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Ardisia gigantifolia

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1 **Bioassay-Guided Isolation and Structural**
2 **Modification of the Anti-TB Resorcinols from *Ardisia***
3 ***gigantifolia***

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32 Tuberculosis (TB) is a highly contagious disease mainly caused by *Mycobacterium*
33 *tuberculosis* H₃₇R_V. Antitubercular (anti-TB) bioassay-guided isolation of the CHCl₃
34 extract of the leaves and stems of the medicinal plant *Ardisia gigantifolia* led to the
35 isolation of two anti-TB 5-alkylresorcinols, 5-(8Z-heptadecenyl) resorcinol (**1**) and 5-
36 (8Z-pentadecenyl) resorcinol (**2**). We further synthesized 15 derivatives based on
37 these two natural products. These compounds (natural and synthetic) were evaluated
38 for their anti-TB activity against *M. tuberculosis* H₃₇R_V. Resorcinols **1** and **2** exhibited
39 anti-TB activity with MIC values at 34.4 μM and 79.2 μM in MABA assay,
40 respectively, and 91.7 μM and 168.3 μM in LORA assay, respectively. Among these
41 derivatives, compound **8** was found to show improved anti-TB activity than its
42 synthetic precursor (**2**) with MIC values at 42.0 μM in MABA assay and 100.2 μM in
43 LORA assay. The active compounds should be regarded as new hits for further study
44 as a novel class of anti-TB agents. The distinct structure–activity correlations of the
45 parent compound were elucidated based on these derivatives.

46 **Keywords:** *Ardisia gigantifolia*; isolation and structure identification; resorcinols;
47 anti-TB activity; *Mycobacterium tuberculosis* H₃₇R_V; structural modification

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49 Tuberculosis (TB) is a highly contagious bacterial disease most commonly
50 manifesting as a pulmonary infection and mainly caused by *M. tuberculosis* (1). The
51 World Health Organization (WHO) estimates that there were about 11 million
52 prevalent cases of TB in 2013, equivalent to 159 cases per 10 million population and
53 lead up to 1.5 million deaths (2).

54 The drugs used for treating TB are more than 40 years old and are far from ideal.
55 Drug-resistant TB (DR-TB) poses a major threat for the control of TB worldwide. In
56 2013, there were an estimated 480,000 new cases of multi-drug-resistant TB (MDR-
57 TB) worldwide and approximately 210,000 deaths (2). In the heavy MDR-TB burden
58 countries, the average duration of hospital stay ranged from 7 to 240 days, with a
59 median of 90 days (2). Two new drugs have been approved for the treatment of MDR-
60 TB under specific conditions: bedaquiline and delamanid in 2012 (3). However, these
61 two drugs are the first compounds to be approved for use in TB treatment in nearly 40
62 years, and the only ones ever to be released specifically for the treatment of MDR-TB
63 (4). This demands our continuous efforts to discover new anti-TB therapeutic agents
64 that improve the treatment of multi-drug-resistant and extensively drug-resistant
65 strains and shortens the total duration of treatment.

66 Plant compounds, known for their enormous numbers and their remarkable structural
67 diversity, are considered an excellent source for exploration of drug lead compounds,
68 and have received considerable attention as potential anti-TB agents (5, 6). *Ardisia*
69 *gigantifolia* Stapf (Primulaceae; previously, Myrsinaceae) collected from Vietnam for
70 the present research (see below) is a shrub growing in the shade and wet places of
71 valley and hillsides and is widely distributed in Southeast Asia including Vietnam,
72 Thailand, Malaysia, Indonesia and Southern provinces of China (7, 8). The whole
73 plant of this species has been used in folk medicine to eliminate blood stasis, disperse
74 swelling, improve blood circulation, and also as an analgesic (9). This plant was
75 investigated as part of our International Cooperative Biodiversity Group (ICBG)
76 project, which was designed to address the related issues of biodiversity conservation,
77 economic growth, and promotion of health through the discovery of anticancer, anti-
78 HIV, antimalarial, and anti-TB natural products through collaboration with
79 institutions in Vietnam, Laos, and the United States (10). This plant was found to be
80 one of the first anti-TB plant leads in our efforts to discover anti-TB agents from

81 plants of the tropical forests of Vietnam and Laos. The current paper describes the
82 isolation, structure elucidation, derivatization of the active natural products and
83 biological evaluation of the pharmacological activities of these compounds.

84 **Experiment**

85 *General Experimental Procedures*

86 NMR spectra were recorded on a Bruker DPX-300 MHz or a Bruker DPX-400 MHz
87 spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent
88 signals (CDCl_3 ; ^1H : 7.24 ppm; ^{13}C : 77.00 ppm), and coupling constants (J) were
89 reported in Hz. All NMR experiments were obtained by using standard pulse
90 sequences supplied by the vendor. Column chromatography was carried out on silica
91 gel (200–400 mesh, Natland International Corporation). Reversed-phase flash
92 chromatography was accomplished with RP-18 silica gel (40–63 μm , EM Science).
93 Thin-layer chromatography was performed on Whatman glass-backed plates coated
94 with 0.25 mm layers of silica gel 60. HR-TOF-MS spectra were recorded on a
95 Micromass QTOF-2 spectrometer. All reagents were purchased from Sigma-Aldrich
96 Chemical Co. and used without further purification. All solvents were reagent grade
97 or HPLC grade.

98 *Plant Material*

99 Leaf and stem sample (SVA0214) of *Ardisia gigantifolia* was recollected in Cuc
100 Phuong National Park, Nho Quan District, Ninh Binh Province, Vietnam, on October
101 21, 2001, from the same location where the original primary active sample (SV0214)
102 was collected on March 20, 1999. The exact location was forest floor at northeast side
103 of Bong at 500 m altitude, in a primary forest on a steep slope, 20° 21' 13" N, 105°
104 35' 48" E. It is a shrub 3 m tall, with the upper leaf surface dark green, lower surface
105 greenish purple, the peduncle green, turning purple toward the tip, bearing purple
106 flower buds with white top set on a purple pedicel. A voucher herbarium specimen of
107 the recollected sample (*Soejarto et al. 11809*) and that of the primary sample
108 (*Soejarto et al. 10628*) have been deposited at each of the following institutions: Cuc
109 Phuong National Park Herbarium (CPNP) in Nho Quan, Ninh Binh, Vietnam;
110 Herbarium of the Department of Botany (HN) of the Vietnam Academy of Science
111 and Technology, Hanoi, Vietnam; and at the J. D. Searle Herbarium of the Field
112 Museum (F), Chicago, USA.

113 *Extraction and Isolation*

114 The dried and milled leaves and stems (5.2 kg) were extracted with CHCl₃ (×3) to
115 yield an extract (37.9 g), which was subsequently defatted with *n*-hexane and
116 partitioned with CHCl₃. The CHCl₃-soluble fraction (33.0 g) was chromatographed
117 over a silica gel column (400 g) and eluted by gradient elution with petroleum
118 ether/EtOAc and EtOAc/MeOH to obtain 8 fractions (F1-F8). Fraction F2 (5.15 g)
119 demonstrated 91 % inhibition against *M. tuberculosis* H₃₇Rv at 50 µg/mL, and was
120 further chromatographed on a silica-gel column (100 g) by gradient elution with
121 CHCl₃ and increasing concentration of Me₂CO to yield 6 fractions (F9-F14). Fraction
122 F11 demonstrated anti-TB activity against *M. tuberculosis* H₃₇Rv with an MIC value
123 of 12.5 µg/ml. This fraction (0.72 g) was subjected to flash column chromatography
124 on a C-18 reverse phase (RP-18, 30 g) column. Subsequent gradient elution with H₂O
125 and increasing concentration of MeCN yielded 5-(8Z-heptadecenyl) resorcinol (**1**,
126 0.15 g) and 5-(8Z-pentadecenyl) resorcinol (**2**, 0.26 g) (Figure 1).

127 *Preparation of the Derivatives (3-14) of Compounds 1 and 2*

128 To a solution of compound **1** or **2** (5.0-8.0 mg) and corresponding selected acyl
129 chloride or *p*-toluenesulfonyl chloride (TsCl) (3 eq) in CH₂Cl₂ (3 mL), triethylamine
130 (TEA) (8 eq) and catalytic amount of 4-dimethylaminopyridine (DMAP) at 0 °C were
131 added. The resulting reaction mixture was stirred at room temperature overnight.
132 Volatile components in the reaction mixtures were removed by evaporation under
133 reduced pressure, and the resulting residue was purified by silica gel column
134 chromatography to afford ester derivatives **3-14**, respectively.

135 *Preparation of Compounds 15-16 (11)*

136 To a stirred suspension consisting of compound **14** (5.0 mg), silver acetate (AgOAc)
137 (4.0 mg) and water (1.6 mg) in glacial acetic acid (5 mL), iodine (2.4 mg) was added.
138 The resultant yellow mixture was stirred for 24 hr at room temperature and then
139 filtered through a cotton wool plug to remove insoluble material. The filtrate was
140 poured into CH₂Cl₂ (20 mL) in a separatory funnel, which was washed successively
141 with H₂O (2×5 mL) and saturated aqueous sodium bicarbonate (NaHCO₃) (5 mL).
142 The aqueous layers were combined and extracted with CH₂Cl₂ (20 mL). The resulting
143 organic layer was added to the original CH₂Cl₂ and further washed with brine (7 mL)

144 and then dried with sodium sulfate (Na₂SO₄). Removal of the solvent under reduced
145 pressure gave an orange residue.

146 A solution of the orange residue, potassium carbonate (K₂CO₃, 5.5 mg) in MeOH and
147 H₂O (5 mL, V_{MeOH} : V_{H₂O} = 10:1) was stirred at room temperature overnight. The
148 solvent was removed under reduced pressure, and the residue was subjected to silica
149 gel column chromatography to give diols **15-16**.

150 *Preparation of compound 17* (12)

151 Sodium periodate (NaIO₄, 1.29 g, 6.0 mmol) was dissolved in 2.5 mL of hot water
152 (~70 °C). To this hot solution, silica gel (230-400 mesh, 5 g) was added with
153 vigorous swirling and shaking to afford a free-flowing powder.

154 To a vigorously stirred suspension of this silica gel-supported NaIO₄ reagent (9 mg) in
155 CH₂Cl₂ (2 mL), a solution of diol **16** (3 mg) in CH₂Cl₂ (3 mL) was added. The
156 mixture was stirred for 30 min and then filtered through a sintered glass funnel. The
157 retained silica gel was thoroughly washed with CH₂Cl₂ (3×10 mL) and added to the
158 filtrate. *In vacuo* removal of the organic solvent from the filtrate afforded aldehyde
159 **17**.

160 Compound **3**, Amount, 2.5 mg; yield, 85%; colourless gum; ¹H NMR (Figure S1)
161 (400 MHz, CDCl₃) δ: 8.04 (4H, d, *J* = 9.1 Hz), 6.93 (3H, brs), 6.69 (4H, d, *J* = 9.1
162 Hz), 5.38-5.30 (2H, m), 3.08 (12H, s), 2.64 (2H, t, *J* = 7.6 Hz), 2.05-1.97 (4H, m),
163 1.68-1.59 (2H, m), 1.38-1.22 (20H, m), 0.88 (3H, t, *J* = 7.0 Hz); ¹³C NMR (Figure S2)
164 (100 MHz, CDCl₃) δ: 165.3, 153.6, 151.5, 145.1, 132.0, 131.2, 129.9, 129.8, 118.9,
165 115.9, 113.2, 110.7, 110.6, 40.0, 35.8, 31.9, 31.8, 31.0, 29.7, 29.5, 29.4, 29.3, 29.0,
166 27.2, 22.6, 14.1; HRTOF positive ESIMS *m/s* 641.4316 [M+1]⁺, (calcd for
167 C₄₁H₅₇N₂O₄, 641.4313).

168 Compound **4**, Amount, 3.0 mg; yield, 90%; colourless gum; ¹H NMR (Figure S3)
169 (400 MHz, CDCl₃) δ: 8.04 (4H, d, *J* = 8.9 Hz), 6.93 (3H, brs), 6.69 (4H, d, *J* = 9.0
170 Hz), 5.38-5.32 (2H, m), 3.08 (12H, s), 2.64 (2H, t, *J* = 7.9 Hz), 2.05-1.97 (4H, m),
171 1.68-1.61 (2H, m), 1.33-1.28 (16H, m), 0.88 (3H, t, *J* = 7.0 Hz); ¹³C NMR (Figure S4)
172 (100 MHz, CDCl₃) δ: 165.3, 153.7, 151.6, 145.0, 132.0, 129.9, 118.9, 115.9, 113.3,
173 110.7, 40.0, 35.8, 31.8, 31.0, 29.8, 29.7, 29.4, 29.3, 29.2, 29.0, 27.2, 22.7, 14.1;
174 HRTOF positive ESIMS *m/s* 613.3973 [M+1]⁺, (calcd for C₃₉H₅₃N₂O₄, 613.4000).

175 Compound **5**, Amount, 2.0 mg; yield, 70%; colourless gum; ¹H NMR (Figure S5)
176 (400 MHz, CDCl₃) δ: 7.15 (2H, dd, *J* = 4.0, 1.8 Hz), 6.91 (3H, brs), 6.88 (2H, dd, *J* =
177 2.2, 2.2 Hz), 6.19 (2H, dd, *J* = 4.1, 2.5 Hz), 5.36-5.32 (2H, m), 3.96 (6H, s), 2.63
178 (2H, t, *J* = 7.6 Hz), 2.08-1.93 (4H, m), 1.69-1.58 (2H, m), 1.38-1.22 (20H, m), 0.88
179 (3H, t, *J* = 6.2 Hz); ¹³C NMR (Figure S6) (100 MHz, CDCl₃) δ: 159.3, 150.8, 145.3,
180 130.6, 129.9, 121.4, 119.2, 119.0, 113.3, 108.3, 36.9, 35.7, 31.7, 31.0, 29.7, 29.6,
181 29.5, 29.4, 29.3, 29.0, 27.2, 22.6, 14.1; HRTOF positive ESIMS *m/s* 561.3660
182 [M+1]⁺, (calcd for C₃₅H₄₉N₂O₄, 561.3687).

183 Compound **6**, Amount, 1.8 mg; yield, 75%; colourless gum; ¹H NMR (Figure S7)
184 (400 MHz, CDCl₃) δ: 7.16 (2H, dd, *J* = 4.0, 1.8 Hz), 6.91 (3H, brs), 6.88 (2H, dd, *J* =
185 2.2, 2.2 Hz), 6.19 (2H, dd, *J* = 4.0, 2.5 Hz), 5.35 (2H, m), 3.96 (6H, s), 2.63 (2H, t, *J* =
186 7.5 Hz), 2.08-1.96 (4H, m), 1.69-1.59 (2H, m), 1.41-1.10 (16H, m), 0.88 (3H, t, *J* =
187 7.0 Hz); ¹³C NMR (Figure S8) (100 MHz, CDCl₃) δ: 159.3, 150.8, 145.2, 130.6,
188 129.9, 129.8, 121.4, 119.2, 119.0, 113.3, 108.3, 36.9, 35.7, 31.7, 30.9, 29.7, 29.3,
189 29.2, 29.0, 27.2, 22.6, 14.1; HRTOF positive ESIMS *m/s* 533.3376 [M+1]⁺, (calcd for
190 C₃₃H₄₅N₂O₄, 533.3374).

191 Compound **7**, Amount, 1.5 mg; yield, 65%; colourless gum; ¹H-NMR (Figure S9)
192 (400 MHz, CDCl₃) δ: 9.40 (2H, d, *J* = 1.5 Hz), 8.87 (2H, dd, *J* = 4.8, 1.5 Hz), 8.51-
193 8.40 (2H, m), 7.48 (2H, dd, *J* = 8.0, 4.8 Hz), 7.05 (1H, d, *J* = 1.9 Hz), 7.03 (2H, brs),
194 5.38-5.30 (2H, m), 2.69 (2H, t, *J* = 7.8 Hz), 2.04-1.98 (4H, m), 1.71-1.62 (2H, m),
195 1.40-1.21 (20H, m), 0.88 (3H, t, *J* = 7.1 Hz); ¹³C NMR (Figure S10) (100 MHz,
196 CDCl₃) δ: 163.5, 154.1, 151.4, 150.7, 146.0, 137.6, 129.9, 129.8, 125.4, 123.5, 119.3,
197 112.7, 35.8, 31.7, 30.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 27.2, 22.6, 14.1;
198 HRTOF positive ESIMS *m/s* 557.3367 [M+1]⁺, (calcd for C₃₅H₄₅N₂O₄, 557.3374).

199 Compound **8**, Amount, 2.0 mg; yield, 60%; colourless gum; ¹H NMR (Figure S11)
200 (400 MHz, CDCl₃) δ: 9.39 (2H, s), 8.87 (2H, d, *J* = 4.8 Hz), 8.46 (2H, d, *J* = 8.0 Hz),
201 7.49 (2H, dd, *J* = 7.8, 4.9 Hz), 7.05 (1H, brs), 7.03 (2H, brs), 5.47-5.20 (2H, m), 2.69
202 (2H, t, *J* = 7.8 Hz), 2.10-1.94 (4H, m), 1.74-1.56 (2H, m), 1.42-1.18 (16H, m), 0.87
203 (3H, t, *J* = 6.3 Hz); ¹³C NMR (Figure S12) (100 MHz, CDCl₃) δ: 163.5, 154.1, 151.3,
204 150.7, 146.0, 137.6, 130.0, 129.7, 125.4, 123.5, 119.3, 112.7, 35.7, 31.7, 30.9, 29.7,
205 29.3, 29.2, 29.1, 28.9, 27.2, 27.1, 22.6, 14.1; HRTOF positive ESIMS *m/s* 529.3061
206 [M+1]⁺, (calcd for C₃₃H₄₁N₂O₄, 529.3062).

207 Compound **9**, Amount, 2.4 mg; yield, 85%; colourless gum; ¹H NMR (Figure S13)
208 (400 MHz, CDCl₃) δ: 8.88 (4H, d, *J* = 5.1 Hz), 8.01 (4H, d, *J* = 5.9 Hz), 7.03 (1H,
209 brs), 7.02 (2H, brs), 5.42-5.26 (2H, m), 2.68 (2H, t, *J* = 7.8 Hz), 2.12-1.91 (4H, m),
210 1.71-1.58 (2H, m), 1.37-1.23 (20H, m), 0.88 (3H, t, *J* = 6.5 Hz); ¹³C NMR (Figure
211 S14) (100 MHz, CDCl₃) δ: 163.4, 150.9, 150.7, 146.2, 136.6, 129.9, 129.8, 123.2,
212 119.3, 112.5, 35.8, 31.8, 30.9, 29.7, 29.5, 29.4, 29.3, 29.2, 29.0, 27.7, 27.2, 22.6, 14.1;
213 HRTOF positive ESIMS *m/s* 557.3371 [M+1]⁺, (calcd for C₃₅H₄₅N₂O₄, 557.3374).

214 Compound **10**, Amount, 1.2 mg; yield, 30%; colourless gum; ¹H-NMR (Figure S15)
215 (400 MHz, CDCl₃) δ: 8.86 (2H, d, *J* = 5.7 Hz), 8.00 (2 H, dd, *J* = 4.4, 1.6 Hz), 6.62
216 (1H, dd, *J* = 1.8, 1.8 Hz), 6.61 (1H, dd, *J* = 2.0, 2.0 Hz), 6.56 (1 H, dd, *J* = 2.2, 2.2
217 Hz), 5.38-5.32 (2H, m), 5.22 (1 H, brs), 2.58 (2H, t, *J* = 7.6 Hz), 2.04-1.98 (4H, m),
218 1.66-1.59 (2H, m), 1.36-1.23 (16H, m), 0.88 (3H, t, *J* = 6.9 Hz); HRTOF positive
219 ESIMS *m/s* 424.2836 [M+1]⁺, (calcd for C₂₇H₃₈NO₃, 424.2846).

220 Compound **11**, Amount, 3.0 mg; yield, 95%; colourless gum; ¹H-NMR (Figure S16)
221 (400 MHz, CDCl₃) δ: 8.20 (4H, d, *J* = 7.6 Hz), 7.64 (2H, dd, *J* = 7.4, 7.4 Hz), 7.52 (
222 4H, dd, *J* = 7.7, 7.7 Hz), 7.00 (1H, d, *J* = 1.7 Hz), 6.99 (2H, brs), 5.45-5.26 (2H, m),
223 2.67 (2H, t, *J* = 7.9 Hz), 2.06-1.96 (4H, m), 1.69-1.62 (2H, m), 1.37-1.23 (20H, m),
224 0.88 (3H, t, *J* = 6.5 Hz); ¹³C-NMR (Figure S17) (100 MHz, CDCl₃) δ: 164.9, 151.2,
225 145.6, 133.6, 130.2, 129.9, 129.4, 128.6, 119.1, 112.9, 35.8, 31.7, 31.0, 29.7, 29.5,
226 29.4, 29.3, 29.0, 27.2, 22.6, 14.1; HRTOF positive ESIMS *m/s* 577.3301 [M+Na]⁺,
227 (calcd for C₃₇H₄₆NaO₄, 577.3288).

228 Compound **12**, Amount, 8 mg; yield, 90%; colourless gum; ¹H-NMR (Figure S18)
229 (400 MHz, CDCl₃) δ: 8.20 (4H, dd, *J* = 7.1, 1.4 Hz), 7.67-7.60 (2H, m), 7.51 (4H, dd,
230 *J* = 7.4, 7.4 Hz), 7.00 (1H, d, *J* = 2.0 Hz), 6.99 (2H, d, *J* = 2.0 Hz), 5.40-5.30 (2H, m),
231 2.69 (2H, t, *J* = 7.7 Hz), 2.07-1.95 (4H, m), 1.70-1.61 (2H, m), 1.41-1.21 (16H, m),
232 0.87 (3H, t, *J* = 6.9 Hz); HRTOF positive ESIMS *m/s* 527.3145 [M+1]⁺, (calcd for
233 C₃₅H₄₃O₄, 527.3156).

234 Compound **13**, Amount, 2.2 mg; yield, 85%; colourless gum; ¹H NMR (Figure S19)
235 (400 MHz, CDCl₃) δ: 7.64 (4H, d, *J* = 8.2 Hz), 7.31 (4H, d, *J* = 8.1 Hz), 6.69 (2H,
236 brs), 6.45 (1H, brs), 5.46-5.28 (2H, m), 2.69-2.40 (8H, m), 2.10-1.94 (4H, m), 1.40-
237 1.20 (22H, m), 0.88 (3H, t, *J* = 6.1 Hz); ¹³C NMR (Figure S20) (100 MHz, CDCl₃) δ:

238 149.4, 146.0, 145.6, 131.9, 129.9, 129.8, 128.4, 121.1, 114.1, 35.3, 31.7, 30.7, 29.7,
239 29.5, 29.4, 29.3, 29.0, 27.2, 22.6, 21.7, 14.1; HRTOF positive ESIMS *m/s* 677.2931
240 [M+Na]⁺, (calcd for C₃₇H₅₀NaO₆S₂, 677.2941).

241 Compound **14**, Amount, 7.5 mg; yield, 90%; colourless gum; ¹H NMR (Figure S21)
242 (400 MHz, CDCl₃) δ: 7.64 (4H, d, *J* = 8.3 Hz), 7.32 (4H, d, *J* = 8.0 Hz), 6.70 (2H, d, *J*
243 = 2.2 Hz), 6.45 (1H, dd, *J* = 2.2, 2.2 Hz), 5.41-5.30 (2H, m), 2.46-2.42 (8H, m), 2.10-
244 1.95 (4H, m), 1.43-1.10 (18H, m), 0.88 (3H, t, *J* = 7.0 Hz); HRTOF positive ESIMS
245 *m/s* 627.2800 [M+H]⁺, (calcd for C₃₅H₄₇O₆S₂, 627.2809).

246 Compound **15**, Amount, 1.1 mg; yield, 25%; colourless gum; ¹H-NMR (Figure S22)
247 (400 MHz, CDCl₃) δ: 7.72 (2H, d, *J* = 8.3 Hz), 7.31 (2H, d, *J* = 8.0 Hz), 6.54 (1H, d, *J*
248 = 1.8 Hz), 6.34 (2H, d, *J* = 1.7 Hz), 5.61 (1H, brs), 3.67-3.56 (2H, m), 2.50-2.39 (5H,
249 m), 2.01-1.85 (2H, m), 1.52-1.41 (4H, m), 1.36-1.19 (16H, m), 0.88 (3H, t, *J* = 6.8
250 Hz); HRTOF positive ESIMS *m/s* 529.2585 [M+Na]⁺, (calcd for C₂₈H₄₂O₆NaS,
251 529.2594).

252 Compound **16**, Amount, 3.5 mg; yield, 35%; colourless gum; ¹H-NMR (Figure S23)
253 (400 MHz, CDCl₃) δ: 7.65 (4H, d, *J* = 8.3 Hz), 7.32 (4H, d, *J* = 8.1 Hz), 6.72 (2H, d, *J*
254 = 2.1 Hz), 6.44 (1H, dd, *J* = 2.2, 2.2 Hz), 3.64-3.57 (2H, m), 2.48-2.41 (8H, m), 1.87-
255 1.78 (2H, m), 1.46-1.38 (4H, m), 1.33-1.18 (16H, m), 0.88 (3H, t, *J* = 6.7 Hz);
256 HRTOF positive ESIMS *m/s* 683.2679 [M+Na]⁺, (calcd for C₃₅H₄₈NaO₈S₂,
257 683.2683).

258 Compound **17**, Amount, 1.5 mg; yield, 95%; colourless gum; ¹H-NMR (Figure S24)
259 (400 MHz, CDCl₃), δ: 9.77 (1H, t, *J* = 1.8 Hz), 7.65 (4H, d, *J* = 8.4 Hz), 7.30 (4H, d,
260 *J* = 8.0 Hz), 6.72 (2H, d, *J* = 2.2 Hz), 6.43 (1H, dd, *J* = 2.2, 2.2 Hz), 2.46-2.41 (10H,
261 m), 1.64-1.60 (2H, m), 1.42-1.36 (2H, m), 1.31-1.23 (6H, m); ¹³C NMR (Figure S25)
262 (100 MHz, CDCl₃) δ: 202.8, 149.5, 145.8, 145.7, 132.0, 129.9, 128.5, 121.2, 114.1,
263 43.9, 35.3, 30.6, 29.1, 29.0, 28.7, 22.0, 21.8; HRTOF positive ESIMS *m/s* 545.1657
264 [M+H]⁺, (calcd for C₂₈H₃₂O₇S₂, 545.1662).

265 *Anti-TB Activity Bioassays*

266 Extracts, fractions, purified compounds and derivatives were subjected to *in vitro*
267 assays. Primary screening was conducted at 100 µg/mL against *M. tuberculosis* H₃₇Rv
268 (ATCC 27294) using the Microplate Alamar Blue Assay (MABA) and Low Oxygen

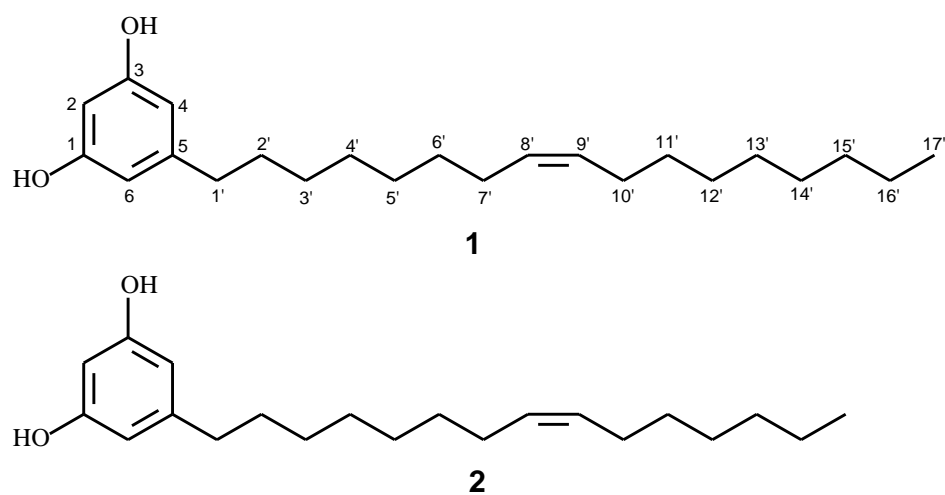
269 Recovery Assay (LORA), according to the procedures described by Collins (13) and
270 Cho (14), respectively. Samples showing $\geq 90\%$ inhibition in the primary screening
271 were considered active and then re-tested at lower concentrations against *M.*
272 *tuberculosis* H₃₇Rv in order to determine the actual MIC. The MIC is defined as the
273 lowest concentration effecting a reduction in fluorescence or luminescence of 90%
274 with respect to untreated controls.

275 Results and Discussion

276 Isolation of Resorcinols **1** and **2**

277 The CHCl₃ extract made from the initially collected leaves and stems of *Ardisia*
278 *gigantifolia* demonstrated anti-TB activity with an MIC value of 25 $\mu\text{g/mL}$. A larger
279 quantity of the leaf and stem samples was subsequently recollected from the same
280 location to isolate the active compounds. The dried sample (5.2 kg) was milled and
281 extracted with CHCl₃, followed by *in vacuo* evaporation to afford a dried extract (37.9
282 g). Through bioassay-guided fractionation of the CHCl₃ extract by repeated column
283 chromatography on silica gel, fraction F11 was identified as the anti-TB fraction, with
284 an MIC value of 12.5 $\mu\text{g/mL}$ against *M. tuberculosis* H₃₇Rv. Further separation of F11
285 using RP-18 silica gel led to the isolation of the anti-TB compounds 5-(8Z-
286 heptadecenyl) resorcinol (**1**) and 5-(8Z-pentadecenyl) resorcinol (**2**) (Figure 1).

287



289 **Figure 1:** Chemical structures of **1** and **2**.

291 **Table 1.** ^1H (300 MHz) and ^{13}C (75 MHz) NMR data for compounds **1** and **2** (in
 292 CDCl_3 , δ in ppm, J in Hz).

Position	1		2	
	δ_{H} (mult) ^a	δ_{C} (mult) ^b	δ_{H} (mult) ^a	δ_{C} (mult) ^b
1		156.9 s		156.4 s
2	6.18 (1H, brs)	100.2 d	6.17 (1H, brs)	100.2 d
3		156.9 s		156.4 s
4	6.24 (2H, brs)	108.1 d	6.25 (2H, brs)	108.1 d
5		146.2 s		146.2 s
6	6.24 (2H, brs)	108.1 d	6.25 (2H, brs)	108.1 d
1'	2.35(2H, t, 7.5)	35.84 t	2.45 (2H, t, 7.5)	35.84 t
2'	1.56 (2H, brs)	31.79 t	1.54 (2H, brs)	31.79 t
3'	1.27 (10×2H, m)	29.31 t	1.29 (8×2H, m)	29.29 t
4'	1.27 (10×2H, m)	29.77 t	1.29 (8×2H, m)	29.41 t
5'	1.27 (10×2H, m)	29.77 t	1.29 (8×2H, m)	29.74 t
6'	1.27 (10×2H, m)	31.06 t	1.29 (8×2H, m)	29.74 t
7'	2.01 (2×2H, m)	27.23 t	1.29 (8×2H, m)	31.05 t
8'	5.35 (2×1H, m)	129.9 d	2.01 (2×2H, m)	27.24 t
9'	5.35 (2×1H, m)	129.9 d	5.34 (2×1H, m)	130.0 t
10'	2.01 (2×2H, m)	27.23 t	5.34 (2×1H, m)	129.8 t
11'	1.27 (10×2H, m)	31.06 t	2.01 (2×2H, m)	27.24 t
12'	1.27 (10×2H, m)	29.77 t	1.29 (8×2H, m)	29.41 t
13'	1.27 (10×2H, m)	29.77 5	1.29 (8×2H, m)	31.94 t
14'	1.27 (10×2H, m)	29.56 t	1.29 (8×2H, m)	22.67 t
15'	1.27 (10×2H, m)	31.94 t	0.88 (3H, t, 6.2)	14.5 q
16'	1.27 (10×2H, m)	22.67 t		
17'	0.88 (3H, t, 6.2)	14.5 q		

293 ^a Multiplicities in parentheses represent: brs (broad singlet), d (doublet), m (multiplicity),
 294 t (triplet),.

295 ^b Multiplicities represent: s (quaternary carbon), d (CH), t (CH₂), and q (CH₃).
 296

299 Compounds **1** and **2** were obtained as colorless gums and showed very similar NMR
300 data (Table 1), suggesting that they have similar structures. Both compounds contain
301 an aromatic ring, a C-C double bond, multi-methylenes and a methyl as evidenced by
302 the ^1H and ^{13}C -NMR spectral data. Compound **1** was shown to have 14 methylenes,
303 two methylenes more than **2** according to the analysis of the HR-TOF-MS (**1**: $[\text{M-H}]^-$
304 m/z 345.2789, calcd. 345.2799, $\text{C}_{23}\text{H}_{37}\text{O}_2$; **2**: $[\text{M-H}]^-$ m/z 317.2483, calcd. 317.2486,
305 $\text{C}_{21}\text{H}_{33}\text{O}_2$) and the NMR data. The coupling patterns in the downfield region [**1**: δ_{H}
306 6.18 (1H, brs, 4-H), 6.24 (2H, brs, 2, 6-H); **2**: δ_{H} 6.17 (1H, brs, 4-H), 6.25 (2H, brs, 2,
307 6-H)] showed that both compounds have a 1, 3, 5-substituted benzene ring.
308 Compounds **1** and **2** were determined to be a 5-alkylresorcinols with a double bond in
309 the side chain, based on the above data. In comparison with the literature data, **1** and **2**
310 were thus identified as 5-(8Z-heptadecenyl) resorcinol and 5-(8Z-pentadecenyl)
311 resorcinol, respectively (11, 15-17).

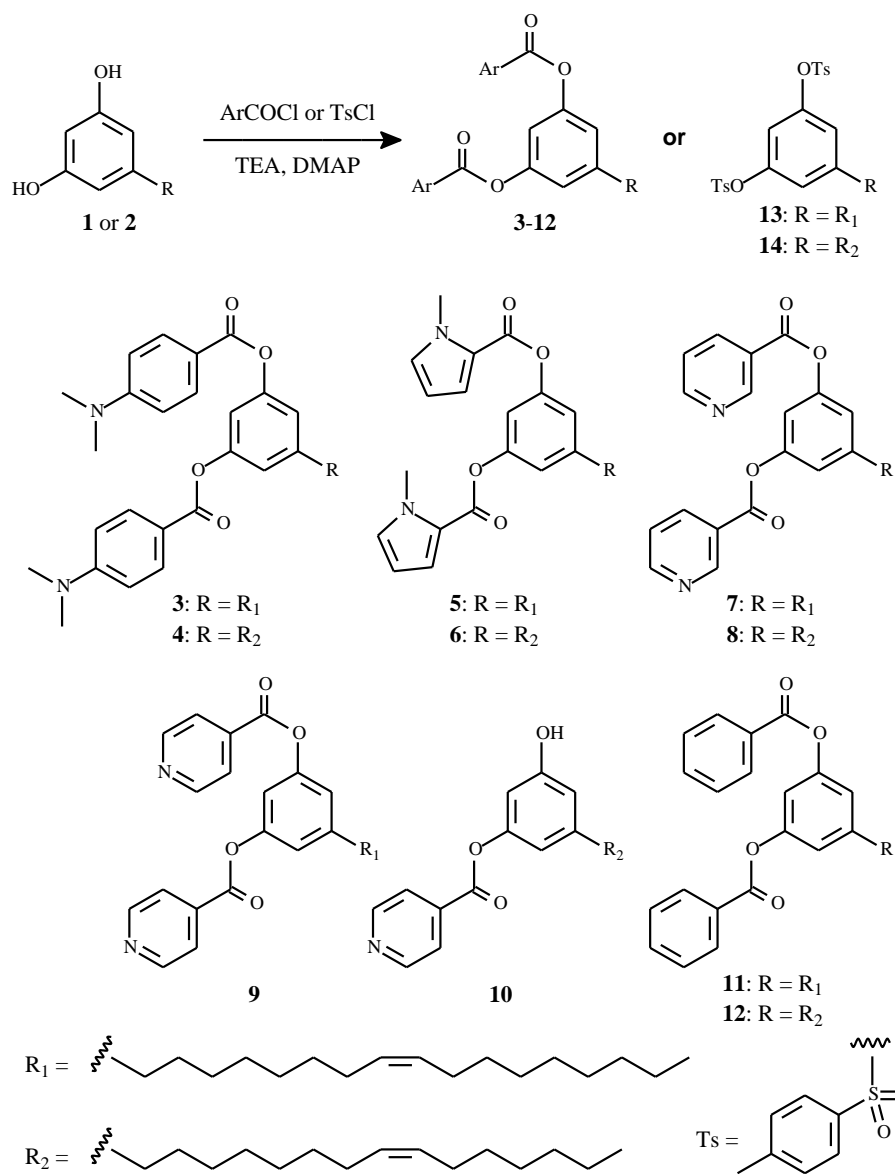
312 *Preparation of Resorcinols 1 and 2 Derivatives*

313 In an attempt to improve the biological activity of the isolated natural resorcinols, we
314 initiated a structural modification effort. To that end, 15 derivatives were prepared by
315 esterification of the phenolic hydroxyl groups and hydroxylation of the double bonds
316 of compounds **1** and **2**. As depicted in Scheme 1, the phenolic hydroxyl groups of the
317 resorcinols were esterified with aromatic acyl chlorides including heterocyclic
318 carbonyl chlorides to afford ester derivatives **3-14** in 30-95% yield. The diester **9** was
319 prepared by treatment of **1** with 3 equivalents of isonicotinic acid chloride, but the
320 monoester **10** was obtained by treatment of the resorcinol with 1.2 equivalents of
321 isonicotinic acid chloride in a yield of 30%.

322 As shown in Scheme 2, compound **14** was subjected to a Woodward-Prevost reaction
323 [14], followed by the subsequent hydrolysis using K_2CO_3 , to yield derivatives **15-16**.
324 Compound **15** was obtained due to the deprotection of the intramolecular hydroxyl
325 groups in the presence of K_2CO_3 . Further oxidative cleavage of the diol group of **16**
326 with NaIO_4 gave aldehyde **17**.

329

330

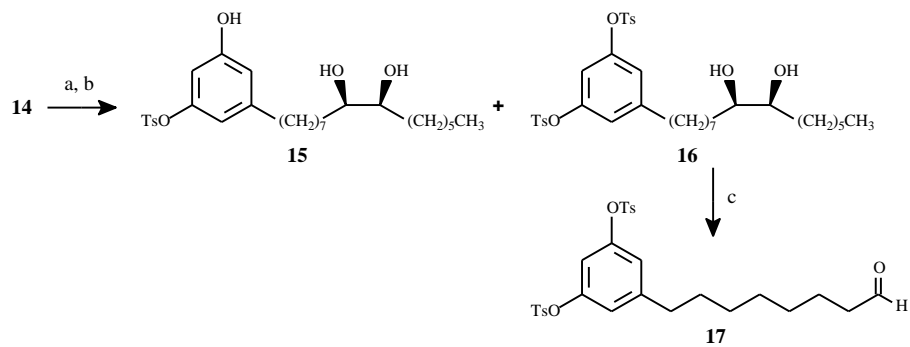


331

332

Scheme 1. Synthesis of the derivatives (**3-14**) of **1** and **2** through esterification.

333



334

335 **Scheme 2.** Synthesis of the diol and aldehyde derivatives of compound **2** through
336 hydroxylation of the double bond. Reagents and reaction conditions: a. AgOAc,
337 I₂, V_{AcOH} / V_{H₂O} = 20:1; b. K₂CO₃, CH₃OH/H₂O; c. NaIO₄·SiO₂, CH₂Cl₂
338

339 *Anti-TB Activity*

340 The source plant extract was identified as an anti-TB lead through our screening
341 effort, and compounds **1-2** were subsequently isolated through bioassay-directed
342 separation by determining MICs against replicating and non-replicating *M.*
343 *tuberculosis* H₃₇Rv using the MABA and LORA, respectively. Compound **1** showed
344 MIC values of 34.4 μM against replicating cultures and 91.7 μM against non-
345 replicating cultures, and **2** had MIC values of 79.2 μM against replicating cultures and
346 168.3 μM in against non-replicating cultures (Table 2).

347 In addition to the natural occurring resorcinols (**1** and **2**), we prepared 15 synthetic
348 derivatives of these molecules for assessment of anti-TB potentials. The synthetic
349 compounds were evaluated for their anti-TB activities against *M. tuberculosis* H₃₇Rv
350 *in vitro* (Table 2). While most of the derivatives displayed little or no inhibitory
351 activity against the bacteria at the concentration of 100 μg/mL, derivative **8** showed
352 equivalent activity to that of compound **1** with the MIC values at 42.0 μM in MABA
353 assay and 100.2 μM in LORA assay. Through analysis of the activity data of Table 2,
354 distinct structure–activity relationships (SARs) have been observed for these
355 resorcinol compounds. Based on the SAR analysis, we obtained some preliminary
356 conclusion: 1) Although the esterification approach did not significantly boost the
357 activity, the slight improvement of the anti-TB activity of **8** in comparison with its
358 parent compound (**2**) indicated that the phenolic hydroxy groups may be used as the
359 functional groups to synthesize other derivatives; 2) Presence of the double bond in
360 the side chain is essential to retain the anti-TB activity for this type of compounds.
361 This effect was observed when the double bond was hydrolyzed as in the cases of
362 compounds **15-16**; 3) the C₂ symmetrical structure may not be important for the anti-
363 TB activity as evidenced by the cases of compounds **9** and **10**.

364 Although the 15 resorcinol derivatives synthesized did not produce a significant
365 improvement in the anti-TB activity of compounds **1** and **2**, the activity profiles of
366 compound **8** verified that the anti-TB activity was marginally enhanced by our present
367 synthetic approach. Further, since **8** contains nitrogen, it can be made water soluble by

368 preparing it as a salt compound, which is worthy for further study as a novel anti-TB
 369 agent.

370

371 **Table 2.** Anti-TB activity of compounds **1-17** ^a.

Compound	Inhibition MABA at 100 μg/mL	Inhibition LORA at 100 μg/mL	MIC MABA μg/mL (μM)	MIC LORA μg/mL (μM)
1	--	--	11.9 (34.4)	31.7 (91.7)
2	--	--	25.2 (79.2)	53.5 (168.3)
3	0%	0%	> 100	> 100
4	4%	0%	> 100	> 100
5	0%	6%	> 100	> 100
6	15%	13%	> 100	> 100
7	88%	64%	> 100	> 100
8	--	--	22.2 (42.0)	52.9 (100.2)
9	87%	61%	> 100	> 100
10	--	--	42.3 (100.0)	91.4 (216.2)
11	0%	9%	> 100	> 100
12	10%	36%	> 100	> 100
13	16%	38%	> 100	> 100
14	26%	46%	> 100	> 100
15	89%	--	> 100	90.5 (178.9)
16	42%	49%	> 100	> 100
17	18%	11%	> 100	> 100
rifampin			(0.06)	(0.24)
isoniazid			(0.47)	(>256)
metronidazole			(>512)	(31.2)
capreomycin			(3.51)	(3.73)
streptomycin			(0.57)	(0.88)

372 ^a Minimum inhibitory concentration (MIC), determined under aerobic (MABA) or
 373 hypoxic (LORA) conditions against *M. tuberculosis* H₃₇Rv. Each value is the mean of
 374 at least three independent determinations.

375

376 There have been only two anti-TB drug introduced in the past 40 years and the rapid
 377 acquisition of drug resistance to the existing drugs necessitates development of new,

378 effective and affordable anti-TB drugs (4). Plant-derived anti-TB compounds provide
379 a great potential for discovery of novel anti-TB agents due to their exceptionally wide
380 diversified structure classes, including terpenoids, alkaloids, phenolic compounds and
381 so on (18). Our bioassay-guided fractionation of the leaves and stems of the medicinal
382 plant *A. gigantifolia* led to the isolation of two active resorcinols (**1** and **2**), which
383 demonstrated inhibitory activity against *M. tuberculosis* (H₃₇R_v) *in vitro* with MIC
384 values at 34.4 μM and 79.2 μM in MABA assay respectively, and 91.6 μM and 168.2
385 μM in LORA assay respectively. Hence medicinal plants remain an important
386 resource to find new therapeutic agents.

387 **Conclusions**

388 In conclusion, anti-TB bioassay-guided fractionation of the CHCl₃ extract of the
389 leaves and stems of *A. gigantifolia* led to the isolation of two 5-alkylresorcinols.
390 Further, 15 synthetic derivatives were prepared from these two lead compounds.
391 These compounds (natural and synthetic) were evaluated for their anti-TB activity
392 against *M. tuberculosis* H₃₇R_v. The distinct structure–activity correlations were
393 elucidated based on these derivatives. Derivative **8** showed equivalent activity to
394 those of the compound **1**, and it displayed improved anti-TB activity as compared
395 with its parent compound (**2**). Since **8** is a nitrogen containing compound, it can be
396 made as a water soluble salt, which is considered as valuable in drug development for
397 the improvement of bioavailability. The compound should be regarded as a lead
398 compound for synthesis of additional resorcinol derivatives in the search of novel
399 anti-TB agents.

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408 **Author Contributions**

409 Dr. Yi-Fu Guan, Mr. Xun Song and Dr. Ming-Hua Qiu performed most of the
410 chemistry related experiments including separation, structure determination, and
411 chemical synthesis of the reported compounds with support of Dr. Hong-Jie Zhang,
412 Dr. Harry H.S. Fong and Dr. Zhen-Dan He. Dr. Shi-Hong Luo did NMR measurement
413 of the synthetic compounds with support of Dr. Sheng-Hong Li. Dr. Nguyen Van
414 Hung performed the extraction of the plant sample. Dr. D. Doel Soejarto and Nguyen
415 Man Cuong collected and authenticated the plant materials. Dr. Bao-Jie Wan
416 performed most of the biology related experiments including anti-TB evaluation with
417 support of Dr. Scott G. Franzblau. Dr. Hong-Jie Zhang, Dr. Harry H.S. Fong, Dr. D.
418 Doel Soejarto and Dr. Scott G. Franzblau designed the bioassay-guided separation
419 study. Dr. Hong-Jie Zhang designed the synthetic study. Dr. Yi-Fu Guan, Mr. Xun
420 Song, Dr. Zheng-Dan He and Dr. Hong-Jie Zhang co-wrote the manuscript with the
421 assistance of Dr. Harry H.S. Fong, Dr. Scott Franzblau and Dr. D. Doel Soejarto. All
422 authors discussed the results and commented on the manuscript.

423 **Conflicts of Interest**

424 The authors declare no conflict of interest.

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478

479 **Supporting Information**

480 Supplementary materials can be found at <http://>

481 **Figure S1.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **3**

482 **Figure S2.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **3**

483 **Figure S3.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **4**

484 **Figure S4.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **4**

485 **Figure S5.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **5**

486 **Figure S6.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **5**

487 **Figure S7.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **6**

488 **Figure S8.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **6**

489 **Figure S9.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **7**

490 **Figure S10.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **7**

491 **Figure S11.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **8**

492 **Figure S12.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **8**

493 **Figure S13.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **9**

494 **Figure S14.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **9**

495 **Figure S15.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **10**

496 **Figure S16.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **11**

497 **Figure S17.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **11**

498 **Figure S18.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **12**

499 **Figure S19.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **13**

500 **Figure S20.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **13**

501 **Figure S21.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **14**

502 **Figure S22.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **15**

503 **Figure S23.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **16**

504 **Figure S24.** ¹H NMR (400 MHz, CDCl₃) spectrum of compound **17**

505 **Figure S25.** ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **17**

506 **Figure S26.** HRTOFMS spectrum of compound **1**

507 **Figure S27.** HRTOFMS spectrum of compound **2**

508 **Figure S28.** HRTOFMS spectrum of compound **3**
509 **Figure S29.** HRTOFMS spectrum of compound **4**
510 **Figure S30.** HRTOFMS spectrum of compound **5**
511 **Figure S31.** HRTOFMS spectrum of compound **6**
512 **Figure S32.** HRTOFMS spectrum of compound **7**
513 **Figure S33.** HRTOFMS spectrum of compound **8**
514 **Figure S34.** HRTOFMS spectrum of compound **9**
515 **Figure S35.** HRTOFMS spectrum of compound **10**
516 **Figure S36.** HRTOFMS spectrum of compound **11**
517 **Figure S37.** HRTOFMS spectrum of compound **12**
518 **Figure S38.** HRTOFMS spectrum of compound **13**
519 **Figure S39.** HRTOFMS spectrum of compound **15**
520 **Figure S40.** HRTOFMS spectrum of compound **16**