

Four Hybrid Flavan–Chalcones, Caesalpinnone A Possessing a 10,11-Dioxatricyclic [5.3.3.0^{1,6}]Tridecane-Bridged System and Caesalpinflavans A-C from Caesalpinia enneaphylla

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S Supporting Information



ABSTRACT: Caesalpinnone A (1), an unprecedented hybrid of flavan and chalcone, possessing a 10,11-dioxatricyclic $[5.3.3.0^{1.6}]$ tridecane-bridged system, and caesalpinflavans A-C (2-4), three new hybrid flavan-chalcones, were isolated from the twigs and leaves of Caesalpinia enneaphylla. Their structures were elucidated by a combination of spectroscopic analyses and single-crystal X-ray diffraction. Caesalpinnone A showed the highest cytotoxicity against the HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines with an IC₅₀ in the range of 0.54–0.87 μ M.

he plant Caesalpinia enneaphylla, belonging to the family Leguminosae, is distributed in Yunnan Province of China, northeast India, Sri Lanka, Burma, Thailand, southern Vietnam, and Malaysia. Because of their characteristic cassane diterpenoids and homoisoflavonoids,¹ the plants of Caesalpinia have drawn wide attention. In our continuing investigation on Caesalpinia plants, four new hybrid flavan-chalcones, caesalpinnone A (1) and caesalpinflavans A-C (2-4), were isolated from the twigs and leaves of C. enneaphylla. It is noteworthy that 1 possesses an unprecedented 10,11-dioxatricyclic- $[5.3.3.0^{1.6}]$ tridecane bridged system. Compound 4 is the hybrid of two flavans and one chalcone.

Caesalpinnone A (1) was obtained as yellow crystals. Its molecular formula was determined to be $C_{30}H_{24}O_6$ (19 degrees of unsaturation) by HR-ESI-MS at m/z 481.1646 [M + H]⁺ (calcd for C₃₀H₂₅O₆, 481.1646). Its UV spectrum absorption maximum at 330 nm and the IR absorption bands at 1688, 1632, and 3425 cm⁻¹ indicated the presence of carbonyl and hydroxyl groups. The ¹³C NMR (125 MHz) and DEPT spectra of 1 displayed the presence of two sp³ methylenes, 16 sp² methines, two sp³ methines, eight sp² quaternary carbons including two $\alpha_{,\beta}$ -unsaturated keto carbonyl carbons, and two sp³ oxygenated quaternary carbons. The ¹H NMR spectrum (Table 1) showed signals for two hydroxyl groups ($\delta_{\rm H}$ 14.07, 2'-OH and 5.53, 10"-OH), a trans double bond ($\delta_{\rm H}$ 7.97 and 8.06, d, J = 15.0 Hz), another two sp² AB proton spin systems, and two monosubstituted benzene rings. The HMBC correlations from proton at $\delta_{\rm H}$ 7.97 (H- α) of a *trans* double bond to a ketone carbonyl ($\delta_{\rm C}$ 192.5), to C- β ($\delta_{\rm C}$ 120.6) and C-2/C-6 ($\delta_{\rm C}$ 129.0), as well as $\delta_{\rm H}$ 8.06 (H- β) to $\delta_{\rm C}$ 192.5 (ketone carbonyl), 144.7 (C- α) and 134.9 (C-1) suggested the presence of a chalcone unit in 1. The HMBC correlations from the proton at $\delta_{\rm H}$ 8.22 (d, J = 9.0 Hz, H-6') to C-2' ($\delta_{\rm C}$ 163.2), C-4' ($\delta_{\rm C}$ 161.6), and ketone carbonyl ($\delta_{\rm C}$ 192.5), from the proton at $\delta_{\rm H}$ 6.48 (d, J = 9.0 Hz, H-5') to C-1' ($\delta_{\rm C}$ 114.2), C-3' $(\delta_{\rm C}$ 111.4), C-4' $(\delta_{\rm C}$ 161.6), and ketone carbonyl $(\delta_{\rm C}$ 192.5), and from the proton of 2'-OH ($\delta_{
m H}$ 14.07) to C-1' ($\delta_{
m C}$ 114.2), C-2' ($\delta_{\rm C}$ 163.2), and C-3' ($\delta_{\rm C}$ 111.4) showed the substituent positions on the B ring of chalcone unit (Figure 1). The remaining part of the structure was established by analysis of the HMBC and ¹H-¹H COSY spectra. The HMBC correlations from H-6" ($\delta_{\rm H}$ 5.93) and H-5" ($\delta_{\rm H}$ 6.84) to C-7" ($\delta_{\rm C}$ 194.6) as well as the ¹H–¹H COSY correlation between H-6" ($\delta_{\rm H}$ 5.93) and H-5" ($\delta_{\rm H}$ 6.84) confirmed another α,β unsaturated keto carbonyl group. At the same time, the HMBC correlations from H₂-8" ($\delta_{\rm H}$ 2.76, 3.36) to C-6" ($\delta_{\rm C}$ 130.5), C-7" ($\delta_{\rm C}$ 194.6), C-9" ($\delta_{\rm C}$ 101.5), and C-10" ($\delta_{\rm C}$ 64.1), from H-6" ($\delta_{\rm H}$ 5.93) to C-8" ($\delta_{\rm C}$ 44.4), C-7" ($\delta_{\rm C}$ 194.6), and C-10" ($\delta_{\rm C}$ 64.1), from H-4" ($\delta_{\rm H}$ 3.74) to C-9" and C-10", and from 10"-OH to C-4", C-9", and C-10" indicated the presence of ring C. The proton spin system of $\delta_{\rm H}$ 4.82 (1H, dd, J = 3.5, 12.2 Hz), 1.91 (1H, dt, J = 13.3, 3.3 Hz) and 2.81 (m), 3.74 (1H, t, J = 3.5 Hz), coupled with the preceding information, showed that

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	1		2		
no.	$\delta_{ m C}$	$\delta_{\rm H} \ ({\rm mult,} \ J)$	$\delta_{ m C}$	δ_{H} (mult, J)	
1	134.9		135.2		
2, 6	129.0	7.90	128.6	7.60 (overlap)	
3, 5	128.9	7.50 (overlap)	129.4	7.45 (overlap)	
4	130.8	7.50 (overlap)	127.8	7.42 (overlap)	
α	144.7	7.97 (d, 15.0)	143.6	7.76 (d, 15.6)	
β	120.6	8.06 (d, 15.0)	121.1	7.38 (overlap)	
γ	192.5		192.2		
1'	114.2		113.4		
2'	163.2		165.3		
3'	111.4		103.3	6.54 (overlap)	
4'	161.6		162.2		
5'	106.5	6.48 (d, 9.0)	125.6		
6'	131.2	8.22 (d, 9.0)	133.3	7.49 (s)	
2″	71.6	4.82 (dd, 3.5, 12.2)	73.9	5.09 (dd, 2.2, 10.3)	
3″	33.6	2.81 (m)	35.9	2.38 (ddd, 5.5, 10.3, 13.9)	
		1.91 (td, 3.3, 13.3)		2.47 (td, 3.0, 13.9)	
4″	32.9	3.74 (t, 3.5)	33.8	4.45 (t, 4.5)	
5″	148.2	6.84 (d, 10.0)	131.4	6.88 (d, 8.2)	
6″	130.5	5.93 (d, 9.8)	109.0	6.54 (overlap)	
7″	194.6		157.8		
8	44.4	2.76 (m), 3.36 (d, 16.0)	103.2	6.54 (overlap)	
9″	101.5		157.0		
10″	64.1		114.6		
1‴	141.3		142.3		
2‴, 6‴	126.2	7.43 (d, 8.5)	126.4	7.36 (overlap)	
3‴, 5‴	128.2	7.35 (t, 7.5)	128.6	7.60 (overlap)	
4‴	127.6	7.28 (t, 7.1)	130.9	7.42 (overlap)	
2'-OH		14.07 (s)	2'-OH	13.18 (s)	
10″-OH		5.53 (s)	4'-OH	9.93	
			7″-OH	8.39	

Table 1. ¹H NMR and ¹³C NMR Spectroscopic Data of Compounds 1 and 2 (Recorded at 500 MHz) (CD₃COCD₃, δ in ppm, J in Hz in Parentheses)



Figure 1. Structures of compounds 1–4.

compound 1 might have a flavan unit bearing a highly modified C-ring. The rings A and D were monosubstituted aromatic rings. The HMBC correlations from H-4" to C-2", C-3", C-5", C-9", C-10", C-2', C- 3', and C-4' indicated the linkage between the flavan moiety and the chalcone unit, and that these two units were attached via C-4 and C-9 of the flavan moiety. The preceding data accounted for 18 degrees of unsaturation, indicating the requirement of an additional ring in the structure of 1. The ring may be an ether linkage between the oxygenated quaternary carbon at $\delta_{\rm C}$ 101.5 and another carbon, which

cannot be decided from 2DNMR because of the presence of multiple quaternary carbons. Finally, a single-crystal X-ray diffraction analysis using Cu K α radiation was carried out to confirm the structure and demonstrate the absolute configuration of **1** according to the Flack parameter 0.07(7),³ shown in Figure 1.

Caesalpinflavan A (2) was obtained as yellow amorphous powder. Its molecular formula was determined to be $C_{30}H_{24}O_5$ on the basis of HR-ESI-MS data ($465.1721 [M + H]^+$, calcd for $C_{30}H_{25}O_5$, 465.1697) with 19 degrees of unsaturation. Its UV spectrum absorption maximum at 343 nm and the IR absorption bands at 1636 and 3426 cm^{-1} indicated the presence of carbonyl and hydroxyl groups. Analysis of the ¹³C NMR (125 MHz) and DEPT spectra of 2 demonstrated the presence of 30 carbons, attributable to 17 sp² methine carbons, two sp³ methines, one sp³ methylene, and 10 sp² quaternary carbons, respectively. The number of sp² carbons suggested that four benzene rings existed in the structure of 2. A coupling constant of 15.6 Hz in the ¹H NMR spectrum suggested a trans double bond in 2. The ¹H NMR spectrum of 2 showed the presence of signals for three hydroxyl protons at $\delta_{\rm H}$ 13.18 (2'-OH), 9.93 (4'-OH), and 8.39 (7"-OH). The HMBC correlations from proton at $\delta_{\rm H}$ 7.76 (H- α) of a *trans* double bond to a ketone carbonyl ($\delta_{\rm C}$ 192.2), to C- β ($\delta_{\rm C}$ 121.1), C-1 ($\delta_{\rm C}$ 135.2), and C-2/C-6 ($\delta_{\rm C}$ 128.6), as well as $\delta_{\rm H}$ 7.38 (H- β) to $\delta_{\rm C}$ 192.2 (ketone carbonyl) suggested the presence of a chalcone unit in caesalpennone B(2). A carbonyl,

2, 67.76 (overlap)7.85 (overlap)3, 57.43 (overlap)7.47 (overlap)47.43 (overlap)7.47 (overlap) α 7.81 (overlap)7.85 (overlap) β 7.81 (overlap)7.92 (d, 15.5)5'6.46 (d, 9.0)6.53 (d, 8.5)6'7.94 (d, 9.0)8.03 (d, 8.5)2"5.53 (dd, 4.2, 5.3)5.55 (dd, 3.5, 6.0)2'"4.76 (d, 10.0)3"2.76 (ddd, 3.9, 8.4, 13.7)2.72 (ddd, 3.5, 9.6, 13.4)3'"2.29 (td, 6.0, 13.7)2.36 (td, 6.5, 13.5)2.09 (m)4"4.48 (t, 7.2)4.50 (t, 6.5)4'"4.6 (dd, 1.9, 5.0)5"6.52 (dd, 0.8, 8.4)6.32 (overlap)6"6.20 (dd, 2.5, 8.4)6.23 (d, 2.5)8"6.39 (d, 2.5)6.23 (d, 2.5)2", 6""7.36 (overlap)7.43 (overlap)3", 5""7.32 (overlap)7.36 (overlap)4"7.30 (overlap)7.30 (overlap)4"4.48	no.	3: $\delta_{\rm H}$ (mult, J)	4: $\delta_{\rm H}$ (mult, J)	no.	4: $\delta_{\rm H}$ (mult, J)
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$	4	7.43 (overlap)	7.47 (overlap)		
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5' $6.46 (d, 9.0)$ $6.53 (d, 8.5)$ $6'$ $7.94 (d, 9.0)$ $8.03 (d, 8.5)$ $2''$ $5.53 (dd, 4.2, 5.3)$ $5.55 (dd, 3.5, 6.0)$ $2'''$ $4.76 (d, 10.0)$ $3''$ $2.76 (ddd, 3.9, 8.4, 13.7)$ $2.72 (ddd, 3.5, 9.6, 13.4)$ $3'''$ $2.21 (ddd, 5.6, 11.2, 13)$ $2.29 (td, 6.0, 13.7)$ $2.36 (td, 6.5, 13.5)$ $2.09 (m)$ $4''$ $4.48 (t, 7.2)$ $4.50 (t, 6.5)$ $4'''$ $4.46 (dd, 1.9, 5.0)$ $5''$ $6.52 (dd, 0.8, 8.4)$ $6.32 (overlap)$ $5'''$ $6.75 (d, 8.0)$ $6'''$ $6.20 (dd, 2.5, 8.4)$ $6.23 (d, 2.5)$ $2'''', 6''''$ $7.36 (overlap)$ $8''$ $6.39 (d, 2.5)$ $7.43 (overlap)$ $2'''', 6''''$ $7.24 (overlap)$ $3''', 5'''$ $7.32 (overlap)$ $7.36 (overlap)$ $3'''', 5''''$ $7.30 (overlap)$ $4'''$ 4.08 4.08 $3'''', 5''''$ $7.30 (overlap)$	β	7.81 (overlap)	7.92 (d, 15.5)		
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6" 6.20 (dd, 2.5, 8.4) 6'" 6.34 (overlap) 8" 6.39 (d, 2.5) 6.23 (d, 2.5) 5000000000000000000000000000000000000	5″	6.52 (dd, 0.8, 8.4)	6.32 (overlap)	5′″	6.75 (d, 8.0)
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2"", 6"" 7.36 (overlap) 7.43 (overlap) 2""', 6""' 7.24 (overlap) 3", 5"" 7.32 (overlap) 7.36 (overlap) 3""', 5""' 7.36 (overlap) 4"" 7.25 (t, 7.0) 7.30 (overlap) 4""' 7.30 (overlap) 2'''H 2''H 2''H 7.30 (overlap)	8″	6.39 (d, 2.5)	6.23 (d, 2.5)		
3"", 5"" 7.32 (overlap) 7.36 (overlap) 3""', 5""' 7.36 (overlap) 4"" 7.25 (t, 7.0) 7.30 (overlap) 4""' 7.30 (overlap) 2'-OH 14.08 2'-OH 14.08 2'-OH 14.08 3""', 5""' 7.36 (overlap)	2‴, 6‴	7.36 (overlap)	7.43 (overlap)	2""', 6""'	7.24 (overlap)
4 ^{'''} 7.25 (t, 7.0) 7.30 (overlap) 4 ^{'''''} 7.30 (overlap) 2'-OH 14.08	3‴, 5‴	7.32 (overlap)	7.36 (overlap)	3""', 5""''	7.36 (overlap)
2'-OH 14.08	4‴	7.25 (t, 7.0)	7.30 (overlap)	4″″′	7.30 (overlap)
			2'-OH 14.08		

Table 2. ¹H NMR Spectroscopic Data of Compounds 3 and 4 (Recorded at 500 MHz) (3 in CD₃OD, 4 in CD₃COCD₃, δ in ppm, J in Hz in Parentheses)

a double bond, and four benzene rings accounted for 18 degrees of unsaturation. The residual one degree of unsaturation revealed the presence of a ring. The proton spin system of $\delta_{\rm H}$ 5.09 (1H, dd, J = 2.2, 10.3 Hz), 2.47 (1H, dt, J = 13.9, 3.0 Hz), 2.38 (1H, ddd, J = 5.5, 10.3, 13.9 Hz), and 4.45 (1H, t, J = 4.5 Hz) and the remaining 15 carbons indicated that compound 2 might have a flavan part. The HMBC correlations also indicated the linkage between a flavan moiety and a chalcone unit, demonstrating correlations from H_2 -3" to C-2", C-4", and C-1" and from H-4" to C-2", C-3", C-5", C-9", C-4', C-5', and C-6'. The HMBC correlations from 7"-OH to C-6", C-7", and C-8", from 2'-OH to C-1', C-2', C-3', C-4', and C-γ, and from 4'-OH to C-3', C-4', and C-5' assisted the assignment of substituent positions on the substituted aromatic rings B and D. On the basis of these data, the structure of caesalpinflavans A (2) was established as a hybrid flavan-chalcone, and assignments of proton and carbon signals for 2 were given in Table 1. A large coupling constant (J = 12.2 Hz) of H-2" (C) indicated an axial configuration of H-2" (C), while small coupling constants (I = 4.5 and 4.5 Hz) of H-4" (C) suggested an equatorial orientation of H-4" (C). The positive Cotton effect of $[\theta]_{218}$ in CD spectrum indicated a 4β -configuration at C-4", which was the same as that of desmosflavan B.² Therefore, the absolute configuration of 2 was established as 2''S and 4''S. The ROESY correlations of H- $2''/H-3''_{equatorial}$, H-3" equatorial/H-4" and H-3" axial/H-4" also supported the configuration of 2.

Caesalpinflavan B (3) had the same molecular formula as that of 2, $C_{30}H_{24}O_5$, deduced from HR-ESI-MS data (465.1661 $[M + H]^+$, calcd for $C_{30}H_{25}O_5$, 465.1697). Careful comparison of the ¹H and ¹³C NMR spectroscopic data (Table 2) of 3 with 2 indicated that their spectroscopic data were quite similar, and compound 3 was also a flavan–chalcone dimer. The main differences in the ¹³C NMR spectrum between 2 and 3 were the slight change of chemical shifts of carbon signals at C-4", C-3', and C-5' and the coupling constant in the E-ring proton spin system in ¹H NMR spectrum. The correlations from H₂-3" to C-2", C-4", C-10", and C-1"" and from H-4" to C-2", C-3", C-5", C-9", C-10", C-2', and C-4' showed the linkage between flavan and chalcone unit shown in Figure 1. The small coupling constant (J = 4.2, 5.3 Hz) of H-2^{*m*} (C) indicated an equatorial configuration of H-2^{*m*} (C), while large coupling constants (J = 7.2 Hz) of H-4^{*m*} (C) suggested an axial orientation of H-4^{*m*} (C). The negative Cotton effect of $[\theta]_{215}$ in CD spectrum indicated a 4 α -configuration at C-4^{*m*}, ² thus establishing 2^{*m*}R and 4^{*m*}R absolute configurations of **3**. This conclusion was also indicated by the ROESY correlations of H-2^{*m*}/H-3^{*m*}_{axiab} H-2^{*m*}/H-3^{*m*}_{equatorial}, and H-3^{*m*}_{equatorial}/H-4^{*m*}.

Caesalpinflavan C (4), yellow amorphous powder, had a molecular formula C45H36O7 with 28 degrees of unsaturation, based on the pesudomolecular ion peak at 711.2359 [M + Na]⁺ (calcd for C45H36O7Na, 711.2353). The IR spectrum showed absorption bands at 1625 and 3425 cm⁻¹, reminiscent the presence of carbonyl and hydroxyl groups. The carbonyl group and a coupling constant of 15.5 Hz in ¹H NMR spectrum showed the presence of a trans chalcone unit. Two sp³ methylenes, two sp³ methines, two sp³-oxygenated methines, and the remaining 24 sp² carbons in the ¹³C NMR spectrum suggested the presence of two flavan units in 4. The HMBC correlations from H- β ($\delta_{\rm H}$ 7.92, d, 15.5 Hz) to C-1, C- α and C- γ , from 2'-OH ($\delta_{\rm H}$ 14.08) to C-1', C-2', C-3', and C-4', from H-6' ($\delta_{\rm H}$ 8.03, d, 8.5 Hz) to C-1', C- γ , C-2', and C-4' confirmed the presence of chalcone unit, and assisted the assignment of substituent positions on the substituted aromatic ring B. The HMBC correlations from H-2" to C-3", C-4", C-9", C-1"", and C-2""/C-6"", from H2-3" to C-2", C-4", C-10", C-3', and C-1"", and from H-4" to C-2", C-3", C-5", C-9", C-10", C-2', C-3', and C-4' confirmed the presence of one of the flavan unit and showed the linkage between flavan and chalcone unit (Figure 1). The HMBC correlations from H-2"" to C-3"", C-4"", C-1""' and C-2""'/ C-6""', from H₂-3"" to C-2"", C-4"", C-10"", C-1""', and C-6", and from H-4"" to C-2"", C-3"", C-5"", C-9"", C-10"", C-5", C-6", and C-7" confirmed the presence of the other flavan unit and showed the linkage between flavan and flavan unit. Thus, compound 4 was decided to be a hybrid of two flavans and one chalcone. In the ¹H NMR spectrum, the coupling constants of the spin systems of E ring and H ring were the same as that of E ring of 3 and 2 respectively. Therefore, the 2"R and 4"R, the 2""S and 4""S absolute configuration of 4 was established, and the ROESY

correlations of H-2"/H-3" axiab H-2"/H-3" equatorial, H-3" equatorial/ H-4", H-2""/H-3"" equatorial, and H-3"" axial/H-4"" were in agreement with the configuration of **4**.

Using the MTS method, compounds 1–4 were tested for their cytotoxicity against the HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines. Compounds 1–4 exhibited potent antitumor activity. The α , β -common unsaturated ketone moiety is likely to be responsible for the biological activities of chalcones.⁴ Caesalpennone A (1) showed the highest cytotoxicity against the five human tumor cell lines with an IC₅₀ value in the range of 0.54–0.87 μ M (Table 3), which indicated that dioxatricyclic[5.3.3.0^{1,6}] tridecane with α , β unsaturated carbonyl moiety might increase the potency for antitumor activity.

Table 3. Cytotoxic Activities of Compounds 1-4 against Tumor Cell Lines a

compd	HL-60	A-549	SMMC-7721	MCF-7	SW480
1	0.54	0.76	0.60	0.87	0.70
2	13.53	14.12	8.40	13.61	8.53
3	3.41	14.11	8.05	4.44	6.11
4	2.88	3.43	3.70	2.99	3.92
DPP ^b	3.86	20.78	33.25	34.75	16.14
taxol ^b	< 0.008	< 0.008	<0.008	< 0.008	< 0.008

^{*a*}Results were expressed as IC_{50} values in μ M. Cell lines: HL-60 acute leukemia; SMMC-7721 liver cancer; A-549 lung cancer; MCF-7 mammary cancer; SW480 colon cancer. ^{*b*}Positive control

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.or-glett.7b01955.

Detailed experimental procedures, method of cytotoxicity test, physicochemical properties, 1D NMR, 2D NMR, MS, UV, IR, and CD spectra of compounds 1–4, X-ray crystal structure of 1 (PDF) X-ray crystallographic data of 1 (CIF)

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Notes

The authors declare no competing financial interest.

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