Labdane Diterpenes from the Aerial Parts of *Curcuma comosa* Enhance Fetal Hemoglobin Production in an Erythroid Cell Line

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Three new labdane diterpenes, curcucomosins A−C (**1**−**3**), four known labdane diterpenes, **4**−**7**, and a known diarylheptanoid, **8**, were isolated from the aerial parts of *Curcuma comosa*. The structures of the new diterpenes were elucidated by spectroscopic data analysis. The fetal hemoglobin (Hb F) induction potency of the isolated compounds was examined using a K562 reporter cell line harboring the enhanced green fluorescence protein (EGFP) gene under the control of a $^{G}\gamma$-globin promoter. Compound **6**, isocoronarin D, exhibited the highest Hb F induction effect of 1.6-fold at 20 μM.

β-Thalassemia is one of the most common inherited disorders affecting hemoglobin synthesis. It is characterized by the absence or reduction of β-globin chain synthesis in human erythroid cells. The continued production of normal levels of the α-globin chain exceeds the binding capacity of the β-globin chain, leading to globin chain imbalance. Excess α-globin chains precipitate in erythroid progenitor cells, resulting in a buildup of reactive oxygen species (ROS), causing cell death, ineffective erythropoiesis, and severe anemia. Untreated, thalassemia is fatal, and patients are dependent on blood transfusions every 3−4 weeks for life. The size of the excess free α-globin chain pool determines the clinical severity in β-thalassemia patients. Factors that reduce the pool of excess α-globin chains such as an increase in the production of γ-globin chains, which combine with the excess α-globin to form fetal hemoglobin (Hb F, $\alpha_2\gamma_2$), result in better conditions and transfusion independence of the patients.[1] The stimulation of γ-globin chain production is an alternative treatment for β-thalassemia and sickle cell anemia patients. The pharmacological agents for Hb F induction, such as 5-azacytidine, hydroxyurea, and butyric acid, have been studied for many years.[2−8] However, they have low efficacy and specificity, and some agents are potential carcinogens and associated with high toxicity.[9−11] There is, therefore, an urgent need to identify new types of pharmacological agents that can induce Hb F with greater efficacy and less toxicity. The discovery of novel inducers of Hb F and their evaluation in biologically relevant in vitro and in vivo model systems should provide early "proof-of-concept" and enable the initiation of preclinical and clinical studies.

The rhizome of *Curcuma comosa* Roxb. (Zingiberaceae) has long been used in indigenous medicine in Thailand as an anti-inflammatory agent. A number of diarylheptanoids with estrogenic activity[12] and a phloracetophenone glucoside with choleretic activity[13] have been isolated. However, investigation on the aerial parts of this plant species has not been reported. As part of our ongoing project in the search for phytochemicals that act as Hb F enhancers with low toxicity, we have screened a number of crude extracts of medicinal plants and found that the *n*-hexane extract of the aerial parts of *C. comosa* enhanced Hb F production. The present work describes the isolation, structural elucidation, and evaluation of Hb F activity of the labdane diterpenes from *C. comosa*.

The *n*-hexane extract of the aerial parts of *C. comosa* exhibited Hb F inducing activity. Column chromatography of the extract resulted in the isolation of three new labdane diterpenes, curcucomosins A−C (**1**−**3**), four known labdane diterpenes, **4**−**7**, and a known diarylheptanoid, **8**. The known compounds were identified as coronarin E (**4**),[14] labda-8(17),11,13-trien-15(16)-olide[15] or villosin[16] (**5**), isocoronarin D (**6**),[15] zerumin (**7**),[17] and (3*S*)-1,7-diphenyl-(6*E*)-6-hepten-3-ol (**8**).[12] It should be noted that although the ^{1}H NMR data of compound **6** and its C-14 epimer, calcaratarin D (**9**),[18] were indistinguishable, the ^{13}C NMR data of these two compounds (data not shown) were different.

Compound **1** was obtained as a white, amorphous solid. The HRTOFMS (ES$^+$) at *m*/*z* 337.1779 [M + Na]$^+$ was compatible with the molecular formula $C_{20}H_{26}O_3$. The IR spectrum indicated the presence of an α,β-unsaturated γ-lactone group (1754 cm^{-1}), a keto group (1701 cm^{-1}), and an exomethylene group (908 cm^{-1}). The ^{1}H NMR spectrum exhibited signals for three methyl groups at δ 1.02, 1.05, and 1.07 and an exomethylene at δ 4.57 (1H, d, J = 1.3 Hz) and 4.81 (1H, d, J = 1.3 Hz), which were characteristic of a labdane-type diterpene. The olefinic protons at δ 6.94 (1H, dd, J = 15.7, 10.1 Hz), 6.10 (1H, d, J = 15.7 Hz), and 7.15 (1H, br s) were attributed to H-11, H-12, and H-14, respectively. Two ^{13}C NMR resonances at δ 172.1 and 216.2 were assigned to the carbonyl carbons of an α,β-unsaturated lactone and a ketone, respectively. The ^{13}C NMR data, which exhibited signals for three methyl groups at δ 25.5, 22.0, and 14.3, a quaternary carbon at δ 147.7, and a methylene carbon at δ 109.6, also provided evidence for **1** being a labdane diterpene. The ^{1}H NMR features of **1** resembled those of the known labdane diterpene **5**. Significant differences include the relative downfield shifts of H-1α, H-1β, H-2α, H-2β, and H-5 (Table 1), suggesting the keto group was located at C-3. The position of the keto group was confirmed by HMBC correlations (see Table 2). In the HMBC spectra, the methylene resonances H-1α, H-1β, H-2α, and H-2β and the C-18 and C-19 methyl resonances showed correlations with the C-3 carbonyl carbon atom at δ 216.2. The α-orientation of H-5 was evident from the large coupling constant (J = 13.5 Hz) with H-6β.

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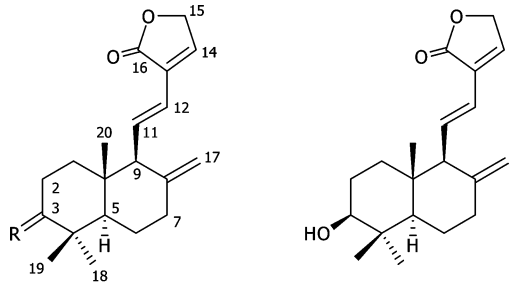
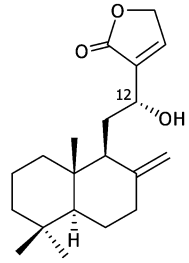


1, R = O
5, R = H_2

2



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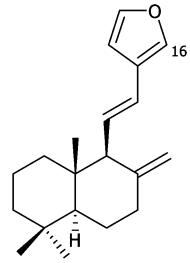

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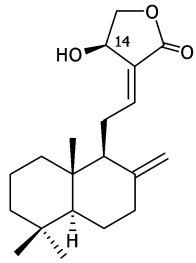

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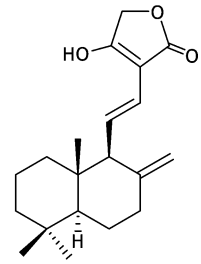

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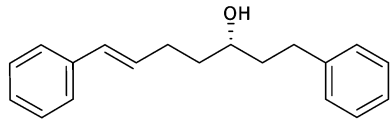

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The diaxial nature of H-6β and H-7α was evident from the large coupling constant between these two protons (see Table 1). The β-orientation of the substituent at C-9 was confirmed by NOE difference experiments, and the key NOE correlations are shown in Figure 1. The NOE correlation of H-5 and H-9 thus confirmed the α-orientation of H-5. The large coupling constant (J = 15.7 Hz) of H-11 and H-12 indicated the *trans* relationship of these two protons. Furthermore, the assignments of H-17a and H-17b signals were confirmed by NOE correlations between H-17a and H-11, and H-17b and H-7β (Figure 1). On the basis of these data, the structure of compound **1** was established as 3-oxolabda-8(17),11,13-trien-15(16)-olide and was named curcucomosin A.

Compound **2** was obtained as a white, amorphous solid. The molecular formula $C_{20}H_{28}O_3$ was deduced from the HRTOFMS (ES^+) spectrum, which showed an $[M + Na]^+$ peak at *m/z* 339.1936. The IR spectrum indicated the presence of a hydroxy group (3478 cm^{-1}), an α,β-unsaturated γ-lactone group (1731 cm^{-1}), and an exomethylene group (911 cm^{-1}). The ^1H NMR features were similar to those of **5**. The significant difference was the presence of a carbinol proton at δ 3.23 (dd, J = 11.4, 4.2 Hz) assignable to H-3. The presence of the hydroxymethine carbon at δ 79.0 in the ^{13}C NMR spectrum was in agreement with the ^1H NMR data. This was further confirmed by the HMBC correlations of H-3 with C-4 (δ 39.2), C-18 (δ 28.3), and C-19 (δ 15.5) and those of H-1, H-2, and H-5 with C-3. The axial nature of H-3 was evident from the large coupling (J = 11.4 Hz) with H-2β. Differentiation between the C-18 and C-19 methyl signals was achieved by NOE experiments (data not shown). Irradiation at the H-3 frequency gave rise to enhancement (7%) of the C-18 methyl signal at δ 1.00, whereas irradiation at the C-18 methyl frequency resulted in enhancement (29%) of the H-3 signal. The spatial arrangement of the C-9 substituent of **2** was established by NOE experiments by analogy with that of compound **1**.

The absolute configuration at C-3 of this labdane diterpene was determined using the modified Mosher's method.[19,20] Thus **2** was subjected to esterification with (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) chloride in dry pyridine[12,21] to yield the corresponding (*S*)-MTPA esters **2x**. Compound **2** was also esterified with (*S*)-(+)-MTPA chloride to yield the corresponding (*R*)-MTPA esters **2y**. It is worthy to note that care must be taken in the assignment of the methyl resonances of an MTPA ester, since the sequence of the chemical shift values of the methyl resonances may change due to the magnetic anisotropy effect of the aromatic

Table 1. ^1H NMR Data of Compounds **1**−**3** and **5** ($CDCl_3$, 400 MHz, J in Hz)[a]

position	1	2	3	5
1α	1.46 (ddd, 13.8, 13.6, 4.5)	1.11 (ddd, 13.4, 13.2, 3.7)	1.06 (td, 12.7, 4.0)	0.97 (ddd, 13.5, 13.5, ca. 2.0)
1β	1.76 (ddd, 13.8, 5.8, 2.9)	1.51[b]	1.69[b]	1.50 (br d, 13.5)
2α	2.24 (ddd, 15.3, 4.4, 3.0)	1.54[b]	1.48[c]	1.34[b]
2β	2.61 (ddd, 15.3, 13.6, 5.8)	1.62[b]	1.53[c]	1.52 (m)
3		3.23 (dd, 11.4, 4.2)	3α: 1.20[c] 3β: 1.38[b]	3α: 1.18 (br t, 12.9) 3β: 1.36[b]
5	1.50 (dd, 13.5, 1.7)	1.06 (dd, 12.5, 2.6)	1.26 (dd, 12.7, 2.7)	1.07 (dd, 12.5, 2.3)
6α	1.64[b]	1.71 (ddt, 13.1, 5.0, 2.6)	1.74[b]	1.68 (ddt, 13.1, 4.8, *ca* 2.3)
6β	1.57 (dddd, 13.6, 13.5, 13.3, 4.5)	1.44 (dddd, 13.1, 13.0, 12.5, 2.4)	1.33 (dddd, 12.9, 12.8, 12.7, 4.2)	1.36[d]
7α	2.06 (ddd, 13.6, 13.3, 4.5)	2.04 (ddd, 13.3, 13.0, 5.0)	2.01[b]	2.05 (ddd, 13.2, 13.1, 4.8)
7β	2.46 (ddd, 13.6, 4.5, 2.5)	2.43 (ddd, 13.3, 2.6, 2.4)	2.40 (ddd, 12.7, 4.2, 2.3)	2.41 (ddd, 13.2, 2.3, 1.8)
9	2.36 (d, 10.1)	2.30 (br d, 10.1)	2.03 (br d, 11.8)	2.34 (br d, 10.0)
11	6.94 (dd, 15.7, 10.1)	6.88 (dd, 15.8, 10.1)	11a: 1.69[c] 11b: 1.86 (ddd, 13.9, 11.3, 2.0)	6.87 (dd, 15.7, 10.1)
12	6.10 (d, 15.7)	6.09 (d, 15.8)	4.53 (br d, 8.5)	6.08 (d, 15.7)
14	7.15 (br s)	7.14 (br s)	7.28 (br s)	7.13 (br s)
15	4.79 (br s)	4.79 (br s)	4.80 (br s)	4.78 (br s)
17a	4.57 (d, 1.3)	4.50 (d, 1.5)	4.68 (br s)	4.48 (br s)
17b	4.81 (d, 1.3)	4.76 (d, 1.5)	4.87 (br s)	4.73 (br s)
18	1.07 (s)	1.00 (s)	0.86 (s)	0.86 (s)
19	1.02 (s)	0.79 (s)	0.78 (s)	0.81 (s)
20	1.05 (s)	0.85 (s)	0.66 (s)	0.85 (s)

[a] Assignments were based on $^1H-^1H$ COSY, HMQC, and NOE experiments; chemical shifts are given in ppm. [b] Obscured signal. [c] Partially overlapping signal. [d] Overlapping signal.

Table 2. ^{13}C NMR Data of Compounds **1**−**3** and **5** ($CDCl_3$, 100 MHz)[a]

	1		**2**		**3**		**5**	
position	δ_C	HMBC	δ_C	HMBC	δ_C	HMBC	δ_C	HMBC
1	38.7	C-2, 3, 5, 9, 10, 20	38.5	C-2, 3, 5, 9, 10, 20	39.0	C-2, 3, 9, 10, 20	40.8	C-2, 3, 9, 10, 20
2	34.8	C-1, 3, 4, 10	27.7	C-1, 3, 4, 10	19.2	C-1, 3, 4	19.0	C-1, 3, 4
3	216.2		79.0	C-4, 18, 19	42.0	C-1, 2, 4, 5, 18, 19	42.2	C-1, 2, 4, 5, 18
4	47.9		39.2		33.5		33.5	
5	54.7	C-4, 6, 9, 10, 19, 20	53.8	C-3, 4, 9, 10, 19, 20	55.4	C-4, 6, 7, 9, 10, 18, 19, 20	54.7	C-4, 6, 7, 9, 10, 18, 19, 20
6	23.8	C-5, 7, 8, 10	22.9	C-3, 5, 10	24.3	C-5, 7, 8	23.3	C-5, 7, 8, 10
7	36.0	C-5, 6, 8, 9, 17	36.4	C-5, 6, 8, 9, 17	38.2	C-5, 6, 8, 9, 17	36.7	C-5, 6, 8, 9, 17
8	147.7		148.8		148.1		149.3	
9	61.0	C-5, 8, 10, 11, 12, 17, 20	61.8	C-5, 8, 10, 17, 20	51.9	C-1, 5, 8, 10, 11, 12, 17, 20	62.1	C-5, 8, 10, 11, 12, 17, 20
10	38.6		39.0		39.2		39.2	
11	135.4	C-8, 9, 10, 13	136.3	C-9, 13	30.3	C-8, 9, 12, 13	136.8	C-9, 10, 13
12	121.4	C-9, 11, 13, 14, 16	120.9	C-9, 11, 13, 14, 16	65.8		120.6	C-9, 11, 13, 14, 16
13	129.0		129.3		138.9		129.5	
14	143.3	C-12, 13, 15, 16	142.8	C-12, 15, 16	143.9	C-12, 13, 15, 16	142.3	C-12, 15, 16
15	69.5	C-13, 14, 16	69.5	C-13, 14, 16	70.4	C-13, 14, 16	69.5	C-13, 14, 16
16	172.1		172.5		171.8		172.2	
17	109.6	C-7, 8, 9	108.8	C-7, 9	107.1	C-7, 8, 9	108.3	C-7, 8, 9
18	25.5	C-3, 4, 5, 19	28.3	C-3, 4, 5, 19	33.5	C-3, 4, 5, 19	33.5	C-3, 4, 5, 19
19	22.0	C-3, 4, 5, 18	15.5	C-3, 4, 5	21.6	C-3, 4, 5, 18	21.9	C-3, 4, 5, 18
20	14.3	C-5, 9, 10	15.0	C-1, 5, 9, 10	14.6	C-1, 5, 9	15.0	C-5, 9, 10

[a] Assignments were based on DEPT, HMQC, and HMBC experiments; chemical shifts are given in ppm.

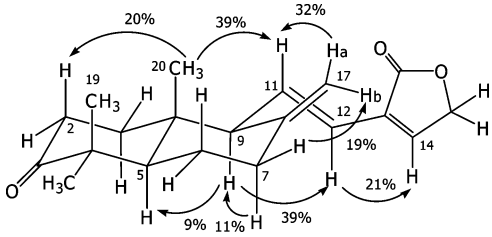
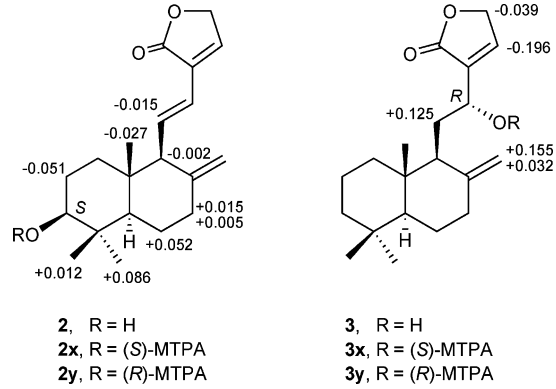


Figure 1. Key NOE correlations and relative stereochemistry for **1**. Arrows indicate identified NOE difference correlations.



2, R = H
2x, R = (*S*)-MTPA
2y, R = (*R*)-MTPA

3, R = H
3x, R = (*S*)-MTPA
3y, R = (*R*)-MTPA

Figure 2. $\Delta\delta = (\Delta\delta_S - \Delta\delta_R)$ values in ppm obtained from the MTPA esters of **2** and **3** in $CDCl_3$.

ring of the ester portion. In the case of the (*R*)-MTPA ester **2y**, a molecular model indicated that the phenyl group of the ester exerted a strong shielding effect to the C-18 methyl group, and this should lead to an upfield shift of this signal. To unambiguously assign the methyl protons of the ester **2y**, NOE experiments were undertaken and NOE correlations between H-3/18 and H-11/20 were observed. The ^1H NMR signals at δ 0.804, 0.827, and 0.887 were therefore assigned to the C-19, C-18, and C-20 methyl groups, respectively. Chemical shift differences, $\Delta\delta$, were determined as shown in Figure 2. Following the MPTA rules,[19] these data indicated an *S* configuration at C-3. The configuration of **2** confirmed the β-orientation of the hydroxy group. On the basis of the above data, compound **2** was characterized as (3*S*)-hydroxylabda-8(17),11,13-trien-15(16)-olide and was named curcucomosin B.

Compound **3** was obtained as a white, amorphous solid. The HRTOFMS (ES^+) showed a molecular ion-sodium adduct at *m*/*z* 341.2093 $[M + Na]^+$, which was used to establish the molecular formula as $C_{20}H_{30}O_3$. The significant IR data of **3** were similar to those of **2**. The ^1H NMR spectrum was characteristic of a labdane-type diterpene with the exomethylene group at δ 4.68 (1H, br s) and 4.87 (1H, br s) and three quaternary methyl groups at δ 0.66, 0.78, and 0.86 (each 3H, s). In addition, the ^1H NMR spectrum of **3** exhibited signals for a proton at δ 4.53 (1H, br d, $J = 8.5$ Hz) on a carbon (δ 65.8) bearing a secondary hydroxy group. The ^1H NMR features and the magnitude of the chemical shift values of the A- and B-ring protons were similar to those of compound **5**. However, the C-20 methyl and the H-9 signals appeared relatively upfield (0.19 and 0.31 ppm, respectively) compared with those of compound **5**. The absence of two *trans*-olefinic proton resonances at C-11−C-12 was in agreement with the upfield shifts of the C-20 methyl and H-9 signals. Placement of the hydroxy group at the 12-position was in agreement with the relatively downfield signal of the allylic carbinol proton at δ 4.53. The C-11 proton signals appeared at δ 1.69 (H-11a) and 1.86 (H-11b). In the HMBC spectra, correlations of H-9, H-11, and H-14 with C-12 were observed.

The absolute configuration at C-12 of compound **3** was determined to be *R* by the modified Mosher's method in the manner described for compound **2** (see Figure 2). The structure of this diterpene was thus elucidated as (12*R*)-hydroxylabda-8(17),13-dien-15(16)-olide and was named curcucomosin C. It should be noted that compound **3** has been reported as an intermediate in the synthesis of a labdane diterpene, but no details were provided.[22]

The orientation of the furan ring in the diterpene **4** was confirmed on the basis of NOE difference experiments (data not shown). Thus, irradiation at the H-11 frequency resulted in enhancement of the H-14 signal and vice versa. NOE differences were also observed for the H-12 and the H-16 signals. By the same analogy, the orientation of the lactone ring in **6** was confirmed by NOE difference experiments (data not shown).

The Hb F induction assay of compounds **1**−**8** was performed by using a stable reporter cell line containing the enhanced green fluorescence protein (EGFP) gene under the control of a γ-globin promoter in the intact human β-globin locus, K562::$\Delta^G\gamma$-$^A\gamma$ EGFP. The diarylheptanoid **8** did not give any Hb F expression at 5−100 μM. The EGFP induction potency of the diterpenes **1**−**7** was compared with known Hb F enhancers. The reporter cells treated with the known Hb F inducers, hemin and cisplatin, showed an increase in EGFP expression (Figure 3). Among the diterpenes

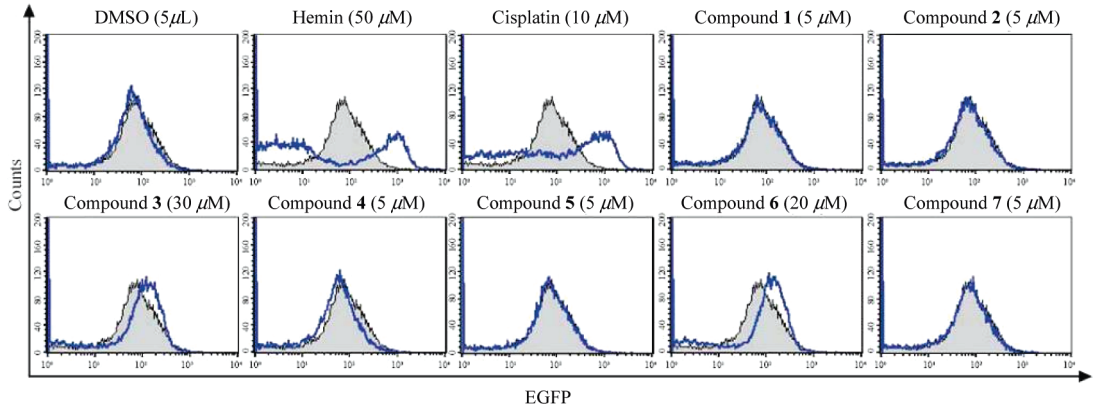


Figure 3. Induction of EGFP expression in the K562::$\Delta^{G}\gamma$-$^{A}\gamma$ EGFP reporter assay by labdane diterpenes **1**−**7**, with hemin and cisplatin as reference drugs. The EGFP expression profiles without inducers (shaded) and with inducers (unshaded).

Table 3. Effect of Labdane Diterpenes of *C. comosa* on EGFP Expression from the K562::$\Delta^{G}\gamma$-$^{A}\gamma$ EGFP Reporter Assay[a]

compound[b]	fold change	% cell viability
DMSO (5 μL)	0.9 ± 0.1	89.1 ± 2.7
hemin (50 μM)	3.6 ± 0.7	44.3 ± 4.0
cisplatin (10 μM)	3.5 ± 1.7	77.6 ± 4.7
1 (5 μM)	1.0 ± 0.1	85.8 ± 1.9
2 (5 μM)	1.0 ± 0.0	87.7 ± 3.4
3 (30 μM)	1.2 ± 0.2	75.8 ± 4.0
4 (5 μM)	0.7 ± 0.0	87.2 ± 2.9
5 (5 μM)	1.0 ± 0.0	88.9 ± 2.7
6 (20 μM)	1.6 ± 0.4	80.7 ± 1.1
7 (5 μM)	1.0 ± 0.1	87.5 ± 3.2

[a] Data represent the mean ± SD of three independent experiments. [b] Concentrations in brackets are those giving the highest Hb F inducing potency.

tested, compound **6** exhibited the highest EGFP inducing potency (Figure 3, Table 3). The EGFP expression was induced in a dose-dependent manner, and the lowest dose that gave the highest inducing effect of 1.6-fold was at 20 μM (Figure 4). Compound **3** had low but measurable EGFP induction activity, 1.2-fold, at 30 μM concentration. The other compounds had no inducing effect at 5−100 μM. The existing data did not permit a clear relationship of the labdane diterpene structure and Hb F inducing potency. However, it seemed that the presence of a hydroxy group, at either the 12- or 14-position, contributed to high biological activity.

The cytotoxicity of the diterpenes was also examined, and the results indicated that, at the concentration that gave the highest EGFP inducing potency, compounds **3** and **6** exhibited less cytotoxicity when compared with hemin (Table 3). When compared with cisplatin, the two compounds had less EGFP inducing effect with comparable toxicity. However, cisplatin has been reported to cause severe side effects including acute and chronic nephrotoxicity, optic neuropathy, and ototoxicity.[23] Some findings have indicated that excess iron from hemin induced the generation of free-radical-related oxidative stress and caused DNA, lipid, and protein damage that led to cell death.[24] The labdane diterpene **6** may serve as an alternative lead compound with Hb F inducing ability.

Experimental Section

General Experimental Procedures. Melting points were determined with an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO-1020 polarimeter. IR spectra were obtained using a Perkin-Elmer FT-IR Spectrum BX spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 FT-NMR spectrometer, operating at 400 (^{1}H) and 100 (^{13}C) MHz. For the spectra taken in $CDCl_3$, the residual nondeuterated solvent signals at δ 7.24 and the solvent signals at δ 77.0 were used as references for ^{1}H and ^{13}C NMR spectra, respectively. ESMS and ES-TOFMS spectra were measured with a Finnigan LC-Q and a Bruker micrOTOF mass spectrometer. Column chromatography was carried out using Merck silica gel 60 (<0.063 mm) and Amersham Biosciences Sephadex LH-20. For TLC, Merck precoated silica gel 60 F_{254} plates were used. Spots on TLC were detected under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating.

Plant Material. The aerial parts of *C. comosa* were collected from Kampaengsaen district, Nakhon Pathom province, Thailand, in January 2008. A voucher specimen (Apichart Suksamrarn, No. 052) is deposited at the Faculty of Science, Ramkhamhaeng University.

Extraction and Isolation. The air-dried aerial parts of *C. comosa* (2.20 kg) were milled and macerated successively with *n*-hexane

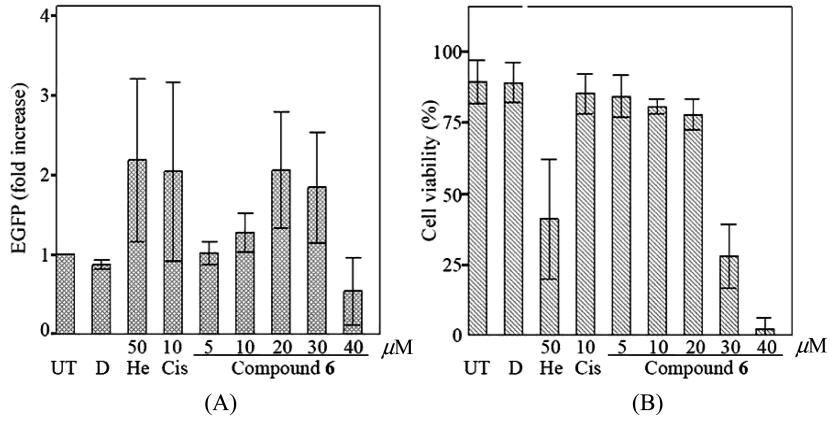


Figure 4. Effect of compound **6** on the K562::$\Delta^{G}\gamma$-$^{A}\gamma$ EGFP reporter assay. EGFP expression induction (A) and cytotoxic effect (B) of compound **6** compared with hemin (He) and cisplatin (Cis). The data represent the mean ± SD of three independent experiments. UT, untreated; D, 5 μL DMSO treated.

and EtOH to yield, after evaporation of the solvents under reduced pressure, the *n*-hexane (171.0 g) and EtOH (193.2 g) extracts, respectively.

The *n*-hexane extract (170.0 g) was fractionated by quick column chromatography (Merck silica gel 60 PF_{254}, 520 g), using a gradient solvent system of *n*-hexane, *n*-hexane−EtOAc, and EtOAc with increasing amounts of the more polar solvent. The eluates were examined by TLC, and five combined fractions (H1−H5) were obtained. Fraction H1 (12.8 g) was subjected to column chromatography twice, using *n*-hexane and *n*-hexane−EtOAc (100:1) to give coronarin E[14] (**4**, 6.3 mg) as a colorless, viscous oil, $[\alpha]_D^{26}$ +23.0 (*c* 0.18, $CHCl_3$). Fraction H4 (15.5 g) was chromatographed, eluting with *n*-hexane, *n*-hexane−EtOAc, and EtOAc in increasing proportions of the more polar solvent, followed by Sephadex LH-20, eluted with MeOH, to yield curcucomosin C (**3**, 4 mg). Fraction H5 (15.1 g) was subjected to repeated column chromatography, using *n*-hexane−EtOAc (70:30), to afford curcucomosin B (**2**, 4.8 mg). Fraction H2 (45.0 g) was chromatographed on silica gel using *n*-hexane−EtOAc (100:1) to afford four subfractions. Subfraction 3 (19.2 g) was repeatedly recrystallized from CH_2Cl_2−*n*-hexane to afford (3*S*)-1,7-diphenyl-(6*E*)-6-hepten-3-ol[12] (**8**, 3.45 g) as colorless needles, mp 47−48 °C (CH_2Cl_2−*n*-hexane) (lit.[12] 46−48 °C), $[\alpha]_D^{27}$ −2.6 (*c* 0.30, EtOH). Fraction H3 (28.0 g) was further fractionated by column chromatography, using an isocratic solvent system of *n*-hexane−EtOAc (80:20), to give five subfractions. Subfraction 2 (3.61 g) was subjected to column chromatography twice, using CH_2Cl_2 and CH_2Cl_2−MeOH (100:1), to furnish labda-8(17),11,13-trien-15(16)-olide[15] (**5**) as a white, amorphous solid (63.3 mg), $[\alpha]_D^{26}$ +10.3 (*c* 0.34, $CHCl_3$). This compound has also been reported as a new labdane diterpene named villosin.[16] For comparison with the new labdane diterpenes, the ^1H and ^{13}C NMR data of compound **5** are given in Tables 1 and 2, respectively. Subfraction 4 was chromatographed by isocratic elution with *n*-hexane−EtOAc (80:20) to give curcucomosin A (**1**, 24.7 mg). Subfraction 5 (7.56 g) was chromatographed three times, using *n*-hexane−EtOAc (85:15), *n*-hexane−EtOAc (80:20), and CH_2Cl_2, to give isocoronarin D (**6**)[15,25] (110.6 mg) as white needles, mp 176−178 °C (CH_2Cl_2−*n*-hexane) (lit.[25] mp 180−182 °C from MeOH), $[\alpha]_D^{26}$ +103.9 (*c* 0.22, $CHCl_3$), and zerumin[17] (**7**, 12.5 mg) as a colorless gum, $[\alpha]_D^{26}$ +32.3 (*c* 0.22, $CHCl_3$).

Curcucomosin A (1): white, amorphous solid; mp 101−103 °C; $[\alpha]_D^{26}$ +29.4 (*c* 0.25, $CHCl_3$); IR (KBr) ν_{max} 2973, 2850, 1754, 1701, 1639, 1458, 1442, 1388, 1346, 1194, 1103, 1054, 993, 908 cm^{-1}; ^1H and ^{13}C NMR data are given in Tables 1 and 2, respectively; ESMS (−ve) *m*/*z* 313 $[M - H]^-$; HRTOFMS (ES^+) *m*/*z* 337.1779 (calcd for $C_{20}H_{26}O_3$ + Na, 337.1780).

Curcucomosin B (2): white, amorphous solid; $[\alpha]_D^{26}$ −7.3 (*c* 0.26, $CHCl_3$); IR (KBr) ν_{max} 3478, 2986, 1731, 1638, 1439, 1393, 1290, 1185, 1129, 1050, 987, 911 cm^{-1}; ^1H and ^{13}C NMR data are given in Tables 1 and 2, respectively; ESMS (+ve) *m*/*z* 655 $[2\ M + Na]^+$, 339 $[M + Na]^+$; HRTOFMS (ES^+) *m*/*z* 339.1936 (calcd for $C_{20}H_{28}O_3$ + Na, 339.1936).

Curcucomosin C (3): white, amorphous solid; $[\alpha]_D^{26}$ +39.2 (*c* 0.30, $CHCl_3$); IR (KBr) ν_{max} 3544, 2934, 1745, 1642, 1447, 1399, 1209, 1039, 908 cm^{-1}; ^1H and ^{13}C NMR data are given in Tables 1 and 2, respectively; ESMS (+ve) *m*/*z* 659 $[2\ M + Na]^+$; ESMS (−ve) *m*/*z* 317 $[M - H]^-$; HRTOFMS (ES^+) *m*/*z* 341.2093 (calcd for $C_{20}H_{30}O_3$ + Na, 341.2093).

Preparation of the MTPA Ester of Curcucomosin B (2) and Curcucomosin C (3). The (*S*)-MTPA ester **2x** was prepared from **2** and (*R*)-(−)-MTPA chloride (Fluka, Switzerland) by the method described previously.[12] By using (*S*)-(+)-MTPA chloride and **2**, the (*R*)-MTPA ester **2y** was similarly prepared. The chemical shift differences of the proton resonances between the (*S*)-MTPA ester **2x** and the (*R*)-MTPA ester **2y**, $\Delta\delta = \delta_{S\text{-MTPA}} - \delta_{R\text{-MTPA}}$, are summarized in Figure 2. Following the above procedure, the absolute configurations of esters **3x** and **3y** were determined, and the results are summarized in Figure 2.

Cell Line and Treatment. K562::$\Delta^G\gamma$-$^A\gamma$ EGFP, a stable reporter assay, is based on the green fluorescence protein gene under the control of $^G\gamma$-globin promoter in the intact human β-globin locus.[26] The induction and measurement of EGFP was performed as previously described.[26]

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**−**3** are shown in Figures S1−S6, respectively. This information is available free of charge via the Internet at http://pubs.acs.org.

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