Cytotoxic activity of quinolinone Alkaloids and acylphloroglucinol from the leaves of Melicope denhamii

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Abstract. Two quinolinone alkaloids, N-methylflindersine (1), flindersine (2) and two acylphloroglucinols, evodionol (3), methylevodionol (4) were isolated from the leaves of Melicope denhamii. Structures of compounds were elucidated using spectroscopic methods such as UV, IR, HRESI MS, 1D and 2D NMR. Compounds 1-4 were evaluated for their cytotoxicity against P-388 cells, compound 2 showed moderate activity with IC50 4.86 μg/ml.

1. Introduction

Melicope locally known as ‘Ki Sampang’ belongs to the Rutaceae family, consisting of about 280 species where widely distributed in Asia, Australia, Africa and Polynesia. Phytochemical studies have shown that the species produce a variety of alkaloids [1-3], flavonoids [4], coumarins [5], and acylphloroglucinols [6], which exhibit various biological activities including antioxidant, anticancer, and antiinflammatory. In continuation of our research for investigation of alkaloid and acylphloroglucinol of Indonesian Melicope plants, we report the isolation of N-methylflindersine (1), flindersine (2) and two acylphloroglucinols, evodionol (3), methylevodionol (4) from the methanol extract of the leaves of Melicope denhamii. The chemical structure of compounds 1-4 were established by UV, IR, HRESIMS, 1D and 2D NMR, as well as by comparison with those related compounds previously reported. The cytotoxic activity against murine leukemia P-388 cells of isolated compounds from this species are also briefly described.

2. Methods

2.1 General experimental

Column chromatography and planar radial chromatography were carried out using silica gel 60 and silica gel 60 PF254 (Merck, Darmstadt, Germany). NMR spectra were measured on a JEOL JNM-ECA 400 MHz FTNMR spectrophotometer (Tokyo, Japan) in CDCl3 with TMS as the internal standard. Mass spectra were measured on an ESI-TOF Waters LCT Premier XE producing pseudo-molecular ions, [M+H]+ positive ion mode (Santa Clara, CA, USA). UV spectra were recorded in MeOH on a Shimadzu series 1800 UV-VIS spectrophotometer (Kyoto, Japan). IR spectra were recorded in KBr on a One Perkin Elmer instrument (Waltham, MA, USA).
2.2 Plant Material
The leaves of *M. denhamii* was collected from the conserved forest of Gunung Salak, Bogor, West Java, Indonesia on November 2016 and was identified by Mr. Ismail Rachman from the Herbarium Bogoriense, Bogor, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

2.3 Extraction and isolation
The air-dried leaves of *M. denhamii* (3.5 kg) was macerated with methanol at room temperature for 24 h two times and then evaporated under reduced pressure to give a dark brown residue (250 g). The extract was redissolved in MeOH-water (9:1) and partitioned with *n*-hexane (201 g) and ethyl acetate (73 g) fractions. A part of ethyl acetate fraction (70 g) was subjected to vacuum liquid chromatography over silica gel and eluted with *n*-hexane-ethyl acetate (from 9:1 to 1:9) to give fractions A-C. Fraction A (5.14 g) was added to column chromatography and eluted with *n*-hexane-ethyl acetate (from 9:1 to 7:3) to produce subfractions A<sub>1</sub>-A<sub>2</sub>. Subfraction A<sub>2</sub> was purified by planar radial chromatography using *n*-hexane-chloroform (from 9:1 to 7:3) to yield compound 3 (68 mg) and 4 (57 mg). Fraction C (625 mg) was refractionated using column chromatography and eluted *n*-hexane-ethyl acetate (from 9:1 to 7:3) to yield subfractions C<sub>1</sub>-C<sub>2</sub>. Subfraction C<sub>1</sub> was purified by planar radial chromatography using *n*-hexane-chloroform (from 9:1 to 1:1) to yield compound 1 (50 mg) and 2 (10 mg).

2.4 Cytotoxic assay
Cytotoxic properties of the isolated compounds 1-4 against murine leukemia P-388 cells was evaluated according to the MTT method as previously described [10-12]. Artonin E was used as the positive control.

3. Result and Discussion
Phytochemical study on the MeOH extract from the leaves of *M. denhamii* yielded N-methylflindersine (1), methylflindersine (2), evodionol (3), and methyl-evodionol (4).

![Compounds 1-4](image)

**Figure 1.** Compounds 1-4 isolated from the leaves of *M. denhamii.*
N-Methylflindersine (1), was isolated as a yellow solid, m.p. 84-86°C, showed a quasimolecular ion [M+H]$^+$ at $m/z$ 242.1180 corresponding to the molecular formula C$_{15}$H$_{15}$NO$_2$. The UV maximum absorption at $\lambda_{\text{max}}$ 226 (4.29), 285 (3.20), 333 (3.65), 348 (3.69) and 365 (3.52) nm typical for a quinolinone skeleton [1]. The IR spectrum of 1 indicated absorptions for conjugated carbonyl (1641 cm$^{-1}$), aromatic (1581 and 1411 cm$^{-1}$) and ether (1188 cm$^{-1}$) groups, respectively. The $^1$H NMR spectrum of 1 (Table 1) showed the presence of four aromatic proton signals corresponding to 1,2-disubstituted benzena ring $[\delta _H 7.95 \text{(1H, } d, J = 7.8; 1.5 \text{ Hz)}, 7.53 \text{(1H, m), } 7.30 \text{(1H, } d, J = 8.5 \text{ Hz)}, 7.21 \text{(1H, } t, J = 7.8 \text{ Hz)}]$, three proton signals of 2,2-dimethylpyrano group $[\delta _H 6.74 \text{(1H, } d, J = 10.0 \text{ Hz)}, 5.56 \text{(1H, } d, J = 10.0 \text{ Hz)}]$, and proton singlet signal of N-methyl at $\delta _H$ 3.68. The $^{13}$C NMR spectrum of 1 showed 15 carbon signals and their assignments were determined by HMQC and HMBC spectra. The $^{13}$C NMR spectrum of 1 corresponding to the quinolinone skeleton. The structure of 1 were confirmed by HMQC and HMBC spectra (Figure 2). The 1D and 2D NMR spectra data are consistent with published data [7].

Flindersine (2), was isolated as a yellow solid, m.p. 197-199°C, showed a quasimolecular ion [M+H]$^+$ at $m/z$ 229.1180 corresponding to the molecular formula C$_{13}$H$_{13}$NO$_2$. The UV spectrum ($\lambda_{\text{max}}$ 222, 282, 331, 347 and 363 nm) and IR spectrum (1651, 1600, 1461 and 1191 cm$^{-1}$) absorptions were similarity to those of 1. The $^1$H NMR spectrum of 2 (Table 1) showed the presence of aromatic proton $[\delta _H 7.89 \text{(1H, } d, J = 8.0 \text{ Hz)}, 7.48 \text{(1H, } t, J = 7.6 \text{ Hz)}, 7.30 \text{(1H, } d, J = 8.2 \text{ Hz)}, 7.19 \text{(1H, } t, J = 7.6 \text{ Hz)}]$, and proton signals of 2,2-dimethylpyrano group $[\delta _H 6.76 \text{(1H, } d, J = 10.0 \text{ Hz)}, 5.56 \text{(1H, } d, J = 10.0 \text{ Hz)}]$, 1.54 (6H, s Hz)]. The $^1$H NMR spectrum of 2 were very similar to those of 1 but the has demethylized at N-methyl. The $^{13}$C NMR spectrum of 2 showed 14 carbon signals and the structure of 2 were confirmed by HMQC and HMBC spectra (Figure 2). The 1D and 2D NMR spectra data are consistent with published data [8].

Table 1. NMR spectroscopic data of compounds 1-2 in CDCl$_3$.

| No. | $\delta_H$ (mult, $J$ in Hz) | $\delta_C$ | HMBC | $\delta_H$ (mult, $J$ in Hz) | $\delta_C$ | HMBC |
|-----|--------------------------|-----------|------|--------------------------|-----------|------|
| 2   | -                        | 78.6      | -    | -                        | 79.2      | -    |
| 3   | 5.52 (d, 9.8)            | 126.2     | C-2; C-4a | 5.56 (d, 10.0) | 126.3 C-2; C-4a; C-11; C-12 |
| 4   | 6.74 (d, 9.8)            | 117.8     | C-2; C-10b | 6.76 (d, 10.0) | 117.2 C-2; C-5; C-10b |
| 4a  | -                        | 105.6     | -    | -                        | 105.8     | -    |
| 5   | -                        | 160.9     | -    | -                        | 162.3     | -    |
| 6a  | -                        | 139.2     | -    | -                        | 137.8     | -    |
| 7   | 7.29 (d, 8.5)            | 113.9     | C-9, C-10a | 7.30 (d, 8.2) | 115.9 C-9, C-10a |
| 8   | 7.52 (t, 7.8)            | 130.8     | C-6a, C-10 | 7.48 (t, 7.6) | 130.9 C-6a, C-10 |
| 9   | 7.21 (t, 7.8)            | 121.6     | C-7, C-10a | 7.19 (t, 7.6) | 122.2 C-7 |
| 10  | 7.95 (d, 7.8)            | 123.0     | C-6a, C-8 | 7.89 (d, 8.0) | 122.7 C-6a, C-8, C-10b |
Evodionol (3), was isolated as a yellow solid, the UV maximum absorption at $\lambda_{\text{max}}$; 217 (3.72), 226 (3.64), 272 (4.21), 295 (3.76), 306 (3.70), and 349 (3.13) nm characteristic for an acylphloroglucinol structure [6]. The $^1$H NMR spectrum of 3 (Table 2) showed the presence of a singlet signal of isolated aromatic proton at $\delta_H$ 5.88 and a singlet of methyl of acetyl at $\delta_H$ 2.59 typical of an acylphloroglucinol with five substituents [7]. The $^1$H NMR spectrum of 3 also showed the presence of a chelated hydroxyl at $\delta_H$ 14.29, a singlet of methoxyl at $\delta_H$ 3.85, and three proton signals of 2,2-dimethylpyrano group [$\delta_H$ 6.64 (1H, d, J = 10.0 Hz), 5.44 (1H, d, J = 10.0 Hz), 1.44 (6H, s Hz)]. The $^{13}$C NMR spectrum of 3 (Table 2) showed 14 carbon signals and confirmed by HMQC and HMBC spectra (Figure 2).

Methylevodionol (4), was isolated as a yellow oil, showed a quasimolecular ion [M+H]$^+$ at m/z 263.1283 corresponding to the molecular formula C$_{15}$H$_{18}$O$_4$. The UV maximum absorption at $\lambda_{\text{max}}$; 237 (3.80), 288 (4.30), 297 (4.31), and 340 (3.77) nm characteristic for an acylphloroglucinol structure [6]. The IR spectrum of 1 indicated absorptions for conjugated carbonyl (1622 cm$^{-1}$), and aromatic (1598 and 1439 cm$^{-1}$) and ether (1185 cm$^{-1}$) groups, respectively [9].

The $^1$H NMR spectrum of 4 were very similar to those of 3 (see Table 2). The major difference, compound 4 showed a methoxy group at C-8. The $^{13}$C NMR spectrum of 2 showed 14 carbon signals and the structure of 4 were confirmed by HMQC and HMBC spectra (Figure 2). The 1D and 2D NMR spectra data are consistent with published data [6].

### Table-2. NMR spectroscopic data of compounds 3-4 in CDCl$_3$.

| No. | $\delta_H$ (mult, $J$ in Hz) | $\delta_C$ | HMBC | $\delta_H$ (mult, $J$ in Hz) | $\delta_C$ | HMBC |
|-----|-----------------------------|------------|------|-----------------------------|------------|------|
| 2   | -                           | 78.2       | -    | -                           | 76.9       | -    |
| 3   | 5.44 (d, 10.0)              | 125.4      | C-2; C-5 | 5.51 (d, 10.0)           | 127.8       | C-2; C-5; C-13/14 |
| 4   | 6.64 (d, 10.0)              | 116.1      | C-2; C-10 | 6.46 (d, 10.0)           | 116.5       | C-2; C-5; C-6; C-10 |
| 5   | -                           | 102.8      | -    | -                           | 108.0       | -    |
| 6   | -                           | 161.9      | -    | -                           | 154.2       | -    |
| 7   | -                           | 105.7      | -    | -                           | 118.3       | -    |
The cytotoxic activity (Table 3) of compounds 1-4 were evaluated for their cytotoxicity by MTT assay against murine leukemia P-388. Artonin E was used as the positive control. Those cytotoxic data for alkaloids suggested that compound 1 was inactive and compound 2 has moderate activity. Methylation at nitrogen on compound 1 can be decreased activity. On the other hand, acylphloroglucinols, compound 4 more active than 3. In this case, methylation of compound 4 enhanced the activity.

Figure 2. Selected HMBC correlations for compounds 1-4
Table 3. Cytotoxic activity data of compounds 1-4 by MTT assay.

| No | Compound               | IC<sub>50</sub> (µg/mL) |
|----|------------------------|-------------------------|
| 1  | N-methylflindersine (1) | 21.06 ± 0.85            |
| 2  | Flindersine (2)        | 4.86 ± 0.30             |
| 3  | Evodionol (3)          | 46.46 ± 1.25            |
| 4  | Methylevodionol (4)    | 11.98 ± 0.65            |
| 5  | Artonin E              | 1.33 ± 0.18             |

4. Conclusions

The phytochemical investigation of the leaves of *M. denhamii* yielded compounds, N-methylflindersine (1), flindersine (2), evodionol (3), methylevodionol (4). Flindersine (2) showed moderate activity against murine leukemia P-388 cells.

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