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Publication Details

Sriyatep, T., Tantapakul, C., Andersen, R. J., Patrick, B. O., Pyne, S. G., Muanprasat, C., Seemakhan, S., Borwornpinyo, S. & Laphookhieo, S. (2018). Resolution and identification of scalemic caged xanthenes from the leaf extract of *Garcinia propinqua* having potent cytotoxicities against colon cancer cells. *Fitoterapia*, 124 34-41.

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Resolution and identification of scalemic caged xanthenes from the leaf extract of *Garcinia propinqua* having potent cytotoxicities against colon cancer cells

Abstract

A new scalemic 8,8a-dihydro caged xanthone (1) was isolated from the leaf extract of *Garcinia propinqua*. Five other known natural products, the three caged xanthenes (2, 5 and 6) and the two neocaged xanthenes, (3 and 4) were also isolated as scalemic mixtures. Their structures were characterized by spectroscopic methods. The enantiomeric ratios (er) of compounds 1-6 ranged from 1:0.7 to 1:0.9. These compounds were also resolved by semipreparative chiral HPLC. The absolute configurations of (+)-2 and (+)-3 were determined by single-crystal X-ray diffraction analysis using Cu K α radiation while the absolute configurations of the other compounds were determined by comparisons of their ECD spectra. Compounds (-)-4, (+)-4, (-)-5, (+)-5, and (-)-6 showed potent cytotoxicities against a colon cancer cell line HCT116 with IC₅₀ values of 2.60, 7.02, 1.47, 3.37, and 4.14 μ M, respectively, which were better than the standard control doxorubicin (IC₅₀ 9.74 μ M).

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

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1 **Resolution and identification of scalemic caged xanthenes from the leaf extract of *Garcinia***
2 ***propinqua* having potent cytotoxicities against colon cancer cells**

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24 Abstract: A new scalemic 8,8a-dihydro caged xanthone (**1**) was isolated from the leaf extract of
25 *Garcinia propinqua*. Five other known natural products, the three caged xanthenes (**2**, **5** and **6**)
26 and the two neocaged xanthenes, (**3** and **4**) were also isolated as scalemic mixtures. Their
27 structures were characterized by spectroscopic methods. The enantiomeric ratios (er) of
28 compounds **1-6** ranged from 1:0.7 to 1:0.9. These compounds were also resolved by
29 semipreparative chiral HPLC. The absolute configurations of (+)-**2** and (+)-**3** were determined by
30 single-crystal X-ray diffraction analysis using Cu K α radiation while the absolute configurations
31 of the other compounds were determined by comparisons of their ECD spectra. Compounds (–)-
32 **4**, (+)-**4**, (–)-**5**, (+)-**5**, and (–)-**6** showed potent cytotoxicities against a colon cancer cell line
33 HCT116 with IC₅₀ values of 2.60, 7.02, 1.47, 3.37, and 4.14 μ M, respectively, which were better
34 than the standard control doxorubicin (IC₅₀ 9.74 μ M).

35 *Keywords:* *Garcinia propinqua*, Scalemic caged xanthenes, Cytotoxicity, Electronic circular
36 dichroism

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47 **1. Introduction**

48 Caged xanthenes are commonly found in *Garcinia* (Clusiaceae) [1-23] and many of them
49 show interesting biological activities, especially cytotoxicity against several cancer cell lines [1-
50 3,5,6,12,13,19]. One of the best-known caged xanthenes is (-)-gambogic acid, a major
51 component from the exuded resin of *G. hanburyi*, first isolated in 1949 by Land and Katz [14]
52 which has been found to be cytotoxic for various cancer cell lines [21]. So far, over 100 caged
53 xanthenes have been isolated and identified from *Garcinia* [1-23]. Some of these have been
54 isolated as scalemic mixtures or reported to have specific rotations of relatively small
55 magnitudes [3,5,9,10,12,20]. In a previous study, we reported the isolation seven new scalemic
56 caged xanthenes from the stem bark extract of *G. propinqua*. Six of them were successfully
57 resolved by chiral HPLC and their absolute configurations were determined [22]. In continuation
58 of our study on the different parts of this plant (Fig. 1), we report herein the resolution and
59 absolute configuration assignment of six scalemic caged xanthenes, **1-6** (Fig. 2), isolated from
60 the leaf extract of *G. propinqua*, collected from Doi Tung, Chiang Rai Province, Thailand. The
61 cytotoxicities of the resolved caged xanthenes against a colon cancer cell line are also reported.

62 **2. Experimental**

63 *2.1. General*

64 Melting points were measured on a Buchi melting point B-540 visual thermometer. The
65 optical rotation values were measured with a Bellingham & Stanley APD440 polarimeter. The
66 UV spectra were recorded with a Perkin-Elmer UV-vis or Varian Cary 5000 UV-Vis-NIR
67 spectrophotometer. Infrared (IR) spectra were recorded on a PerkinElmer FTS FT-IR or
68 PerkinElmer Frontier Optica FT-IR spectrophotometers. Electronic circular dichroism spectra
69 were recorded on a JASCO J-815 spectrometer. The 1D and 2D NMR spectra data were obtained

70 on a 600 MHz Bruker AV-600 spectrometers in CDCl₃ with TMS as the internal standard.
71 Chemical shifts are reported in parts per million (δ), and coupling constants (J) are expressed in
72 hertz. Low- and high-resolution MS were recorded using ESI ionization and a TOF mass
73 analyzer. Chiral HPLC was performed on a CHIRALPAK IA 15 column of 10 \times 250 mm or
74 CHIRALCEL OD-H column of 4.6 \times 250 mm and attached to Waters 2487 Dual λ Absorbance
75 detector. Quick column chromatography (QCC) and column chromatography (CC) were carried
76 out on silica gel 60 H (5-40 μ m, SiliCycle Inc.) and silica gel 100 (63-200 μ m, SiliCycle Inc.),
77 respectively. Sephadex LH-20 was also used for CC. Precoated plates of silica gel 60 F₂₅₄ were
78 used for analytical purposes.

79 *2.2 Plant material*

80 The leaves of *G. propinqua* were collected from Doi Tung, Chiang Rai Province,
81 Thailand in September 2011. The plant was identified by Mr. Matin Van de Bult (Doi Tung
82 Development Project, Chiang Rai, Thailand), and the specimen (MFU-NPR0090) was deposited
83 at the Natural Products Research Laboratory, School of Science, Mae Fah Luang University.

84 *2.3. Extraction and isolation*

85 Chopped and dried leaves of *G. propinqua* (2.5 kg) were extracted with MeOH (10 L) for
86 three days at room temperature. Removal of the solvent under reduced pressure provided the
87 crude extract (196 g), which was partitioned between water and CH₂Cl₂. The CH₂Cl₂ extract (96
88 g) was subjected to QCC over silica gel (100% hexanes to 100% EtOAc) to give 24 fractions
89 (P1-P24). Fraction P3 (300 mg) was further fractionated by QCC (100% hexanes to 100%
90 EtOAc) to afford seven subfractions (P3A-P3G). Subfraction P3D (100 mg) was further
91 fractionated by CC (3:7 CH₂Cl₂/hexanes) to provide five subfractions (P3D1-P3D5). Compound
92 **7** (2.3 mg) were obtained from subfraction P3D2 (10 mg) by PLC (1:19 EtOAc/hexanes).

93 Subfraction P3C (10 mg) was further separated by PLC (1:19 EtOAc/hexanes) to give
94 compounds **16** (2.3 mg) and **8** (2.9 mg). Compounds **5** (13.9 mg), **3** (8.7 mg), **6** (14.4 mg), and
95 **11** (4.8 mg) were obtained from fraction P14 (62 mg), P15 (55 mg), P11 (50 mg), and P7 (30
96 mg), respectively, by washing with hexanes. Fraction P8 (500 mg) was separated CC (1:9
97 acetone/hexanes) to give three subfractions (P8A-P8C). Compound **12** (6.0 mg) was obtained
98 from subfraction P8C (42 mg), whereas compound **9** (10.8 mg) was isolated from subfraction
99 P8B (30 mg) by Sephadex LH-20 (100% MeOH). Compound **4** (9.5 mg) was obtained from
100 subfraction P8A (50 mg) by CC (3:2 CH₂Cl₂/hexanes). Fraction P21 (500 mg) was further
101 separated by Sephadex LH-20 (100% MeOH) to give compound **13** (13.0 mg). Fraction P9 (200
102 mg) was fractionated by Sephadex LH-20 (100% MeOH) to give three subfractions (P9A-P9C).
103 Compound **2** (28.7 mg) was isolated from subfraction P9B (40 mg) by CC (1:9 acetone/hexanes).
104 Fraction P18 (300 mg) was washed with hexanes to give compound **14** (4.5 mg) and the mother
105 liquor of P18 was further separated by Sephadex LH-20 (100% MeOH) to give three
106 subfractions (P18A-P18C). Compound **1** (16.2 mg) was isolated from subfraction P18A (50 mg).
107 Finally, fraction P16 (200 mg) was separated by CC (1:9 acetone/hexanes) to give compounds **10**
108 (**4.3 mg**), **17** (5.3 mg), and **15** (3.4 mg).

109 *2.4. Compound 1*

110 White solid; m.p. 221–223 °C; UV (MeOH) λ_{\max} (log ϵ) 219 (3.81), 297 (3.65), and 337
111 (2.92) nm; IR (neat) ν_{\max} 3436, 1740, 1638 cm⁻¹; ¹H NMR and ¹³C NMR data, see **Tables 1 and**
112 **2**; ESITOFHRMS m/z 497.2547 [M+H]⁺ (calcd for C₂₉H₃₇O₇, 497.2539).

113 *2.5 Resolution of compound 1 and ECD spectroscopic data of (-)-1 and (+)-1*

114 Resolution of the two enantiomers of **1** (15.0 mg) was performed by semi-preparative
115 HPLC on a chiral column (CHIRALPACK IA 15 μ L, 10 \times 250 mm, flow rate 1 mL/min, *n*-

116 hexane/*i*PrOH, 99:1 v/v). Compound (–)-**1** (t_R 90 min) [(7.3 mg), $[\alpha]_D^{24}$ –66.6 (*c* 0.1, CHCl₃);
117 ECD (MeOH) λ_{max} ($\Delta\epsilon$) 224 (+0.85 × 10²) and 306 (–0.88×10²) nm] and (+)-**1** (t_R 95 min) [(4.7
118 mg), $[\alpha]_D^{24}$ +56.5 (*c* 0.1, CHCl₃); ECD (MeOH) λ_{max} ($\Delta\epsilon$) 224 (–0.92 × 10²) and 306 (+0.99 ×
119 10²) nm] were obtained.

120 2.6. Compound 2

121 Yellow solid; m.p. 180–184 °C; UV (MeOH) λ_{max} (log ϵ) 216 (3.16), 310 (2.64), 328
122 (2.83), and 355 (2.96) nm; IR (neat) ν_{max} 3462, 1739, 1635 cm^{–1}; ¹H and ¹³C NMR data, see
123 **Tables 1 and 2**; ESITOFHRMS m/z 487.2094 [M+Na]⁺ (calcd for C₂₈H₃₂NaO₆, 487.2097).

124 2.7 Resolution of compound 2 and ECD spectroscopic data of (–)-**2** and (+)-**2**

125 Resolution of two enantiomers of **2** (10.0 mg) was performed by semi-preparative HPLC
126 on a chiral column (CHIRALCEL OD-H, 4.6 × 250 mm, flow rate 1 mL/min, *n*-hexane/*i*PrOH,
127 98:2 v/v). Compound (–)-**2** (t_R 45 min) [(3.5 mg), $[\alpha]_D^{24}$ –24.2 (*c* 0.07, CHCl₃); ECD (MeOH)
128 λ_{max} ($\Delta\epsilon$) 209 (+0.32 × 10²), 219 (–0.04 × 10²), 236 (+0.09 × 10²), 262 (+0.31 × 10²), 280 (+0.42
129 × 10²), 306 (+0.39 × 10²), and 350 (–1.13 × 10²) nm] and (+)-**2** (t_R 48 min) [(2.9 mg), $[\alpha]_D^{24}$
130 +38.2 (*c* 0.06, CHCl₃); ECD (MeOH) λ_{max} ($\Delta\epsilon$) 208 (–0.64 × 10²), 227 (+0.03 × 10²), 239 (–0.12
131 × 10²), 263 (–0.30 × 10²), 280 (–0.40 × 10²), 306 (–0.35 × 10²), and 350 (+0.88 × 10²) nm].

132 2.8. Compound 3

133 Yellow solid; m.p. 178–182 °C; UV (MeOH) λ_{max} (log ϵ) 213 (3.23), 252 (2.61), and 342
134 (3.02) nm; IR (neat) ν_{max} 3406, 1749, 1634 cm^{–1}; ¹H and ¹³C NMR data, see **Tables 1 and 2**;
135 ESITOFHRMS m/z 501.2254 [M+Na]⁺ (calcd for C₂₉H₃₄NaO₆, 501.2253).

136 2.9. Resolution of compound 3 and ECD spectroscopic data of (–)-**3** and (+)-**3**

137 Resolution of the two enantiomers of **3** (8.4 mg) was performed by the method described
138 for **1**, to give compounds (–)-**3** (t_R 70 min) [(4.0 mg), $[\alpha]_D^{24} -27.7$ (c 0.08, MeOH); ECD (MeOH)
139 $\lambda_{\max} (\Delta\epsilon)$ 232 ($+0.82 \times 10^2$), 260 ($+0.52 \times 10^2$), 310 (-0.53×10^2), 348 ($+0.13 \times 10^2$), and 360
140 ($+0.14 \times 10^2$) nm] and (+)-**3** (t_R 75 min) [(3.6 mg), $[\alpha]_D^{24} +34.4$ (c 0.07, MeOH); ECD (MeOH)
141 $\lambda_{\max} (\Delta\epsilon)$ 231 (-1.11×10^2), 258 (-0.67×10^2), 304 ($+0.65 \times 10^2$), 316 ($+0.72 \times 10^2$), 345 (-0.08
142 $\times 10^2$) and 358 (-0.25×10^2) nm].

143 2.10. Compound **4**

144 Yellow solid; m.p. 187–192 °C; UV (MeOH) $\lambda_{\max} (\log \epsilon)$ 211 (3.48), 257 (2.94), 306
145 (2.89), and 343 (3.23) nm; IR (neat) ν_{\max} 3387, 1750, 1637 cm^{-1} ; ^1H and ^{13}C NMR data, see
146 **Tables 1 and 2**; ESITOFHRMS m/z 463.2125 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{28}\text{H}_{31}\text{O}_6$, 463.2121).

147 2.11. Resolution of compound **4** and ECD spectroscopic data of (–)-**4** and (+)-**4**

148 Resolution of the two enantiomers of **4** (13.0 mg) was performed by the method
149 described for **2**, to give compounds (–)-**4** (t_R 18 min) [(5.2 mg), $[\alpha]_D^{24} -28.3$ (c 0.1, MeOH); ECD
150 (MeOH) $\lambda_{\max} (\Delta\epsilon)$ 238 ($+0.61 \times 10^2$), 262 ($+0.49 \times 10^2$), 301 (-0.34×10^2), and 341 (-0.22×10^2)
151 nm] and (+)-**4** (t_R 21 min) [(4.8 mg), $[\alpha]_D^{24} +37.4$ (c 0.1, MeOH); ECD (MeOH) $\lambda_{\max} (\Delta\epsilon)$ 237
152 (-0.72×10^2), 262 (-0.57×10^2), 301 ($+0.39 \times 10^2$), and 341 ($+0.24 \times 10^2$) nm].

153 2.12. Compound **5**

154 Yellow solid; m.p. 140–144 °C; UV (MeOH) $\lambda_{\max} (\log \epsilon)$ 218 (3.20), 297 (2.75), 326
155 (2.68), and 350 (2.75) nm; IR (neat) ν_{\max} 3456, 1737, 1636 cm^{-1} ; ^1H and ^{13}C NMR data, see
156 **Tables 1 and 2**; ESITOFHRMS m/z 501.2249 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{29}\text{H}_{34}\text{NaO}_6$, 501.2253).

157 2.13. Resolution of compound **5** and ECD spectroscopic data of (+)-**5** and (–)-**5**

158 Resolution of the two enantiomers of **5** (20.0 mg) was performed by the method
159 described for **2**, to give compounds (-)-**5** (t_R 5 min) [(4.6 mg), $[\alpha]_D^{24}$ -74 (c 0.1, MeOH); ECD
160 (MeOH) λ_{max} ($\Delta\epsilon$) 210 ($+0.44 \times 10^2$), 234 (-0.13×10^2), 260 ($+0.12 \times 10^2$), 285 ($+0.20 \times 10^2$),
161 313 ($+0.29 \times 10^2$), and 350 (-0.60×10^2) nm] and (+)-**5** (t_R 7 min) [(4.9 mg), $[\alpha]_D^{24}$ +58 (c 0.1,
162 MeOH); ECD (MeOH) λ_{max} ($\Delta\epsilon$) 235 ($+0.04 \times 10^2$), 264 (-0.09×10^2), 283 (-0.10×10^2), 315
163 (-0.12×10^2), and 350 ($+0.34 \times 10^2$) nm].

164 2.14. Resolution of compound **6** and ECD spectroscopic data of (+)-**6** and (-)-**6**

165 Resolution of the two enantiomers of **6** (20.0 mg) was performed by the method
166 described for **2**, to give compounds (-)-**6** (t_R 15 min) [(3.0 mg), $[\alpha]_D^{24}$ -83 (c 0.06, MeOH); ECD
167 (MeOH) λ_{max} ($\Delta\epsilon$) 211 ($+1.10 \times 10^2$), 229 (-0.42×10^2), 263 ($+0.47 \times 10^2$), 282 ($+0.67 \times 10^2$),
168 310 ($+0.69 \times 10^2$), and 352 (-1.65×10^2) nm] and (+)-**6** (t_R 17 min) [(3.5 mg), $[\alpha]_D^{24}$ +96 (c 0.07,
169 MeOH); ECD (MeOH) λ_{max} ($\Delta\epsilon$) 209 (-0.87×10^2), 229 ($+0.29 \times 10^2$), 265 (-0.38×10^2), 282
170 (-0.51×10^2), 310 (-0.53×10^2), and 352 ($+1.30 \times 10^2$) nm].

171 2.15. X-ray crystallographic analysis of compounds (+)-**2**, (+)-**3** and scalemic mixture **4**

172 Single-crystal X-ray diffraction analysis was collected on a Bruker APEX DUO
173 diffractometer with cross-coupled multilayer optics Cu-K α radiation. Data were corrected for
174 absorption effects using the multi-scan technique (SADABS). The structure was solved by direct
175 methods.

176 Single-crystal X-ray data for (+)-**2**: Yellow tablet crystal of C₂₈H₃₂O₆, $M = 464.53$,
177 crystal system orthorhombic with $a = 10.0612(7)$ Å, $b = 11.6588(8)$ Å, $c = 19.8385(13)$ Å, $\alpha =$
178 90° , $\beta = 90^\circ$, $\gamma = 90^\circ$, $v = 2327.1(3)$ Å³, and chiral group $P2_12_12_1$, $z = 4$. The X-ray diffraction
179 analysis using Cu-K α radiation values were 7.50 cm^{-1} , 22624 reflections measured, 4027

180 independent reflections ($R_{\text{int}} = 0.035$). Final R indices: $R_1 = 0.029$ and $wR_2 = 0.074$. The standard
181 deviation of an observation of unit weight was 1.03. The absolute configurations of (+)-**2** was
182 assigned as $5S, 7R, 10aR, 22R$ (Fig. 4) with a Flack x -parameter of 0.06(5).

183 Single crystal X-ray data for (+)-**3**: Yellow tablet crystal of $C_{29}H_{34}O_6$, $M = 478.56$, crystal
184 system monoclinic with $a = 11.7599(7)$ Å, $b = 36.717(2)$ Å, $c = 17.2160(9)$ Å, $\alpha = 90^\circ$, $\beta =$
185 $90.767(3)^\circ$, $\gamma = 90^\circ$, $v = 7433.0(7)$ Å³, and chiral group $P2_1$, $z = 12$. The x-ray diffraction analysis
186 using Cu- $K\alpha$ radiation values were 7.19 cm⁻¹, 97789 reflections measured, 26173 independent
187 reflections ($R_{\text{int}} = 0.070$). Final R indices: $R_1 = 0.056$ and $wR_2 = 0.142$. The standard deviation of
188 an observation of unit weight was 1.04. The absolute configurations of compound (+)-**3** was
189 assigned as $6S, 7R, 10aS, 22S$ (Fig. 4) with a Flack x -parameter of -0.12(8).

190 Single-crystal X-ray data for scalemic mixture **4**: Yellow irregular crystal of $C_{28}H_{32}O_6$, M
191 = 464.53, crystal system orthorhombic with $a = 19.5660(8)$ Å, $b = 8.0604(3)$ Å, $c = 14.3543(6)$
192 Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, $v = 2263.81(6)$ Å³, and chiral group $Pca2_1$, $z = 4$. The x-ray
193 diffraction analysis using Cu- $K\alpha$ radiation values were 7.71 cm⁻¹, 12938 reflections measured,
194 3570 independent reflections ($R_{\text{int}} = 0.039$). Final R indices: $R_1 [I > 2.00\sigma(I)] = 0.032$ and $wR_2 =$
195 0.083 , respectively. The standard deviation of an observation of unit weight was 1.06.

196 The X-ray Crystallographic data for compounds (+)-**2**, (+)-**3** and scalemic mixture **4** have
197 been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC
198 1539175 for (+)-**2**, CCDC 1539176 for (+)-**3** and CCDC 1539178 for scalemic mixture **4**. These
199 data can be obtained free of charge from the Cambridge Crystallographic Data Centre via
200 www.ccdc.cam.ac.uk/data_request/cif.

201 *2.16. Cytotoxic Assay*

202 Determination of the cytotoxicities of compounds on colon cancer cell (HCT116,
203 American Type Culture Collection (ATCC), Manassas, VA, USA) was performed by the
204 previous method [22]. Briefly, HCT116 cells (1×10^4 cells/well) were cultured in 96 well plate
205 at 37 °C for 24 h which were treated with compounds (10 μ M in DMSO) in DMEM medium for
206 24 h. The medium was removed and fresh DMEM containing 0.5 mg/mL of MTT solution was
207 added for 2 h. After removal of medium, the violet formazan crystals were dissolved in DMSO
208 (100 μ L) and the absorbance was measured at 570 nm using a microplate reader. Doxorubicin
209 (IC_{50} 9.74 μ M) was used as a positive control.

$$210 \quad \% \text{cell viability} = \frac{\text{absorbance of treated well}}{\text{absorbance of control well}} \times 100$$

$$211 \quad \% \text{cytotoxicity} = 100 - \% \text{cell viability}$$

212

213 3. Results and discussion

214 Compound **1** was obtained as a white solid with m.p. 221–223 °C. Its molecular formula
215 was determined as $C_{29}H_{36}O_7$, from its protonated molecular ion at m/z 497.2547 $[M+H]^+$ (calcd
216 for 497.2539) in the ESITOFHRMS. The UV spectrum showed absorption bands at λ_{max} 219,
217 297, and 337 nm. The IR spectrum contained absorption bands at 3436, 1638, and 1740 cm^{-1}
218 indicating the presence of a hydroxy group and conjugated carbonyl and unconjugated carbonyl
219 groups. The ^1H and ^{13}C NMR spectra of compound **1** (Table 1), which were similar to those of
220 doitunggarcinone F^{22} isolated from the stem bark of *G. propinqua*, displayed resonances for: a
221 H-bonded hydroxy proton [δ_{H} 12.09 (1H, s, OH-1)], an isolated aromatic proton [δ_{H} 6.08 (1H, s,
222 H-2)/ δ_{C} 99.5], a methoxy group [δ_{H} 3.29/ δ_{C} 55.7], three methine protons [δ_{H} 4.35 (1H, dd, J =
223 4.4, 1.4 Hz, H-8)/ δ_{C} 74.6, δ_{H} 2.88 (1H, dd, J = 4.5, 5.9 Hz, H-7)/ δ_{C} 44.7, δ_{H} 3.30 (1H, d, J = 1.4
224 Hz, H-8a)/ δ_{C} 47.2], a 1,1-dimethylallyl moiety [δ_{H} 6.24 (1H, dd, J = 10.5, 17.9 Hz, H-12)/ δ_{C}

225 149.2, δ_{H} 5.35 (1H, d, $J = 17.9$ Hz, H-13a)/ δ_{C} 113.4, δ_{H} 5.30 (1H, d, $J = 10.5$ Hz, H-13b)/ δ_{C}
226 113.4, δ_{H} 1.66 (3H, s, H₃-15)/ δ_{C} 27.3, and δ_{H} 1.62 (3H, s, H₃-14)/ δ_{C} 30.3], a (–OC(Me)₂–
227 CHCH₂–C–) unit [δ_{H} 2.54 (1H, d, $J = 8.7$ Hz, H-22)/ δ_{C} 43.9, δ_{H} 1.97 (1H, dd, $J = 6.3, 14.7$ Hz,
228 H-21a)/ δ_{C} 20.2, δ_{H} 1.39 (3H, s, H₃-25)/ δ_{C} 30.5, δ_{H} 1.38 (1H, dd, $J = 8.7, 14.7$ Hz, H-21b)/ δ_{C}
229 20.2, and δ_{H} 1.12 (3H, s, H₃-24)/ δ_{C} 27.4] and a prenyl group [δ_{H} 5.29 (1H, m, H-17)/ δ_{C} 117.8, δ_{H}
230 2.86 (1H, dd, $J = 14.6, 5.9$ Hz, H-16a), δ_{H} 2.69 (1H, dd, $J = 14.6, 9.1$ Hz, H-16b)/ δ_{C} 28.1, δ_{H}
231 1.66 (3H, s, H₃-20)/ δ_{C} 26.0 and δ_{H} 1.62 (3H, s, H₃-19)/ δ_{C} 18.0]. The only difference between the
232 structures of compound **1** and doitunggarcinone F is that compound **1** had a C-3 hydroxy group
233 (δ_{H} 6.96, s) while the latter compound had a C-3 methoxy group. In the HMBC spectrum, the C-
234 3 hydroxy group showed cross-peaks with C-3 (δ_{C} 165.5) and C-4 (δ_{C} 111.4) (Fig. 3). Compound
235 **1** was therefore identified as doitunggarcinone L.

236 Similar to doitunggarcinones F-K, previously isolated from the stem bark of this plant
237 [22], doitunggarcinone L (**1**) could be resolved by semipreparative chiral HPLC to give
238 compounds (–)-**1** (t_{R} 90 min), $[\alpha]_{\text{D}}^{24} -66.6$ (c 0.1, CHCl₃) and (+)-**1** (t_{R} 95 min), $[\alpha]_{\text{D}}^{24} +56.5$ (c
239 0.1, CHCl₃) in a ratio of ca 1:0.9 (Fig. S31, Supplementary material). The absolute
240 configurations of (–)-**1** and (+)-**1** were identified by comparisons of their ECD spectra with those
241 of (–)-doitunggarcinone F and (+)-doitunggarcinone F [22]. The ECD spectrum of (–)-**1** showed
242 a positive Cotton effect around 224 and a negative effect around 306 nm, while (+)-**1** exhibited
243 opposite signs of Cotton effects at those same wavelengths (Fig. 5), which were similar to those
244 of (–)-doitunggarcinone F²² and (+)-doitunggarcinone F [22], respectively. Thus, the absolute
245 configuration of (–)-**1** was assigned as *5R, 7S, 8S, 8aR, 10aR, 22S* and the configuration of (+)-**1**
246 was assigned as *5S, 7R, 8R, 8aS, 10aR, 22R*. Compounds (–)-**1** and (+)-**1** were assigned trivial
247 names as (–)-doitunggarcinone L and (+)-doitunggarcinone L, respectively.

248 Compounds **2-5** were identified as isobractatin (**2**) [5], 3-*O*-methylneobractatin (**3**) [10],
249 neobractatin (**4**) [10] and 3-*O*-methylbractatin (**5**) [10], by spectroscopic methods including
250 NMR, ESITOFHRMS, UV, and IR as well as comparisons with literature data. The structure of
251 compound **4** was also confirmed by single crystal X-ray diffraction analysis (Fig. 4). Compounds
252 **2-5** were previously isolated from *G. bracteata*, with all having specific rotations of relatively
253 small magnitudes. Isobractatin (**2**), $[\alpha]_D -3$ (*c* 0.58, CHCl₃), was first isolated from the leaf
254 extract of *G. bracteata* in 2000 by Thoison *et al.* [5] and was shown to be a scalemic mixture by
255 analytical chiral HPLC analysis [5]. Compounds **3-5** were first isolated from the twig extract of
256 *G. bracteata* by Na *et al.* in 2010 [10]. They had specific rotations of $[\alpha]_D^{26} +5.4$ (*c* 0.17, MeOH)
257 for **3** [10], $[\alpha]_D^{27} +9.4$ (*c* 0.24, MeOH) for **4** [10], and $[\alpha]_D^{24} -1.3$ (*c* 0.44, MeOH) for **5** [10]. In
258 this study, compounds **2-5** were resolved by semipreparative chiral HPLC. All of them were
259 scalemic mixtures with enantiomeric ratios (er) ranging from 1:0.7 to 1:0.9: Compound **2** (er =
260 1:0.8; (-)-**2**, t_R 45 min, $[\alpha]_D^{24} -24.2$ (*c* 0.07, CHCl₃)/(+)-**2**, t_R 48 min, $[\alpha]_D^{24} +38.2$ (*c* 0.06,
261 CHCl₃)), compound **3** (er = 1:0.9; (-)-**3**, t_R 70 min, $[\alpha]_D^{24} -27.7$ (*c* 0.08, MeOH)/(+)-**3**, t_R 75
262 min, $[\alpha]_D^{24} +34.4$ (*c* 0.07, MeOH), compound **4** (er = 1:0.7; (-)-**4**, t_R 18 min, $[\alpha]_D^{24} -28.3$ (*c* 0.1,
263 MeOH)/(+)-**4**, t_R 21 min, $[\alpha]_D^{24} +37.4$ (*c* 0.1, MeOH) and compound **5** (er = 1:0.8; (-)-**5**, t_R 5
264 min, $[\alpha]_D^{24} -74$ (*c* 0.1, MeOH)/(+)-**5**, t_R 7 min, $[\alpha]_D^{24} +58$ (*c* 0.1, MeOH) (Fig. S31,
265 Supplementary material). Each pair of enantiomers displayed identical ¹H and ¹³C NMR spectra
266 (Tables 1 and 2). Compounds (+)-**2** and (+)-**3** produced single crystals from CH₂Cl₂/MeOH (1:3
267 v/v) which were subjected to X-ray diffraction analysis using Cu K α radiation to determine their
268 absolute configurations.

269 From the X-ray data analysis, the absolute configuration of (+)-**2** (Fig. 4) was established
270 as *5S*, *7R*, *10aR*, *22R* with a Flack x-parameter of 0.06(5). The ECD spectrum of (+)-**2** (Fig. 5)

271 displayed a strong positive Cotton effect around 350 nm and negative Cotton effects around 208
272 and 280 nm. Thus, the absolute configuration of (-)-**2**, was assigned as *5R, 7S, 10aS, 22S*
273 because of its opposite signs of ECD Cotton effects (Fig. 5) as well as specific rotation, $[\alpha]_D^{24} -$
274 24.2 (*c* 0.07, CHCl₃), to that of (+)-**2**, $[\alpha]_D^{24} +38.2$ (*c* 0.06, CHCl₃). The trivial names (+)-
275 isobractatin and (-)-isobractatin were given for (+)-**2** and (-)-**2**, respectively.

276 The absolute configuration *6S, 7R, 10aS, 22S* of (+)-**3**, $[\alpha]_D^{24} +34.4$ (*c* 0.07, MeOH), was
277 established on the basis of single-crystal X-ray diffraction analysis (Fig. 4) with a Flack *x*-
278 parameter of -0.12(8). Compound (-)-**3**, $[\alpha]_D^{24} -27.7$ (*c* 0.08, MeOH), presented reverse
279 absorption curves in the ECD spectrum (Fig. 5) indicating the absolute configuration of *6R, 7S,*
280 *10aR, 22R*. Thus, (+)-3-*O*-methylneobractatin and (-)-3-*O*-methylneobractatin were assigned for
281 compounds (+)-**3** and (-)-**3**, respectively.

282 The absolute configuration *6R, 7S, 10aR, 22R* of (-)-**4** and *6S, 7R, 10aS, 22S* of (+)-**4**
283 were established by the comparisons of their ECD spectra (Fig. 5) and specific rotations ($[\alpha]_D^{24} -$
284 28.3 (*c* 0.1, MeOH) for (-)-**4** and ($[\alpha]_D^{24} +37.4$ (*c* 0.1, MeOH) for (+)-**4**) to those of (-)-**3** and (+)-
285 **3**. Therefore, compound (+)-**4** and (-)-**4** were assigned names as (+)-neobractatin and (-)-
286 neobractatin, respectively.

287 Compound (-)-**5** displayed ECD Cotton effects (Fig. 5) and specific rotation, $[\alpha]_D^{24} -74$
288 (*c* 0.1, MeOH), similar to those of (-)-cochinchinone C.²⁴ Thus, its absolute configuration was
289 assigned as *5R, 7S, 10aS, 22S*. The absolute configuration *5S, 7R, 10aR, 22R* assigned to (+)-**5**
290 was identified from the opposite signs of its ECD Cotton effects (Fig. 5) and specific rotation,
291 $[\alpha]_D^{24} +58$ (*c* 0.1, MeOH), with those of (-)-**5**. The names of (+)-**5** and (-)-**5** were assigned as (+)-
292 3-*O*-methylbractatin and (-)-3-*O*-methylbractatin, respectively.

293 Bractatin (**6**) was previously resolved into its two enantiomers but their absolute
294 configurations were not determined [5]. In this study, bractatin was resolved by semipreparative
295 chiral HPLC which showed two peaks, t_R 15 min for (–)-**6** ($[\alpha]_D^{24} -83$ (c 0.06, MeOH)) and 17
296 min for (+)-**6** ($[\alpha]_D^{24} +96$ (c 0.07, MeOH)), (Fig. S31, Supplementary material) with an
297 approximately enantiomeric ratio ca 1:0.7 ((–)-**6**/(+)-**6**). The ECD spectra of (+)-**6** and (–)-**6** (Fig.
298 5) were similar to those of (+)-**5** and (–)-**5**, respectively, indicating their absolute configurations
299 were *5S*, *7R*, *10aR*, *22R* for (+)-**6** and *5R*, *7S*, *10aS*, *22S* for (–)-**6**.

300 Possible biosynthetic pathways for the generation of the caged xanthenes **1-6** are shown
301 in Scheme 1. The intermediate **13.1** could be obtained from xanthone **13** via double *O*-
302 prenylation at C-5 and C-6 [22]. Claisen rearrangement of **13.1** could generate intermediate **13.2**
303 (carbonyl group at C-6) or intermediate **13.3** (carbonyl group at C-5). These intermediates could
304 then produce caged molecules having different bridge-head structures. Using the dextroisomer
305 (+)-**6** for example, containing a caged bridge-head at C-5/C-7/C-10a, this molecule could be
306 obtained from intermediate **13.2** via an intramolecular Diels-Alder reaction. The cyclization of
307 the 3-OH onto the C-12 olefinic moiety of (+)-**6** would give compound (+)-**2**, whereas *O*-
308 methylation at C-3 would produce compound (+)-**5**. In addition, the oxidation of (+)-**6** at C-8/C-
309 8a and then *O*-methylation at C-8 would give compound (+)-**1**. While caged compound (+)-**3**
310 with a different bridge-head, at C-6/C-7/C-10a, (called a neocaged xanthone) could obtain from
311 intermediate **13.3** via an intramolecular Diels-Alder reaction. *O*-methylation at C-3 of compound
312 (+)-**3** would then give compound (+)-**4**.

313 The remaining compounds were identified as doitunggarcinone A (**7**) [25, 26],
314 doitunggarcinone B (**8**) [25, 26], morusignin J (**9**) [27], 1,3,5-trihydroxy-6',6'-dimethylpyrano
315 (2'-2',6,7)-4-(1,1-dimethylprop-2-enyl)-xanthone (**10**) [28], gartanin (**11**) [29], allanxanthone A

316 (12) [30], gerontoxanthone I (13) [31], 1,3,5,6-tetrahydroxy-4(1,1-dimethylprop-2-enyl)-7-(3-
317 methylbut-2-enyl)-xanthone (14) [28], 1,3,7-trihydroxy-2,4-diisoprenylxanthone (15) [32],
318 blancoxanthone (16) [33], 10-*O*-methylmacluraxanthone (17) [34], (Fig. 2) by spectroscopic
319 methods as well as comparisons with the literature.

320 Caged xanthenes 1-6 were evaluated for their cytotoxicities against the colon cancer cell
321 line (HCT116). Compounds (-)-4, (+)-4, (-)-5, (+)-5, and (-)-6, showed potent inhibitory
322 cytotoxicities with IC₅₀ values of 2.60, 7.02, 1.47, 3.37, and 4.14 μM, respectively, better than
323 that of the positive control doxorubicin (IC₅₀ 9.74 μM). Compound (-)-2 had weak cytotoxicity
324 (IC₅₀ > 50 μM) while all remaining resolved caged xanthenes ((-)-1, (+)-1, (+)-2, (-)-3, (+)-3,
325 and (+)-6) were found to be inactive. It is interesting to note that the levorotatory compounds
326 showed cytotoxicity greater than their dextrorotatory counterparts. The structural difference
327 between compounds 3 and 4 is only at C-3. Compound 3 contained a C-3 methoxy group
328 whereas compound 4 had a C-3 hydroxy group which played an important role in the
329 cytotoxicity. Also, the double bond at C-8/C-8a of compounds 5 and 6 might be a crucial
330 structural element for cytotoxicity as indicated by the different potencies of the compounds 1 and
331 2 [22].

332 4. Conclusions

333 Previous phytochemical studies of the stem bark [22], root [23] and twig [25] of *G.*
334 *propinqua*, have resulted in the isolation of benzophenones, xanthenes, and caged xanthenes. In
335 our studies, the latter two types of compounds were the major compounds of this plant. All caged
336 xanthenes previously isolated from this plant were scalemic mixtures. In the present study, we
337 isolated an additional six scalemic caged xanthenes (1-6) with enantiomeric ratios ranging from
338 1:0.7 to 1:0.9. Interestingly, none of these compounds were isolated from the stem bark [22] and

339 twig [25] of this plant. These compounds were resolved by chiral HPLC and their absolute
340 configurations were identified using X-ray crystallography and ECD spectroscopy. Five
341 enantiomeric caged xanthonoids (–)-**4**, (+)-**4**, (–)-**5**, (+)-**5**, and (–)-**6** showed potent cytotoxicities
342 against a colon cancer cell line which may have potential for further development as anticancer
343 lead compounds.

344 **Conflicts of interest**

345 The authors declare no conflict of interest.

346 **Acknowledgements**

347 This work was supported by the Thailand Research Fund through the Advanced Research
348 Scholar (BRG5580008), Direct Basic Research Grant (DBG5980001), the NSFC-TRF coproject
349 (Grant 8156114801) and Mae Fah Luang University through the graduate student research grant.
350 We also thank the Thailand Research Fund through the Royal Golden Jubilee Ph.D. to provide a
351 full Ph.D. scholarship (PHD/0153/2556) to T. Sriyatep. Mae Fah Luang University and the
352 University of British Columbia were also acknowledged for laboratory facilities. We would like
353 to thank Mr. Matin Van de Bult, Doi Tung Development Project, Chiang Rai, Thailand for plant
354 collection and identification. S. Laphookhieo thanks the Australian Government via the
355 Endeavour Award 2016 for a research fellowship.

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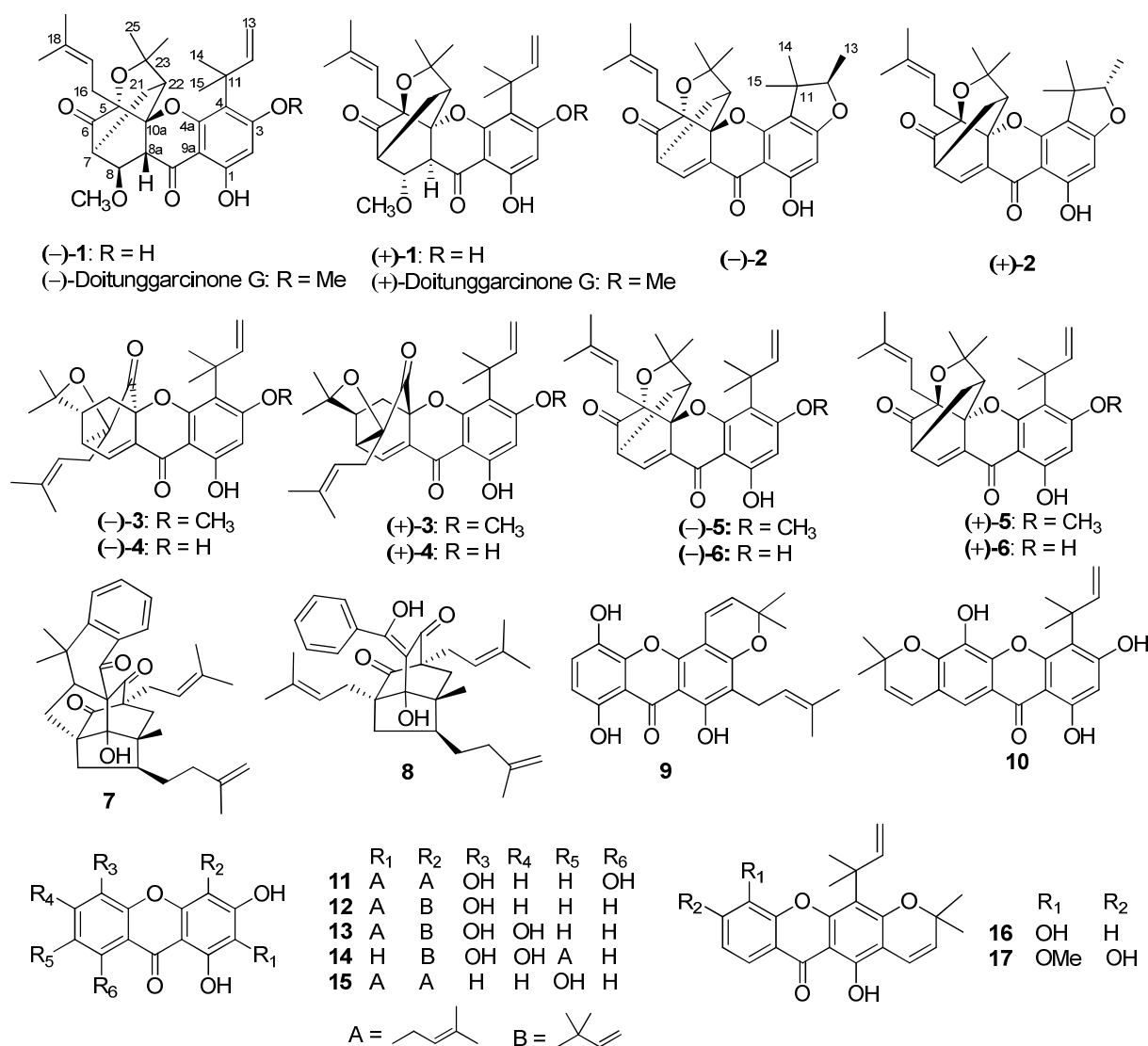
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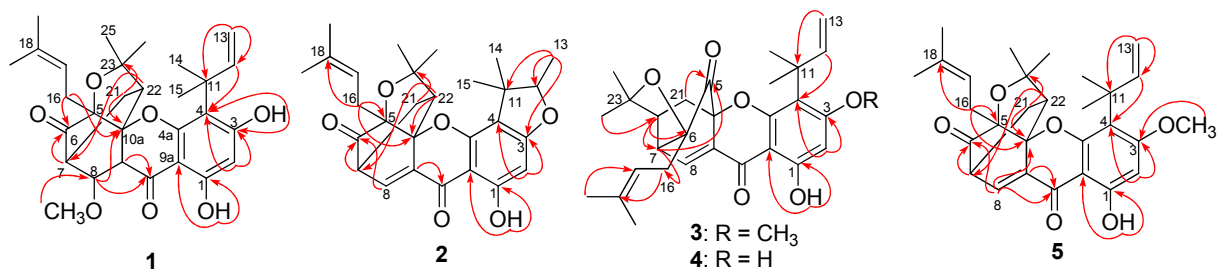
Fig. 1. *Garcinia propinqua* (These photos were taken by Surat Laphookhieo).



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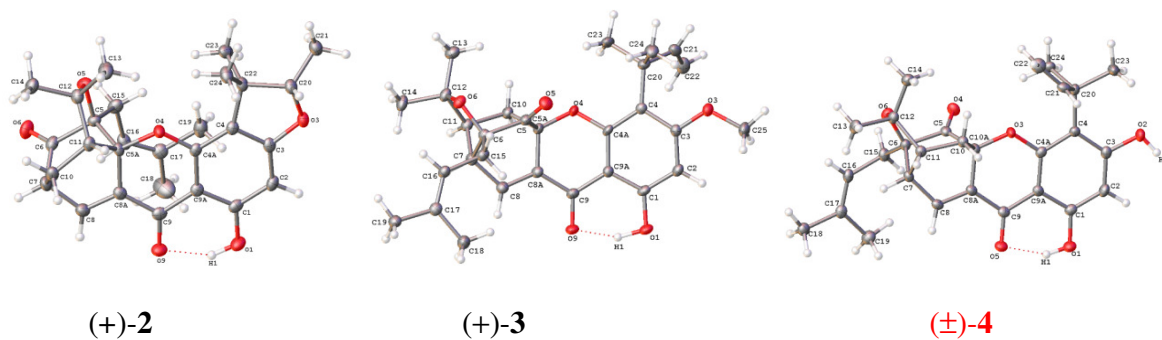
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Fig. 2. Compounds isolated from the leaves of *G. propinqua*.

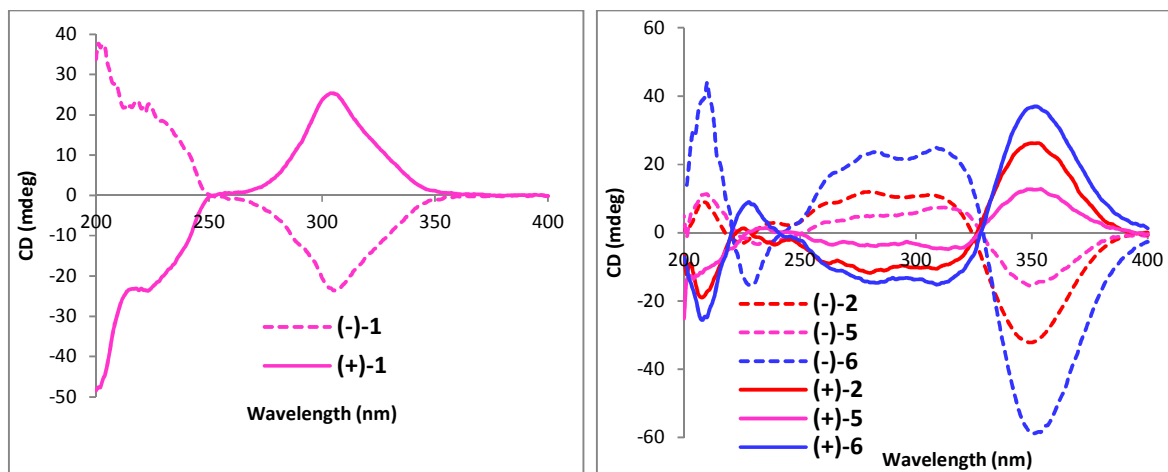


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454 **Fig. 3.** Selected HMBC correlations of compounds 1-5.

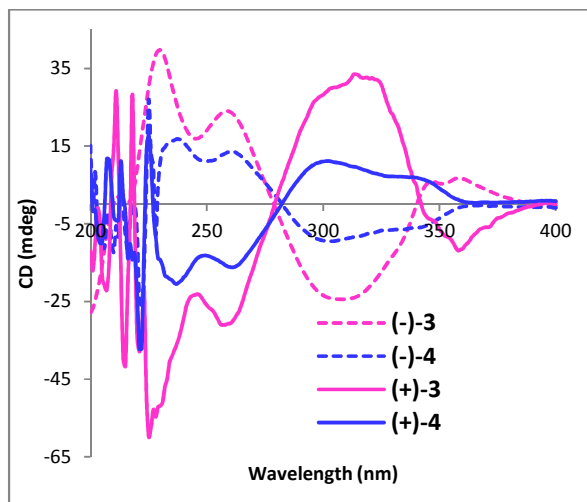
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459 **Fig. 4.** X-ray ORTEP diagrams of compounds (+)-2, (+)-3 and (±)-4.



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462 **Fig. 5.** ECD spectra of resolved compounds **1-6** in MeOH.

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479 **Table 1.** ¹H NMR spectroscopic data of compounds **1–5** (600 MHz in CDCl₃).

Position	1	2	3	4	5
1	-	-	-	-	-
2	6.08, s	6.02, s	6.08, s	6.02, s	6.12, s
3	-	-	-	-	-
4	-	-	-	-	-
4a	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	2.88, dd (4.5, 5.9)	3.50, dd (4.9, 6.8)	3.73, m	3.76, dd (4.8, 6.7)	3.49, dd (5.1, 7.0)
8	4.35, dd (4.4, 1.4)	7.47, d (6.8)	7.16, d (6.1)	7.19, d (6.7)	7.48, d (7.0)
8a	3.30, d (1.4)	-	-	-	-
9	-	-	-	-	-
9a	-	-	-	-	-
10a	-	-	-	-	-
11	-	-	-	-	-
12	6.24, dd (10.5,17.9)	4.54, q (6.7)	6.32, dd (11.1,17.1)	6.47, dd (10.4,17.8)	6.14, dd (10.6,17.3)
13	5.35, d (17.9)	1.30, d (6.7)	4.86, d (17.1)	5.47, d (17.8)	4.76, d (17.3)
	5.30, d (10.5)		4.75, d (11.1)	5.36, d (10.4)	4.69, d (10.6)
14	1.62, s	1.42, s	1.60, s	1.72, s	1.66, s
15	1.66, s	1.51, s	1.57, s	1.61, s	1.63, s
16	2.86, dd (5.9, 14.6)	2.64, m	2.50, m	2.49, m	2.63, dd (7.7)
	2.69, dd (9.1, 14.6)		2.07, dd (8.5, 14.4)	2.09, dd (8.1, 14.7)	
17	5.29, m	4.35, m	5.02, t (7.6)	5.02, t (7.6)	4.38, t (7.7)
18	-	-	-	-	-
19	1.62, s	1.38, s	1.72, s	1.73, s	1.40, s
20	1.66, s	1.07, s	1.59, s	1.59, s	1.09, s
21	1.97, dd (6.3, 14.7)	2.34, dd (4.9, 13.5)	2.50, m	2.49, m	2.33, dd (4.9, 13.4)
	1.38, dd (8.7,14.7)	1.39, m	1.79, m	1.86, dd (9.9, 13.2)	1.31, dd (9.1, 13.4)
22	2.54, d (8.7)	2.55, dd (10.8, 14.3)	2.16, m	2.19, dd (4.8, 9.9)	2.49, d (9.1)
23	-	-	-	-	-
24	1.12, s	1.27, s	1.34, s	1.35, s	1.23, s
25	1.39, s	1.74, s	1.35, s	1.36, s	1.69, s
OH-1	12.09, s	13.15, s	12.77, s	12.54, s	13.27, s
OH-3	6.96, s	-	-	7.52, s	-
OMe-3	-	-	3.79, s	-	3.77, s
OMe-8	3.29, s	-	-	-	-

Table 2. ^{13}C NMR spectroscopic data of compounds **1–5** (150 MHz in CDCl_3).

Position	1	2	3	4	5
1	162.5	166.3	163.8	163.3	164.2
2	99.5	92.6	94.1	99.2	94.0
3	165.5	168.2	168.3	165.7	168.3
4	111.4	112.4	114.4	111.5	113.6
4a	158.0	156.5	158.3	159.8	159.3
5	86.7	84.6	199.9	199.9	85.1
6	209.1	203.9	79.4	79.2	204.3
7	44.7	46.7	44.9	44.9	47.5
8	74.6	134.4	134.2	134.2	134.2
8a	47.2	135.0	135.0	134.3	133.5
9	194.6	178.9	178.8	178.6	180.2
9a	103.1	101.3	102.0	102.4	101.7
10a	88.7	90.8	83.7	83.9	91.5
11	41.4	43.7	40.9	41.4	41.6
12	149.2	91.5	151.6	149.9	150.9
13	113.4	15.9	106.1	113.3	106.7
14	30.3	25.1	29.5	28.3	29.0
15	27.3	19.6	29.0	27.6	28.0
16	28.1	28.5	30.4	30.4	29.4
17	117.8	117.5	117.6	117.4	118.2
18	133.9	133.7	136.4	136.5	135.3
19	18.0	25.5	18.3	18.3	25.7
20	26.0	16.3	25.9	26.1	17.1
21	20.2	27.8	32.8	32.9	26.6
22	43.9	48.9	42.5	42.4	49.5
23	81.1	83.0	83.6	83.9	83.3
24	27.4	28.6	26.9	26.9	31.0
25	30.5	30.5	29.7	29.7	31.4
OMe-3	-	-	55.6	-	55.5
OMe-8	55.7	-	-	-	-

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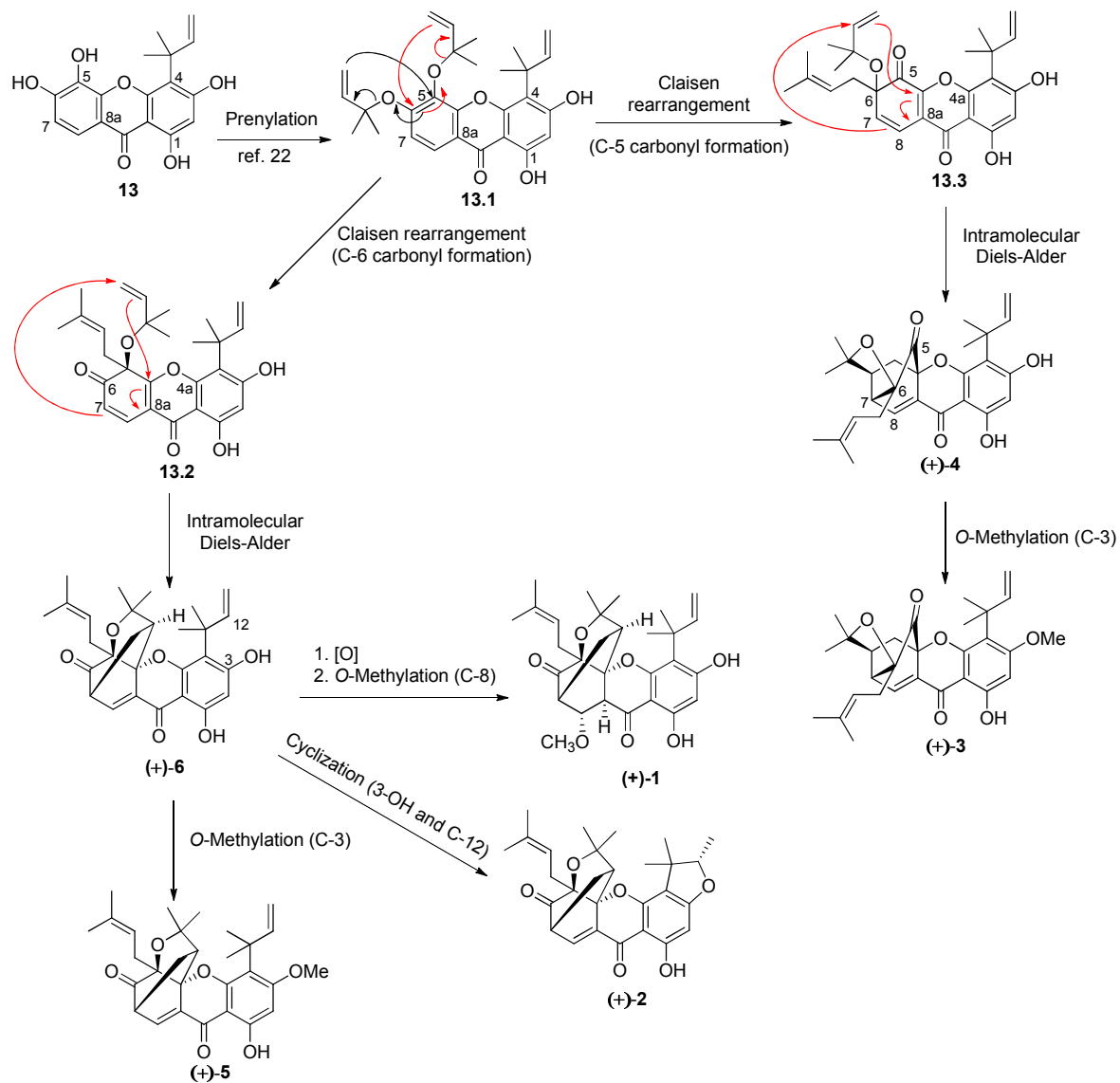
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492 Scheme 1. Plausible biosynthetic pathway of compounds 1-6.

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