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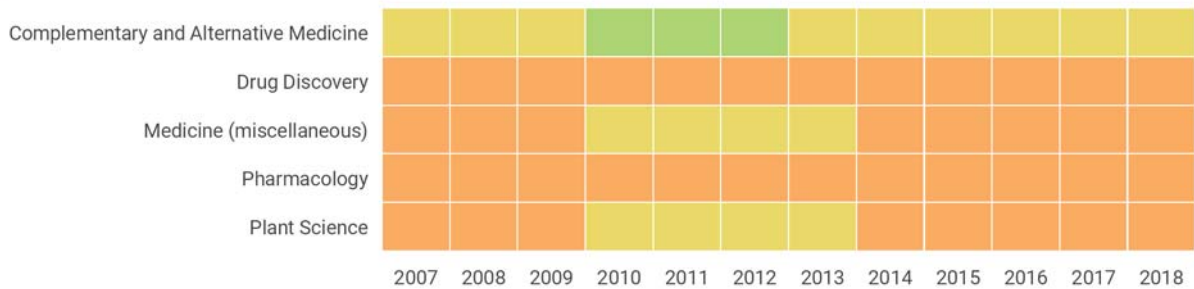
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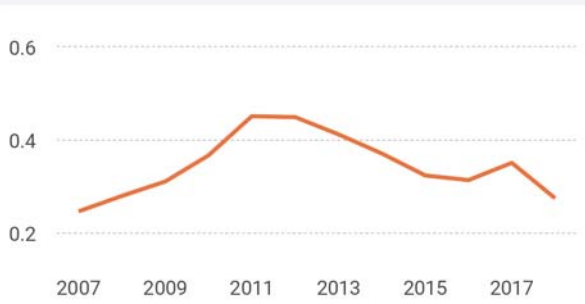
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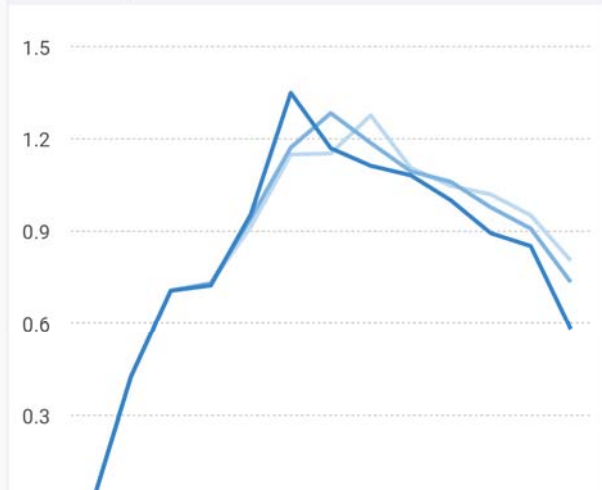
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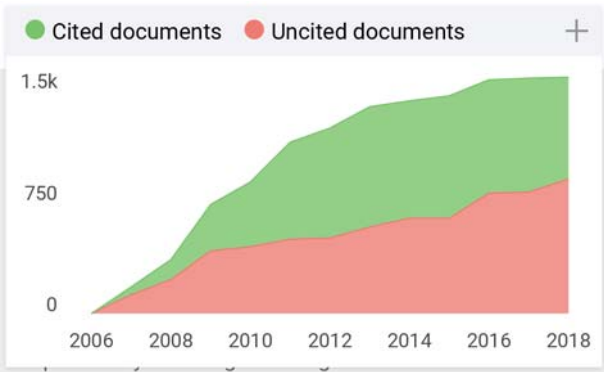
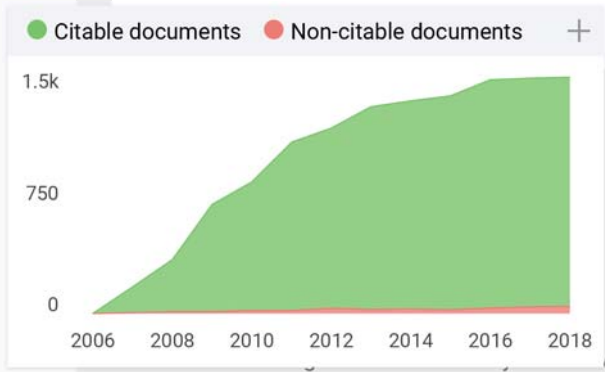
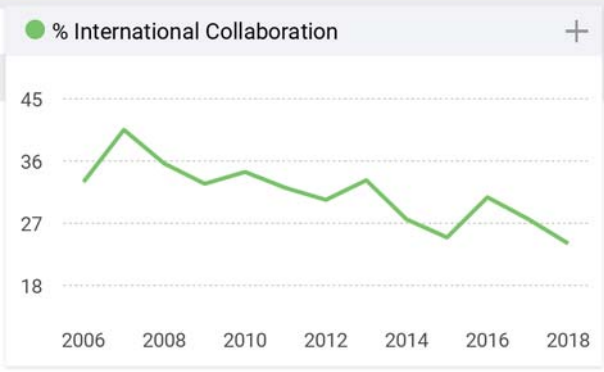
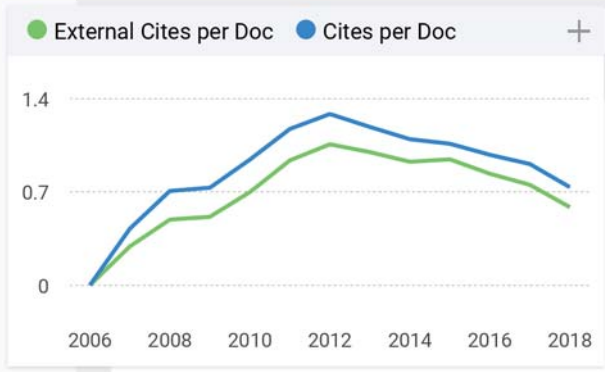


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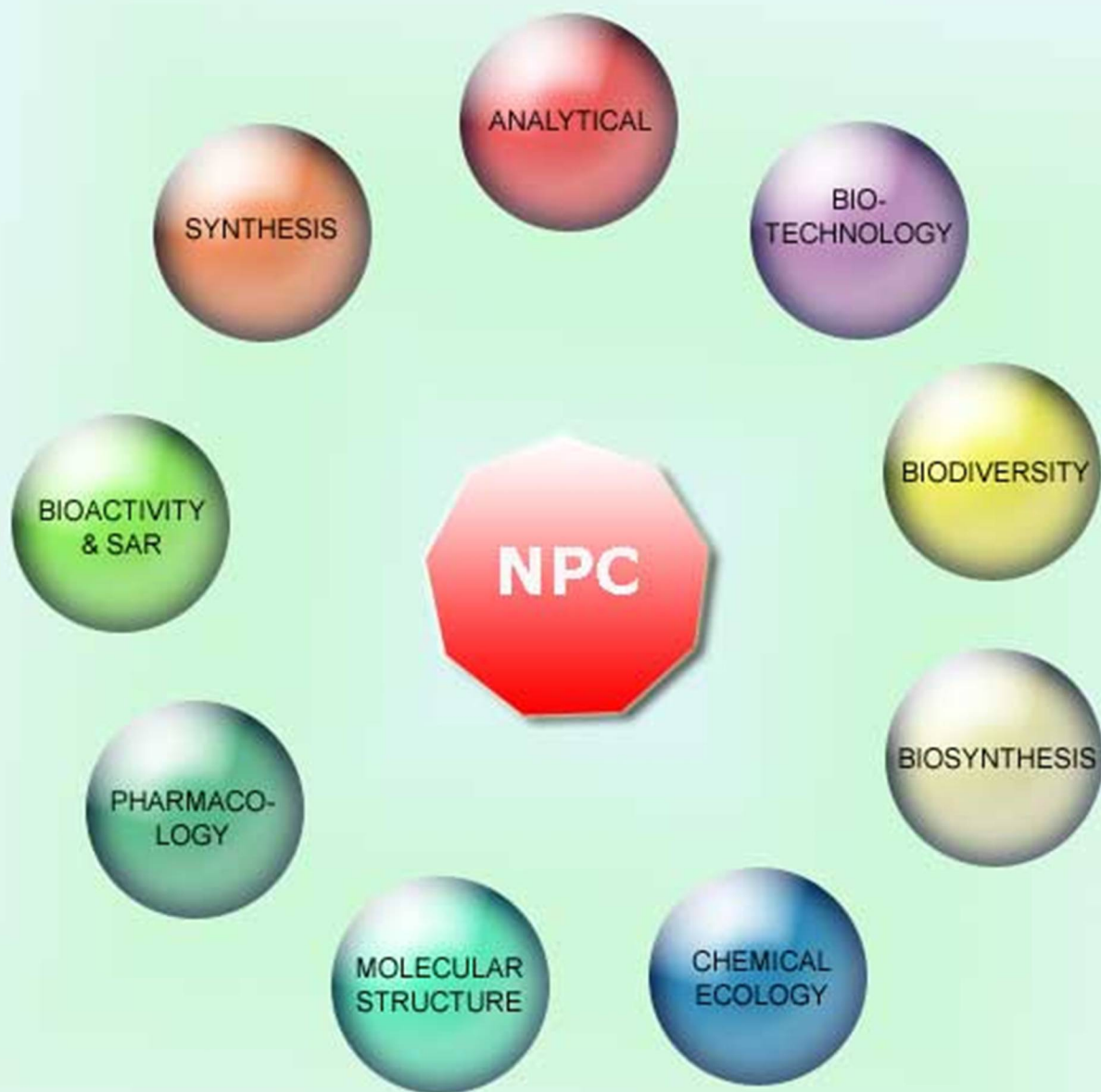
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Dihydroflavonol and Flavonol Derivatives from *Macaranga recurvata*Mulyadi Tanjung<sup>a</sup>, Euis H. Hakim<sup>b</sup>, Elfahmi<sup>c</sup>, Jalifah Latip<sup>d</sup> and Yana M. Syah<sup>b\*</sup><sup>a</sup>Chemistry Department, Airlangga University, Jalan Darmawangsa Dalam, Surabaya 60222, Indonesia<sup>b</sup>Organic Chemistry Division, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia<sup>c</sup>School of Pharmacy, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia<sup>d</sup>Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

yana@chem.itb.ac.id

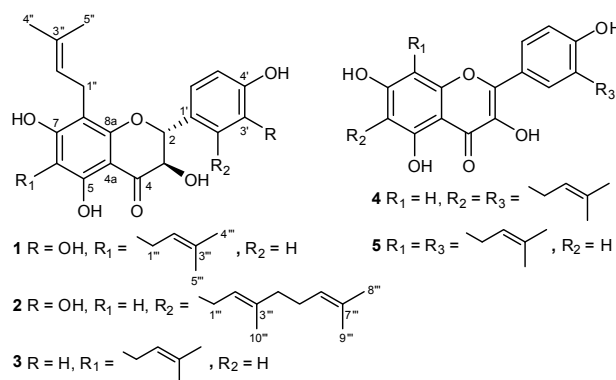
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Two new dihydroflavonol derivatives, macarecurvatins A and B, have been isolated from the leaves of *Macaranga recurvata* (Euphorbiaceae), along with the known compounds diisoprenylaromadendrin, glyasperin A and brousoflavonol F. The structures of the new compounds were determined on the basis of spectroscopic evidence. Upon cytotoxic evaluation against P-388 cells, macarecurvatin B showed strong activity with an IC<sub>50</sub> of 0.83 μM.

**Keywords:** Macarecurvatins A and B, Flavanol, Dihydroflavonol, *Macaranga recurvata*, Euphorbiaceae, P-388 cells.

In our previous reports, we disclosed the presence of isoprenylated and geranylated flavonoids [1-4], and phenolic derivatives containing an irregular sesquiterpenyl side chain, from Indonesian *Macaranga* [3,5]. In continuation of these chemical investigations, we have examined *M. recurvata* Gage and succeeded in isolating two new dihydroflavonols, trivially named as macarecurvatins A (**1**) and B (**2**), together with the known compounds 6,8-diisoprenylaromadendrin (**3**) [6], glyasperin A (**4**) [7], and brousoflavonol F (**5**) [8]. This paper discusses the structure elucidation of the new compounds. Also, cytotoxic properties of compounds **1-5** against murine leukemia P-388 cells are briefly described.

Macarecurvatin A (**1**), obtained as a yellowish solid,  $[\alpha]_D^{20} +40.4$  (*c* 0.32, MeOH), showed a quasimolecular ion  $[M+H]^+$  at *m/z* 441.1902 corresponding to the molecular formula C<sub>25</sub>H<sub>28</sub>O<sub>7</sub>. The UV spectrum of **1** exhibited absorption maxima at λ<sub>max</sub> 204 and 291 nm, and showed bathochromic shifts on addition of NaOH, AlCl<sub>3</sub>, and NaOAc solution. In the <sup>1</sup>H NMR spectrum, the presence of a pair of doublets at δ<sub>H</sub> 4.97 and 4.56, as well as a singlet of a chelated -OH group at δ<sub>H</sub> 11.97, are reminiscent of a 2,3-dihydroflavonol structure. This was substantiated by the presence of a conjugated carbonyl group (δ<sub>C</sub> 198.8) and two methines of oxycarbons (δ<sub>C</sub> 84.3 and 73.3). The presence of five signals of oxyaryl carbons (δ<sub>C</sub> 162.7, 159.7, 158.7, 146.4, and 145.7) suggested that **1** has the basic structure of taxifolin (= 5,7,3',4'-tetrahydroxy-2,3-dihydroflavonol). Furthermore, by the observation of four methyl singlets in the <sup>1</sup>H NMR spectrum (δ<sub>H</sub> 1.75, 1.64, 1.60, and 1.55), together with two vinyl (δ<sub>H</sub> 5.17 and 5.13) and two methylene (δ<sub>H</sub> 3.32 and 3.24) signals, this compound should contain two isoprenyl groups. In the aromatic region of <sup>1</sup>H NMR spectrum, three signals at δ<sub>H</sub> 7.07, 6.91, and 6.85 were observed with multiplicities consistent with the structural unit of the ring B of taxifolin, and, consequently, the isoprenyl groups must be located at C-6 and C-8. Key <sup>1</sup>H-<sup>13</sup>C long range correlations found in the HMBC spectrum, particularly from the chelated -OH (δ<sub>H</sub> 11.97) and the methylene (δ<sub>H</sub> 3.32 and 3.24) signals confirmed the assignment of structure **1** for macarecurvatin A. From the coupling constant of H-2/H-3 (11.5 Hz, *trans*) and the sign and value of its specific optical rotation, the stereochemistry at C-2 and C-3 was determined to be 2*R*,3*R* [9].



Macarecurvatin B (**2**), obtained also as a yellowish solid, has the molecular formula C<sub>30</sub>H<sub>36</sub>O<sub>7</sub>, deduced from the  $[M+H]^+$  ion at *m/z* 509.2534. The UV absorptions of **2** had very similar characteristics to those of **1**, and the NMR parameters in **2** (Table 1) also showed characteristics of the taxifolin structure. The presence of geranyl and isoprenyl groups in **2** was indicated by the <sup>1</sup>H NMR signals of five methyl singlets (δ<sub>H</sub> 1.58, 1.54, 1.51, and 1.51), together with three methine vinyl (δ<sub>H</sub> 5.20, 5.12, and 5.02), and four methylene (δ<sub>H</sub> 3.58, 3.16, 1.98 and 1.95) signals. Furthermore, a singlet (δ<sub>H</sub> 6.07) of aromatic signals was found, suggesting that one of the side chain groups must be located either at C-6 or C-8. In the HMBC spectrum, the chelated-OH signal (δ<sub>H</sub> 11.65) was correlated with an oxyaryl (δ<sub>C</sub> 162.6), a quarternary (δ<sub>C</sub> 101.4), and a methine (δ<sub>C</sub> 96.6) carbon signals, showing that C-6 is unsubstituted. The methylene signal that showed long-range correlations with the oxyaryl carbon signals (δ<sub>C</sub> 165.4 and 161.1) in the A-ring was a doublet at δ<sub>H</sub> 3.16, which, from its COSY spectrum, is part of the isoprenyl group (δ<sub>H</sub> 5.12, 3.16, 1.58, 1.51). Consequently, the geranyl group must be the side chain of ring B. From the presence of a pair of *ortho*-coupled doublets (*J* = 8.4 Hz) at δ<sub>H</sub> 7.05 and 6.82, this group should be located at C-2'. Analysis of HMQC and HMBC spectra confirmed the assignment of structure **2**. By the same argument used for **1** (*J*<sub>H-2/H-3</sub> = 11.7 Hz;  $[\alpha]_D^{20} +35.3$  (*c* 0.24, MeOH), the stereochemistry at C-2 and C-3 was also determined to be 2*R*,3*R* [9].



**Table 1:** NMR spectroscopic data of macarecurvatins A (1) and B (2).

	1, $\delta_H$ (mult., J in Hz)	1, $\delta_C$	2, $\delta_H$ (mult., J in Hz)	2, $\delta_C$
2	4.97 (d, 11.5)	84.4	5.33 (d, 11.7)	80.8
3	4.56 (d, 11.5)	73.3	4.71 (d, 11.7)	73.0
4	-	198.8	-	198.8
4a	-	101.6	-	101.4
5	-	159.7	-	162.6
6	-	109.0	6.07 (s)	96.6
7	-	162.7	-	165.4
8	-	108.2	-	108.5
8a	-	158.7	-	161.1
1'	-	130.1	-	128.4
2'	7.07 (d, 1.9)	115.7	-	129.2
3'	-	145.7	-	143.8
4'	-	146.4	-	145.4
5'	6.91 (d, 8.1)	115.8	6.82 (d, 8.4)	113.3
6'	6.85 (dd, 8.1, 1.9)	120.6	7.05 (d, 8.4)	119.6
1''	3.25 (d, 7.1)	21.8	3.16 (d, 7.3)	22.0
2''	5.13 (tm, 7.1)	123.1	5.12 (tm, 7.3)	123.4
3''	-	132.2	-	131.1
4''	1.60 (s)	25.8	1.58 (s)	25.7
5''	1.55 (s)	17.9	1.51 (s)	17.7
1'''	3.32 (d, 7.0)	22.2	3.58 (d, 6.7)	25.1
2'''	5.17 (tm, 7.0)	123.1	5.20 (tm, 6.7)	124.3
3'''	-	132.2	-	135.2
4'''	1.64 (s)	25.8	1.93 (brt, 6.8)	40.4
5'''	1.75 (s)	17.9	1.98 (brq, 6.8)	27.5
6'''	-	-	5.02 (tm, 6.8)	125.0
7'''	-	-	-	131.3
8'''	-	-	1.58 (s)	25.9
9'''	-	-	1.51 (s)	17.8
10'''	-	-	1.65 (s)	16.4
5-OH	11.96 (s)	-	11.65 (s)	-

<sup>1</sup>H and <sup>13</sup>C NMR signals were assigned from COSY, NOE1D, HMQC and HMBC spectra.

Preliminary cytotoxic evaluation of compounds **1–5** was carried out against murine leukemia P-388 cells using MTT assay [10]; IC<sub>50</sub> values were 6.07, 0.83, 4.83, 17.02, 7.05  $\mu$ M, respectively. It is worth noting that the dihydroflavonols **1–3** tend to be more active than the flavonols **4–5**.

## Experimental

**General:** UV spectra were measured with a UV/VIS Varian Cary 100 Conc. Optical rotations were determined with a Perkin Elmer Polarimeter Model 341. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded with a JEOL JNM ECA400 (<sup>1</sup>H: 400 MHz; <sup>13</sup>C: 100 MHz). HRESIMS were obtained with a Waters LCT Premier XE (positive mode). VLC (vacuum liquid chromatography) and radial chromatography were carried out using Merck silica gel 60 GF<sub>254</sub>, respectively.

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**Plant materials:** Samples of *M. recurvata* were collected in August 2008 from the conserved forest of Rimba Pati, Pasaman, West Sumatera, Indonesia. The plant was identified by the staff at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia. and a voucher specimen had been deposited at the herbarium.

**Extraction and isolation:** The dried and powdered leaves (1.3 kg) of *M. recurvata* were macerated with MeOH (2 times) at room temperature to obtain, after solvent evaporation, a gummy MeOH extract (85 g). The extract was redissolved in MeOH-water (9:1) and was successively partitioned into *n*-hexane (53 g) and EtOAc (17 g) fractions. The EtOAc fraction was then fractionated using VLC eluting with mixtures of *n*-hexane-EtOAc (4:1, 7:3, and 1:1) to give 4 major fractions A-D. Fraction A (200 mg), purified using radial chromatography eluted with *n*-hexane-EtOAc = (9:1 and 4:1), yielded **4** (17 mg) and **5** (4 mg). Using the same method (eluent *n*-hexane-CHCl<sub>3</sub> = 2:3, 1:4), purification of fraction B (370 mg) gave **3** (27 mg), while fraction C (eluent *n*-hexane-CHCl<sub>3</sub> = 2:3, 1:4, and 0:1) yielded **2** (41 mg), and fraction D (twice, eluent *n*-hexane-EtOAc = 4:1 and 7:3; *n*-hexane-CHCl<sub>3</sub> = 1:4, CHCl<sub>3</sub>, and CHCl<sub>3</sub>-EtOAc = 9:1) afforded **1** (35 mg).

### Macarecurvatin A (1)

UV (MeOH):  $\lambda_{max}$  (log  $\epsilon$ ): 207 (4.51), 294 (3.95), 340 (3.43) nm; (+NaOH): 207 (4.68), 249 (3.99), 295 (sh, 3.76), 333 (4.04) nm; (+AlCl<sub>3</sub>): 210 (4.54), 316 (4.00), 403 (3.34) nm; (+NaOAc): 207 (4.79), 297 (4.22) nm.

<sup>1</sup>H and <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>): Table 1.

HRESIMS: m/z [M+H]<sup>+</sup> calc. for C<sub>25</sub>H<sub>28</sub>O<sub>7</sub> 441.1913; found: 441.1902.

### Macarecurvatin B (2)

UV (MeOH):  $\lambda_{max}$  (log  $\epsilon$ ): 204 (4.47), 228 (sh, 4.21), 291 (3.99), 348 (3.34) nm; (+NaOH): 211 (4.71), 245 (sh, 4.00), 288 (3.81), 344 (4.00) nm; (+AlCl<sub>3</sub>): 206 (4.53), 226 (sh, 4.29), 308 (4.03), 370 (3.34) nm; (+NaOAc): 206 (4.57), 293 (3.97), 348 (3.22) nm.

<sup>1</sup>H and <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>): Table 1.

HRESIMS: m/z [M+H]<sup>+</sup> calc. for C<sub>30</sub>H<sub>36</sub>O<sub>7</sub> 509.2539; found: 509.2534.

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