SUPPLEMENTARY MATERIAL

A new cytotoxic biflavonoid from the rhizome of *Wikstroemia indica*

Meng Shao ^{a, 1}, Xiao-Jun Huang ^{b, 1}, Jun-Shan Liu ^a, Wei-Li Han ^c, Hong-Bing Cai ^a,
Qing-Fa Tang ^{a,*} and Qin Fan ^{a,*}

^a School of Traditional Chinese Medicine, Southern Medical University, Guangzhou 510515, China; ^b Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China; ^c Hygiene Detection Center, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou 510515, China

Corresponding authors,

E-mail: fqin@163.com, Phone: +86-20-62789493;

E-mail: tangqf96@163.com, Phone: +86-20-62648265.

One new chalcone-flavone biflavonoid, 3'-hydroxydaphnodorin A (**1**), together with twelve known biflavonoids (**2-13**), was isolated from the rhizome of *Wikstroemia indica*. Their structures were established on the basis of extensive spectroscopic methods. Eight isolated compounds **1-3**, **6**, **7**, **9**, **12** and **13** were evaluated for their cytotoxic activities against cancer-derived cell lines Hep3B, HepG2 and CNE2, and **1** was found to possess moderate cytotoxicity against HepG2 and CNE2 cell lines with the IC₅₀ values of 65.5 ± 11.4 and 53.6 ± 10.1 μM, respectively.

Keywords: *Wikstroemia indica*; biflavonoid; 3'-hydroxydaphnodorin A; cytotoxicity

*Corresponding author. Email: fqin@163.com, tangqf96@163.com

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Computation methods

The plausible stable conformers of **1** were obtained by random conformation search in Sybly8.1 with MMFF94s force field and charged with MMFF94. The conformers were further optimized in Gaussian09 software [1] by using DFT method (B3LYP/6-31G(d)), and screened according to the energy window of 3 kcal/mol with respect to the global minima. Six conformers were selected for further optimization and OR calculation at B3LYP/6-311+G(d) and B3LYP/aug-cc-pVDZ levels. The overall calculated OR values were averaged according to Boltzmann weighting, and compared to the experimental one.

Table S1. Calculated and experimental specific rotation values of **1**

Conformers	ΔE (kcal/mol)	p %	calcd		exptl
			B3LYP/6-311+G(d)	B3LYP /aug-cc-pVDZ	
a	0.00	0.37	-78.71	-75.03	-30.4
b	0.00	0.37	-78.84	-75.29	
c	0.20	0.26	89.57	86.28	
d	3.57	0.00	-117.98	-116.71	
e	3.96	0.00	-167.60	-182.13	
f	4.10	0.00	-1081.34	-1097.03	
Averaged			-35.0	-33.2	

Geometry and coordinates of structure **1a**

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	-1.963418	4.293724	0.243592
2	6	0	-2.938013	3.268852	-0.351491
3	6	0	-2.703535	1.895795	0.285108
4	8	0	-1.357394	1.452048	0.010615
5	6	0	-3.647527	0.805571	-0.187209

6	6	0	-5.000761	0.850780	0.167419
7	6	0	-5.878515	-0.158902	-0.236875
8	6	0	-5.407538	-1.238973	-0.980691
9	6	0	-4.048068	-1.294544	-1.332691
10	6	0	-3.184832	-0.268661	-0.957498
11	8	0	-6.252232	-2.248242	-1.352209
12	6	0	-0.557362	3.733884	0.272531
13	6	0	-0.347914	2.361283	0.121180
14	6	0	0.963406	1.851641	0.046367
15	6	0	2.029561	2.749358	0.158590
16	6	0	1.882222	4.119157	0.351285
17	6	0	0.567617	4.587058	0.401804
18	8	0	3.213648	2.081706	0.036151
19	6	0	1.556687	0.553309	-0.174028
20	6	0	2.914165	0.742533	-0.184239
21	6	0	4.061471	-0.138597	-0.361312
22	6	0	3.942968	-1.535577	-0.246050
23	6	0	5.040070	-2.369466	-0.435707
24	6	0	6.293823	-1.821415	-0.734468
25	6	0	6.434514	-0.431712	-0.839215
26	6	0	5.332757	0.394153	-0.656360
27	6	0	0.798111	-0.676749	-0.559259
28	6	0	0.166414	-1.566752	0.404781
29	6	0	0.319467	-1.472006	1.822907
30	6	0	-0.337048	-2.342055	2.683608
31	6	0	-1.171996	-3.339008	2.163008
32	6	0	-1.355709	-3.478499	0.784669
33	6	0	-0.692056	-2.611545	-0.074206
34	8	0	0.686519	-0.882931	-1.792111
35	8	0	7.407787	-2.585121	-0.926946
36	8	0	1.138350	-0.512223	2.311082
37	8	0	-1.778159	-4.150784	3.058151
38	8	0	-0.909199	-2.769744	-1.402450
39	8	0	0.283725	5.915419	0.570556
40	8	0	-3.663411	-2.400028	-2.049442
41	1	0	-2.288783	4.573739	1.255632
42	1	0	-1.980824	5.217620	-0.344552
43	1	0	-3.974114	3.578076	-0.182139
44	1	0	-2.794000	3.178814	-1.435350
45	1	0	-2.794962	1.999313	1.377121
46	1	0	-5.379917	1.669824	0.773317
47	1	0	-6.927885	-0.130724	0.041473
48	1	0	-2.144305	-0.314228	-1.251718
49	1	0	-5.705498	-2.918935	-1.801200

50	1	0	2.735250	4.783607	0.441894
51	1	0	2.989446	-1.984089	0.012619
52	1	0	4.926317	-3.446634	-0.339371
53	1	0	7.410272	-0.014648	-1.068511
54	1	0	5.452625	1.468281	-0.746809
55	1	0	-0.208819	-2.263135	3.758538
56	1	0	-2.003604	-4.245171	0.372254
57	1	0	7.176124	-3.523634	-0.831096
58	1	0	1.152015	-0.564880	3.282013
59	1	0	-2.328180	-4.804172	2.593665
60	1	0	-0.298442	-2.107789	-1.859935
61	1	0	1.114223	6.416757	0.613183
62	1	0	-2.697779	-2.544607	-1.941507

Table S2. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) data of compound **1** in CD₃OD

No.	δ_{H}	δ_{C}	No.	δ_{H}	δ_{C}
2	4.76 (1H, d, 8.0)	78.9	2"		150.0
3	2.70 (2H, m)	21.5	3"		119.3
4	2.15 (1H, m)	31.5	4"		197.6
	1.81 (1H, m)				
4a		106.5	5"		108.4
5		155.5	6"		166.8
6	6.51 (1H, s)	90.8	7"	6.12 (1H, d, 1.6)	96.3
7		150.7	8"		168.0
8		112.5	9"	6.13 (1H, d, 1.6)	96.0
8a		155.0	10"		166.7
1'		135.2	11"		124.1
2'	6.62 (1H, s)	114.1	12"	7.46 (1H, d, 7.0)	128.8
3'		146.2	13"	6.74 (1H, d, 7.0)	116.9
4'		145.8	14"		159.2
5'	6.61 (1H, d, 6.4)	116.8	15"	6.74 (1H, d, 7.0)	116.9
6'	6.39 (1H, dd, 6.4, 1.6)	118.6	16"	7.46 (1H, d, 7.0)	128.8

Table S3. Cytotoxic activities of compounds **1-3, 6, 7, 9, 12, 13**

Compounds	IC ₅₀ (μM)		
	Hep3B	HepG2	CNE2
1	>100	65.5 ± 11.4	53.6 ± 10.1
3	>100	67.4 ± 7.1	13.8 ± 3.5
6	>100	>100	55.8 ± 6.7
7	59.8 ± 6.4	62.8 ± 6.1	48.2 ± 6.5
9	41.2 ± 4.7	36.4 ± 2.1	11.6 ± 4.2
2, 12, 13	>100	>100	>100

Figure S4. ^1H -NMR spectrum of compound **1**

Figure S5. ^{13}C -NMR spectrum of compound **1**

Figure S6. DEPT-135 spectrum of compound **1**

Figure S7. ^1H - ^1H COSY spectrum of compound **1**

Figure S8. HSQC spectrum of compound **1**

Figure S9. HMBC spectrum of compound **1**

Figure S10. ROESY spectrum of compound **1**

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