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(R)-3-(8'-Hydroxyfarnesyl)-indole and other chemical constituents from the flowers of *Anomianthus dulcis* and their antimalarial and cytotoxic activities

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Abstract

A new farnesylindole, (R)-3-(8'-hydroxyfarnesyl)-indole (1), as a scalemic mixture (33% ee) along with nine known compounds (2-10), including one farnesylindole, three flavanones, three flavone derivatives and two chalcone derivatives were isolated from the methanolic crude extract of the flowers from *Anomianthus dulcis*. All compounds were purified by appropriate chromatographic techniques and their structures elucidated by spectroscopic methods. Compounds 1, 2 and 8 showed moderate antiplasmodial activities against TM4/8.Two and K1CB1 strains of which compound 2 displayed the best activity with IC₅₀ values of 27.9 ± 2.57 and 21.4 ± 1.68 μ M, respectively. In addition, compound 1 also presented modest cytotoxicity against a KB cell line with an IC₅₀ value of 22.3 ± 0.39 μ M. None of these compounds showed cytotoxicity against Vero cells.

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**(R)-3-(8'-Hydroxyfarnesyl)-indole and other chemical constituents
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(R)-3-(8'-Hydroxyfarnesyl)-indole and other chemical constituents from the flowers of *Anomianthus dulcis* and their antimalarial and cytotoxic activities

ABSTRACT

A new farnesylindole, (*R*)-3-(8'-hydroxyfarnesyl)-indole (**1**), as a scalemic mixture (33 % *ee*) along with nine known compounds (**2-10**), including one farnesylindole, three flavanones, three flavone derivatives and two chalcone derivatives were isolated from the methanolic crude extract of the flowers from *Anomianthus dulcis*. All compounds were purified by appropriate chromatographic techniques and their structures elucidated by spectroscopic methods. Compounds **1**, **2** and **8** showed moderate antiplasmodial activities against TM4/8.2 and K1CB1 strains of which compound **2** displayed the best activity with IC₅₀ values of 27.9 ± 2.57 and 21.4 ± 1.68 μ M, respectively. In addition, compound **1** also presented modest cytotoxicity against a KB cell line with an IC₅₀ value of 22.3 ± 0.39 μ M. None of these compounds showed cytotoxicity against Vero cells.

Keywords: *Anomianthus dulcis*; farnesylindole; antiplasmodial activities; cytotoxicity

1. Introduction

Anomianthus dulcis (synonym *Uvaria dulcis* or “Nom Maew Son” in Thai) belongs to the Annonaceae family which are distributed in Northeastern and Southern of Thailand (Smitinand T. 2014). The water decoction of the stem and roots of this plant has been used for fever treatment and as a galactagogue in traditional medicine (Sapchareun P. 2006). The chemical constituents of the twigs, stems and leaves have been identified as polyoxygenated cyclohexenes (Kaweetripob et al. 2015), acetogenins (Sinz et al. 1998a) phenylpropanoid amides (Sinz et al. 1999), flavonoids (Chantrapromma et al. 2001; Ubonopas et al. 2014) and alkaloids (Sinz et al. 1998b; Ubonopas et al. 2014). The crude extract and some secondary metabolites from this plant showed antibacterial (Ubonopas et al. 2014; Kadchumsang et al. 2015), anticancer, anti-Herpes Simplex Virus I (Ubonopas et al. 2014), antispasmodic, (Wiya et al. 2018) and antioxidant activities (Kadchumsang et al. 2015). Furthermore, previous investigations of plants from the *Uvaria* genus have found several types of secondary metabolites such as polyoxygenated cyclohexenes (Ho et al. 2015; Okpekon et al. 2015; Hsu et al. 2016; Auranwiwat et al.

2017; Awale et al. 2017; Auranwiwat et al. 2019a; Auranwiwat et al. 2019b), flavonoids (Salae et al. 2017; Auranwiwat et al. 2018), alkaloids (Auranwiwat et al. 2018), naphthalenes (Auranwiwat et al. 2017; Salae et al. 2017), xanthenes (Macabeo et al. 2014) and lignan glycosides (Nguyen et al. 2015). Some of these chemical constituents have shown significant bioactivities, including anticancer (Macabeo et al. 2014; Ho et al. 2015; Awale et al. 2017), anti-inflammatory (Hsu et al. 2016), antimalarial (Auranwiwat et al. 2017; Salae et al. 2017), antimicrobial (Okpekon et al. 2015), antiglycation and antioxidant activities (Thomas and Essien 2018). The crude extract of *U. angolensis* inhibited HIV-1 RNase H function and RDDP activity (Mfopa et al. 2017). Herein, we report on the phytochemicals from the flower extracts of *A. dulcis* and their antimalarial activities and cytotoxicities.

2. Results and discussion

The dried flowers of *A. dulcis* were extracted with methanol and the crude extract was separated using various chromatographic techniques which led to the isolation of a new indole alkaloid, (*R*)-3-(8'-hydroxyfarnesyl)-indole (**1**), and nine known compounds; 3-farnesylandole (**2**) (Nkunya et al. 1987), ($\pm$)-pinostrobin (**3**) (Chou et al. 2010), (+)-dihydrowogonin (**4**) (McNulty et al. 2009), (–)-pinocembrin (**5**) (Liu et al. 2016), chrysin (**6**) (Benabderrahmane et al. 2018), 2',3',4',5',6'-pentahydroxychalcone (**7**) (Xia et al. 2010), luteolin (**8**) (Jung et al. 2004), 5-*O*-caffeoylshikimic acid (**9**) (Wada et al. 1988) and kaempferol-3- β -D-(6-*O*-*trans*-*p*-coumaroyl)glucopyranoside (**10**) (Tsukamoto et al. 2004) as shown in Figure 1. The structures of these compounds were determined by spectroscopic methods, including 1D and 2D NMR spectroscopy, UV-vis spectrophotometry, IR and mass spectrometry.

Compound **1** $\{[\alpha]_D^{27} -6.9$ (*c* 0.15, CH₂Cl₂) $\}$ was obtained as a yellow gum with a molecular formula of C₂₃H₃₁NO from the HRESI-MS ion peak at *m/z* 360.2313 [M+Na]⁺ (calculated for C₂₃H₃₁NONa, 360.2303) which indicated an oxygenated derivative of **2**. The UV spectrum showed maximum absorption bands at λ_{max} 222 and 283 nm which corresponded to an indole derivative (Sangster et al. 1964). The IR spectrum showed stretching bands for a hydroxy group at 3415 cm⁻¹ and an alkene C=C at 1617 cm⁻¹. The former IR band indicated **1** was a hydroxy derivative of **2**. The ¹H NMR spectrum of compound **1** (Table S2) showed resonances similar to those of 3-farnesylandole (**2**),

except that one of the allylic methylene resonances in compound **2** was replaced by that of an oxymethine (δ_{H} 3.97) resonance. Compared to compound **2**, the C-6' olefinic proton resonance in compound **1** (δ_{H} 5.41) was observed at lower field (δ_{H} 5.14 in **2**) indicating the presence of a nearby hydroxy group substituent. The hydroxy group was attached at C-8' (δ_{C} 77.4) from the HMBC correlations of H-8' (δ_{H} 3.97) to C-6' (δ_{C} 126.2), C-7' (δ_{C} 136.9), **C-7'CH₃** (δ_{C} 11.8), C-9' (δ_{C} 34.3) and C-10' (δ_{C} 120.3). The configuration at C-8' was designated based on an analysis of its Mosher ester derivatives (Ohtani et al. 1991). Compound **1** was reacted with the Mosher reagents; (*R*)-(-)-MTPA-Cl and (*S*)-(+)-MTPA-Cl which gave the (*S*)-MTPA (**1a**) and (*R*)-MTPA (**1b**) esters, respectively. These reactions produced a 2:1 and 1:2 mixture of diastereomeric ester derivatives indicating that **1** was a scalemic mixture of 33 % *ee*. The ^1H NMR chemical shift differences ($\Delta\delta^{\text{SR}}$) of H-2', H-6', H_a-9' and H-10' were used to assign the (*R*)-configuration of the major enantiomer of **1** (Table S1). Therefore, the structure of **1** was assigned as (*R*)-(-)-3-(8'-hydroxy-3',7',11'-trimethyldodeca-2',6',10'-trienyl)-1*H*-indole with 33 % *ee*.

All isolated compounds, except for compounds **4** and **7**, which were not available in sufficient quantities, were evaluated for their antimalarial activities against *Plasmodium falciparum* (TM4/8.2 and K1CB1) and cytotoxicities against human mouth epidermal carcinoma cells (KB) and Vero cells (Table S3). The farnesylindoles **1** and **2** exhibited significant but modest activities against the *P. falciparum* strains, which were much better than the flavonoid derivatives. Compound **2** had IC₅₀ values of 27.9 ± 2.57 and 21.4 ± 1.68 μM against the TM4/8.2 and K1CB1 strains, respectively while compound **1** was slightly less active with IC₅₀ values of 30.3 ± 4.10 and 25.1 ± 1.61 μM , respectively. In terms of cytotoxicity, compound **1** (IC₅₀ = 22.3 ± 0.39 μM) displayed the highest activity against the KB cell line in comparison to the other isolated compounds. In addition, flavone **8** showed moderate activity against the TM4/8.2 and K1CB1 strains with IC₅₀ values at 36.3 ± 7.29 and 27.2 ± 6.20 μM , respectively. The tested compounds displayed little cytotoxicity against Vero cell with IC₅₀ values > 50 μM in these assays.

3. Experimental

For the details of the isolation of all compounds see the Supplementary material.

(*R*)-3-(8'-Hydroxyfarnesyl)-indole (**1**): yellow gum; $[\alpha]_{\text{D}}^{27}$ -6.9 (*c* 0.15, CH₂Cl₂); UV (MeOH) λ_{max} (log ϵ): 222 (4.3), 283 (3.6) nm; IR (neat) ν : 3415, 2924, 1617 cm⁻¹; ^1H

NMR (400 MHz, CDCl₃): δ_{H} 7.60 (*d*, *J* = 7.8 Hz, 1H, H-4), 7.35 (*d*, *J* = 7.8 Hz, 1H, H-7), 7.19 (*t*, *J* = 7.8 Hz, 1H, H-6), 7.12 (*t*, *J* = 7.8 Hz, 1H, H-5), 6.96 (*d*, *J* = 1.2 Hz, 1H, H-2), 5.47 (*t*, *J* = 6.3 Hz, 1H, H-2'), 5.41 (*t*, *J* = 6.5 Hz, 1H, H-6'), 5.09 (*t*, *J* = 7.7 Hz, 1H, H-10'), 3.97 (*dd*, *J* = 7.5 & 5.8 Hz, 1H, H-8'), 3.48 (*d*, *J* = 7.1 Hz, 2H, H-1'), 2.12–2.29 (*m*, 6H, H-4', 5', 9'), 1.78 (*s*, 3H, H-3'CH₃), 1.72 (*s*, 3H, H-11'CH₃), 1.64 (*s*, 3H, H-12'), 1.63 (*s*, 3H, H-7'CH₃); ¹³C NMR (100 MHz, CDCl₃): δ_{C} 136.9 (C-7'), 136.6 (C-8), 135.3 (C-3'), 134.8 (C-11'), 127.6 (C-9), 126.2 (C-6'), 123.4 (C-2'), 122.0 (C-6), 121.4 (C-2), 120.3 (C-10'), 119.2 (C-5), 119.1 (C-4), 116.0 (C-3), 111.2 (C-7), 77.4 (C-8'), 39.4 (C-4'), 34.3 (C-9'), 26.2 (C-5'), 26.0 (C-12'), 24.1 (C-1'), 18.1 (C-11'CH₃), 16.2 (C-3'CH₃), 11.8 (C-7'CH₃); (HRESI-MS *m/z* [M+Na]⁺ 360.2313 (calcd for C₂₃H₃₁NONa, 360.2303)).

4. Conclusions

A chemical investigation of the flower extracts of *Anomianthus dulcis* led to the isolation and identification of a new farnesylindole (**1**), which was a scalemic mixture (33 % *ee*), and nine known compounds (**2**–**10**). The farnesylindoles **1** – **2**, and compounds **9** – **10** were discovered for the first time from this plant. The farnesylindoles **1** and **2** and flavone **8** showed moderate biological activity against the *P. falciparum* strains (TM4/8.2 and K1CB1). Furthermore, compound **1** also showed significant cytotoxicity against a KB cell line. None of these compounds were cytotoxic to Vero cells.

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Disclosure statement

The authors declare no conflict of interest

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Figure 1. Structures of all isolated compounds (**1-10**).