11]. The transformation of monooxyprenylated chalcone (1) was carried out under the following reaction conditions: The compound 1 (102 mg; 0.3 mmol) and ZnCl$_2$ (41 mg; 0.3 mmol) as a catalys were dissolved into toluene (20 mL) and then stirred and refluxed (150°C) for 24 hours [12]. Furthermore, the resulting product was evaporated and obtained the product of 113.1 mg. The product was separated and purified by radial chromatography using a mixture of eluents n-hexane:EtOAc at a ratio of 97.5:2.5; 95:5:0.5; 9:1; 8:2. The radial chromatography purification has obtained four compounds, which were prenylated chalcone derivatives. The purity of the compounds was monitored by TLC and visualization was performed with UV lamp (254 nm) or sprayed with CeSO$_4$ (1.5% b/v) followed by heating in an oven with a temperature of 60°C.

2.3. Antibacterial test
The antibacterial test against five bacterial strains, i.e. Escherichia coli O157:H7, Staphylococcus aureus ATCC 29737, Klebsiella pneumoniae ATCC 13733, Proteus mirabilis ATCC 21100, and Bacillus subtilis ATCC 6633 were used microdilution method as recommended by Clinical and Laboratory Standards Institute (CLSI) [13]. Cultures of the bacteria were preserved on Mueller-Hinton Agar (Oxoid). In vitro antibacterial tests were carried out on 96-well microtiter plates to determine the MICs of compounds (2-5) using the standard broth microdilution method with an inoculum of 5-10$^5$ CFU/ml. A total of 100 µL with concentration 10$^{-4}$ µL of inoculum was inserted into the microplate starting from column number 12 to 2, while column number 1 was only filled with sterile MHB media (without inoculum). The dilutions were started from the wells in column 12 of the microtiter plates. Thus, column 12 conceived the highest concentrations and column 3 contained the lowest concentrations of the tested compounds (concentrations from 500 to 0.97 µg/mL). Column 2 provided as the positive control for all compounds and column 1 was the negative control (DMSO). The plates were incubated aerobically at 37°C for 24h. The chlorhexidine was used as positive control and each test compounds was run in duplicate. The MIC is the concentration of the lowest antimicrobial agent that can inhibit bacterial growth.
3. Results and Discussion

3.1. Transformation of monooxyprenylated chalcone (1)

The transformation reaction of monooxyprenylated chalcone (1) was outlined in figure 1. Figure 1 shows that the transformation reaction resulted in four compounds. The four compounds were monocyclicprenylated chalcone (2), monocyclicprenylated pinostrobin (3), monoprenylated chalcone (4), and monoprenylated pinostrobin (5). The compound 2 and 3 were 10.84% and 9.15% yields, respectively. Meanwhile, the compound 4 and 5 gave 11.34% and 4.60% yields, respectively. The transformation of compound 1 has produced various derivatives, the prenyl group undergoes a cyclization reaction forming the pyran ring for the compounds 2 and 3, while for compounds 4 and 5 the prenyl group was substituted on C-5’ and C-6 atoms. Based on the resulting product, the transformation reaction of compound 1 due to regioselectivity.

![Figure 1. Scheme of transformation reaction of monooxyprenylated chalcone (1).](image)

3.2. The elucidation structure of compounds 2-5

The structures of compounds 2-5 were confirmed by NMR and HRMS data. They were monocoicprenylated chalcone (2), monocyclicprenylated pinostrobin (3), monoprenylated chalcone (4), and monoprenylated pinostrobin (5). The NMR and HRMS data of these compounds were:

**Monocyclicprenylated chalcone (2).** Yield 10.84% as yellow solid; mp135-136°C; **1H NMR** Spectrum (CDCl3), δ (ppm): 14.16 (1H, s, OH-6’); 8.06 (1H, d, J=16.0 Hz, H-α); 7.73 (1H, d, J=15.6 Hz, H-β); 7.34-7.41 (5H, m, H-2, H-3, H-4, H-5, H-6); 6.04 (1H, s, H-5’); 3.85 (1H, s, 4’-OCH3); 2.58 (1H, t, H-1’); 1.81 (1H, t, H-2’); 1.44 (6H, s, 3H-4’, 3H-5’). ESI-HRMS (+) m/z: Anal. calc. for C21H22O5 (M+H)+:339.1596; found: 339.1608.

**Monocyclicprenylated pinostrobin (3).** Yield 9.15% as white solid; mp119-120°C; **1H NMR** Spectrum (CDCl3), δ (ppm): 7.36-7.48 (5H, m, H-2’, H-3’, H-4’, H-5’, H-6’); 6.11 (1H, d, J=13.4 Hz, H-2); 3.82 (3H, s, 7-OCH3); 3.02 (1H, dd, J=13.4; 16.5 Hz, H-3ax); 2.76 (1H, dd, J=2.8; 16.5 Hz, H-3eq); 2.56 (1H, t, H-1’); 1.78 (1H, t, H-2’); 1.41 (3H, s, H-5’); 1.38 (3H, s, H-4’). **13C NMR** Spectrum (CDCl3), δ (ppm): 189.1 (C-4’); 163.3 (C-7’); 163.2 (C-8a); 156.3 (C-5’); 139.3 (C-1’); 128.9 (C-3’, C-5’); 128.6 (C-4’); 126.2 (C-2’, C-6’); 106.0 (C-4a); 103.8 (C-6); 91.1 (C-8); 179.4 (C-2); 75.6 (C-3’); 55.8 (OCH3); 46.1 (C-3’); 31.6 (C-2’); 26.9 (C-5’); 26.6 (C-4’); 16.8 (C-1’). ESI-HRMS (+) m/z: Anal. calc. for C39H29O11 (M+H)+:339.1596; found: 339.1591.

**Monoprenylated chalcone (4).** Yield 11.34% as yellow solid; mp123-124°C; **1H NMR** Spectrum (CDCl3), δ (ppm): 12.06 (1H, s, OH-6’); 8.02 (1H, d, J=15.5 Hz, H-β); 7.81 (1H, d, J=15.5 Hz, H-α); 7.38-7.61 (5H, m, H-2, H-3, H-4, H-5, H-6); 6.07 (1H, s, H-3’); 5.20 (1H, t, H-2’); 3.83 (1H, s, 4’-OCH3); 3.36 (2H, d, H-1’); 1.84 (3H, s, H-4’); 1.77 (3H, s, H-5’). **13C NMR** Spectrum (CDCl3), δ (ppm): 193.6 (C-α); 166.1 (C-3’); 164.0 (C-6’); 163.5 (C-2’); 159.4 (C-1’); 142.9 (C-3’); 135.6 (C-1); 130.3 (C-β); 129.0 (C-2, C-6); 128.7 (C-3, C-4, C-5); 127.7 (C-α); 121.9 (C-2’); 105.9 (C-1’); 94.8 (C-3’);
92.4 (C-5’); 56.0 (OCH3, C-4’); 26.0 (C-4’’); 18.1 (C-5’”). ESI-HRMS (+) m/z: Anal. calc. for C21H22O4 (M+H)+: 339.1596; found: 339.1598.

**Monoprenylated pinostrobin (5):** Yield 4.60% as white solid; mp100-102 ºC; 1H NMR Spectrum (CDCl3), δ (ppm): 12.07 (1H, s, OH-5); 7.41-7.49 (5H, m, H-2’, H-3’, H-4’, H-5’, H-6’); 6.11 (1H, d, H-8); 5.42 (1H, dd, J=3.45; 13.25 Hz, H-2); 5.21 (1H, t, H-2’”); 3.85 (3H, s, 7-OCH3); 3.28 (2H, d, H-1’); 3.10 (1H, dd, J=13.25; 17.10 Hz, H-3ax); 2.83 (1H, dd, J=2.95; 17.10 Hz, H-3eq); 1.84 (3H, s, H-5’’); 1.77 (3H, s, H-4”). 13C NMR Spectrum (CDCl3), δ (ppm): 196.3 (C=O, C-4’); 165.9 (C-7); 161.9 (C-5’); 160.6 (C-8a); 138.9 (C-1’); 132.0 (C-3’’); 129.5 (C-2’, C-4’, C-6’); 126.7 (C-3’, C-5’’); 122.7 (C-2’’); 103.4 (C-4a); 110.8 (C-6); 80.1 (C-2); 91.4 (C-8); 56.3 (OCH3-7); 44.0 (C-3); 26.2 (C-4’’); 21.5 (C-1’’); 18.1 (C-5’”). ESI-HRMS (+) m/z: Anal. calc. for C21H22O4 (M+H)+: 339.1596; found: 339.1603.

### 3.3. Antibacterial activity

The compounds 2-5 were evaluated against five bacterial strains, mainly *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 29737, *Klebsiella pneumoniae* ATCC 13733, *Proteus mirabilis* ATCC 21100, and *Bacillus subtilis* ATCC 6633. The antibacterial activity was shown by the MICs value, which was the lowest concentration that can inhibit bacterial growth. The results are presented in table 1.

**Table 1. Antibacterial activity of compounds 2-5.**

| MIC µg/mL | B. subtilis ATCC 6633 | S. aureus ATCC 29737 | P. mirabilis ATCC 21100 | K. pneumoniae ATCC 13733 | E. coli O157:H7 |
|-----------|----------------------|----------------------|------------------------|--------------------------|-----------------|
| 1         | >500                 | >500                 | >500                   | >500                     | >500            |
| 2         | >500                 | >500                 | >500                   | >500                     | >500            |
| 3         | >500                 | >500                 | >500                   | >500                     | >500            |
| 4         | 7.8                  | 31.3                 | 15.6                   | 62.5                     | 250             |
| 5         | >500                 | >500                 | >500                   | >500                     | >500            |
| Chlorhexidine | 3.9                  | 0.5                  | 0.5                    | 0.5                      | 0.5             |

a The concentration of compounds 1-5 were 1000 µg/mL.

b The concentration of chlorhexidine was 500 µg/mL.

The results of the MIC determination showed no antibacterial activity for compounds 1, 2, 3, and 5 (MIC >500 µg/mL). Meanwhile, the derived compound 4 indicated a significant antibacterial activity. The compound 4 (monoprenylated chalcone) had the highest activity against Gram-(+) *B. subtilis* bacteria (MIC of 7.8 µg/mL) and had moderate activity against *S. aureus* bacteria with MIC values 31.3 µg/mL. Monoprenylated chalcone (4) also had moderate activity against Gram-(+) *P. mirabilis* and *K. pneumoniae* bacteria with MIC values of 15.6 µg/mL and 62.5 µg/mL, respectively, but had weak activity against Gram-(+) *E. coli* bacteria with MIC values 250 µg/mL [14]. According to this results, compound 4 exhibited valuable antibacterial activity.

The mechanism of action of flavanoid group compounds in inhibiting growth or killing microbes is not yet widely known. The mechanism is estimated in various ways, namely damaging the cytoplasmic membrane, inhibiting protein synthesis, disrupting metabolism, inhibiting cell wall synthesis and inhibiting membrane cell synthesis [15].

### 4. Conclusion

Transformation of monooxyprenylated chalcone of pinostrobin derivative was resulted four compound derivatives i.e monocyclicprenylated chalcone (2), monocyclicprenylated pinostrobin (3), monoprenylated chalcone (4), and monoprenylated pinostrobin (5). The evaluation in vitro antibacterial test of these compounds was showed that monoprenylated chalcone (4) was a significant antibacterial activity and could be an alternative to a new antibacterial compound.
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