CHAPTER IV
RESULTS AND DISCUSSION

In this study, the dried and powdered whole plant of *Dendrobium brymerianum* (2.8 kg) was macerated with methanol. The methanol extract was concentrated under reduced pressure to give 100 g of a crude extract. This methanol crude extract showed cytotoxicity against KB cancer cells with approximately 70% growth inhibition at a concentration of 50 µg/mL. It was further separated by vacuum liquid chromatography to yield seven fractions. Fraction F showed the most potent cytotoxicity against KB cell line (68.50% inhibition at 50 µg/mL). This fraction was separated using several chromatographic techniques to give eight pure compounds [**DB1-DB8**] inclucing, 3 bibenzyls, 3 fluorenones and 2 phenanthrenes. The structures of these compounds were evaluated by spectroscopic techniques, including UV, IR, MS and NMR. They were, also, evaluated for their cytotoxicity against KB cells and anti-migration activity against H460 lung cancer cells.

1. Structure characterization of isolated compounds

1.1 Structure determination of compound DB1

Compound DB1 was obtained as a brown amorphous solid. The ESI mass spectrum (Figure 3) showed a pseudomolecular ion $[M+H]^+$ at *m/z* 305, suggesting the molecular formular $C_{17}H_{20}O_5$.

The IR spectrum (Figure 4) exhibited absorption bands at 3437 (hydroxyl), at 3012, 1611, 1455 (aromatic) and at 1219, 1114 (C-O) cm^{-1}. Its UV spectrum (Figure 5) showed characteristic absorptions for a bibenzyl skeleton at λ_{max} 220 and 281 nm (Zhang *et al.*, 2008a).

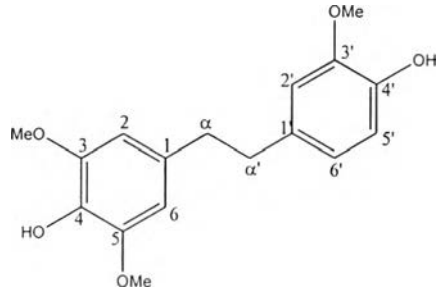
The ^{1}H NMR spectrum (Figure 6 and Table 2) showed signals for three aromatic methoxyl groups at δ_H 3.76 (6H, s) and δ_H 3.78 (3H, s) and for four benzylic methylene protons of a bibenzyl derivative at δ_H 2.78 (4H, s, H_2-α, H_2-α′). The ^{1}H NMR spectrum also revealed signals for five aromatic protons, two of which appeared as a two-proton singlet at δ_H 6.48. The relative up-field position of these protons corresponded to the two *meta*-coupled protons of a 3,4,5-trioxygenated benzyl moiety. The three remaining aromatic protons resonated at 6.78 (1H, d, *J* = 2.0 Hz), 6.71 (1H, d, *J* = 8.0 Hz) and 6.64 (1H, dd, *J* = 8.0, 2.0 Hz). The chemical shifts and the splitting patterns of these protons were typical of H-2′, H-5′ and H-6′, respectively, of a 3′,4′-dioxygenated benzyl moiety. The above mentioned spectral data suggested a 3,3′,4,4′,5-pentaoxygenated bibenzyl structure for moscatilin.

From the NOESY spectrum (Figure 7), the cross-peaks between 3-OMe (5-OMe) and H-2 (H-6), and between 3′-OMe and H-2′ indicated that the three methoxyl groups were linked to C-3, C-5 and C-3′, respectively.

The ^{13}C NMR spectrum (Figure 8 and Table 2) exhibited only fourteen carbon signals, including two signals for three methoxyl groups at δ_C 56.2 and 56.6, one signal for two quarternary carbons (C-3 and C-5) at δ_C 148.5, one signal for two methine carbons (C-2 and C-6) at δ_C 106.9, five signals for quarternary carbons (C-1, C-1′, C-3′, C-4 and C-4′) and three signals for methine carbons (C-2′, C-5′ and C-6′). These NMR data suggested that the two methoxyl groups were symmetrically substituted on one aromatic ring. Moreover, the ^{13}C NMR data showed signals for two methylene carbons at δ_C 38.5 and 39.0, which, together with the methylene protons signal at δ_H 2.78, displayed characteristic signals for a bibenzyl.

From the above data and through comparison of its ^{1}H, ^{13}C NMR, MS, IR and UV data with previously reported data (Majumder and Sen, 1987), DB1 was identified as moscatilin [**59**].

Moscatilin was a bibenzyl derivative firstly isolated from *D. moscatum* and later found in *D. amoenum, D. aurantiacum var. denneanum, D. chrysanthum, D. densiflorum, D. gratiotissimum, D. loddigesii, D. longicornu and D. secundum* (Majumder and Sen, 1987; Majumder *et al.*, 1999; Fan *et al.*, 2001; Yang *et al.*, 2006a; Yang *et al.*, 2006b; Hu *et al.*, 2008a; Zhang *et al.*, 2008a; Ito *et al.*, 2010).



Moscatilin [**59**]

Table 2 NMR spectral data of compound DB1 (in acetone-d_6) and moscatilin (in $CDCl_3$)

Position	Compound DB1		Moscatilin[a]	
	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
1	-	133.2	-	132.84
2	6.48 (s)	106.9	6.36 (s)	105.19
3	-	148.5	-	146.77
4	-	135.0	-	133.53
5	-	148.5	-	146.77
6	6.48 (s)	106.9	6.36 (s)	105.19
α	2.78 (s)	39.0	2.89 (s)	38.28
α′	2.78 (s)	38.5	2.89 (s)	37.75
1′	-	134.2	-	132.76
2′	6.78 (d, 2.0)	113.0	6.65 (d, 2.0)	111.18
3′	-	148.0	-	146.14
4′	-	145.6	-	143.69
5′	6.71 (d, 8.0)	115.5	6.94 (d, 8.0)	114.07
6′	6.64 (dd, 8.0, 2.0)	121.7	6.75 (dd, 8.0, 2.0)	120.98
3-OMe	3.76 (s)	56.6	3.81 (s)	56.15
5-OMe	3.76 (s)	56.6	3.81 (s)	56.15
3′-OMe	3.78 (s)	56.2	3.81 (s)	55.76

[a] Majumder and Sen, 1987.

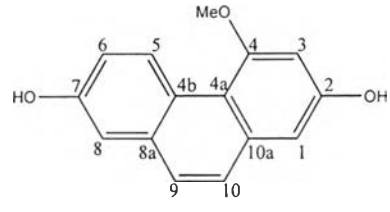
1.2 Structure determination of compound DB2

Compound DB2 was obtained as a brown amorphous solid. The ESI mass spectrum (Figure 9) showed a molecular ion $[M+H]^+$ at *m/z* 241, suggesting the molecular formula $C_{15}H_{12}O_3$. The IR spectrum (Figure 10) showed absorption peaks at 3307, 3010, 1613, 1433 cm^{-1}, indicating the presence of the hydroxyl group and aromatic ring, respectively. The UV spectrum (Figure 11) of this compound exhibited maximal absorption at 256 nm, typical of phenanthrene derivative (Zhang *et al.*, 2008b).

The ^1H NMR spectrum (Figure 12 and Table 3) showed signals for one aromatic methoxyl group at δ_H 4.09 (3H, s) and seven aromatic protons at δ_H 6.99 (1H, d, *J* = 2.4 Hz, H-1), δ_H 6.86 (1H, d, *J* = 2.4 Hz, H-3), δ_H 7.46 (1H, d, *J* = 9.3 Hz, H-5), δ_H 7.26 (1H, dd, *J* = 1.5, 9.3 Hz, H-6), δ_H 7.43 (1H, d, *J* = 1.5 Hz, H-8), δ_H 7.63 (1H, d, *J* = 9.0 Hz, H-9) and δ_H 7.50 (1H, d, *J* = 9.0 Hz, H-10). The pair of doublets at δ_H 7.50 and δ_H 7.63 was typical of the *ortho*-coupled H-9 and H-10 of a phenanthrene derivative. Furthermore, the splitting pattern of the signals between δ_H 7.26 to 7.46 having *J* values corresponding to *ortho*- and *meta*-coupled aromatic protons implied that while C-6 of the compound was unsubstituted, its C-7 should contain a substituent. Accordingly, the signal at δ_H 7.26 was assigned to H-6, which was splitted by both H-5 (δ_H 7.46) and H-8 (δ_H 7.43). A methoxyl group at δ_H 4.09 should be located at C-4 because the methoxyl protons exhibited NOESY interaction with H-3, but not with H-1 (Figure 13). The ^1H NMR spectrum also revealed *meta*-coupled signals at δ_H 6.86 and 6.99, indicating the presence of tetrasubstituted phenyl group.

The ^{13}C NMR spectrum (Figure 14 and Table 3) showed fifteen carbon signals, including, seven aromatic quarternary carbons, one methoxyl carbon and seven aromatic methine carbons.

Through comparison of its ^{1}H NMR, ^{13}C NMR, UV and MS data with those previously reported in the literature (Majumder and Banerjee, 1990a), DB2 was identified as flavanthrinin (4-methoxyphenanthrene-2,7-diol) [**176**], which was first isolated from *Eria flava* and later found in *Dendrobium nobile* (Zhang *et al.*, 2008b).



Flavanthrinin [**176**]

Table 3 NMR spectral data of compound DB2 (in $CDCl_3$) and flavanthrinin (in $CDCl_3$)

Position	Compound DB2		Flavanthrinin[a]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	6.99 (d, 2.4)	107.4	6.96 (d, 2.5)	107.4
2	-	154.3	-	154.3
3	6.86 (d, 2.4)	101.6	6.84 (d, 2.5)	101.7
4	-	155.4	-	155.5
4a	-	114.4	-	114.4
4b	-	118.6	-	118.8
5	7.46 (d, 9.3)	127.0	7.47 (d, 7.6)	127.1
6	7.26 (dd, 9.3, 1.5)	116.6	7.22 (dd, 7.6, 1.5)	116.6
7	-	153.9	-	154.0
8	7.43 (d, 1.5)	120.7	7.40 (d, 1.5)	120.7
8a	-	134.1	-	134.2
9	7.63 (d, 9.0)	129.5	7.62 (d, 8.8)	129.5
10	7.50 (d, 7.5)	125.8	7.43 (d, 8.8)	125.8
10a	-	136.1	-	136.1
4-OMe	4.09 (s)	58.4	4.08 (s)	58.5

[a] Zhang *et al.*, 2008b

1.3 Structure determination of compound DB3

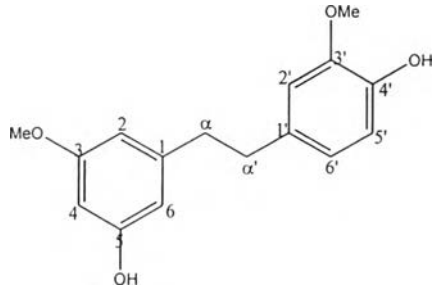
Compound DB3 was obtained as a brown amorphous solid. The ESI mass spectrum (Figure 15) showed a molecular ion $[M+H]^+$ at *m/z* 275, suggesting the molecular formula $C_{16}H_{18}O_4$. The IR spectrum (Figure 16) showed characteristic bands for hydroxyl (3400 cm^{-1}), aromatic (3050, 1613, 1598, 1461 cm^{-1}) and C-O for ether (1272, 1150 cm^{-1}) functionalities (Juneja *et al.*, 1985). Its UV spectrum (Figure 17) showed absorption maxima at 222 and 281 nm.

The ^{1}H NMR (Figure 18 and Table 4) showed a characteristic proton signal for a bibenzyl skeleton at δ_H 2.78 which correlated to two methylene carbons at δ_C 37.9 and 39.0 ppm in the ^{13}C NMR spectrum (Figure 19). In addition, the ^{1}H NMR data exhibited signals for six aromatic protons at δ_H 6.22 (1H, t, *J* = 2.0 Hz, H-2), 6.28 (1H, t, *J* = 2.0 Hz, H-4), 6.30 (1H, t, *J* = 2.0 Hz, H-6), 6.64 (1H, dd, *J* = 8.0, 1.5 Hz, H-6′), 6.70 (1H, d, *J* = 8.0 Hz, H-5′) and 6.79 (1H, d, *J* = 1.5 Hz, H-2′). The ^{1}H NMR spectrum also revealed the presence of two methoxyl groups at δ_H 3.69 (3H) and 3.78 (3H). Their positions were assigned by the NOESY spectrum (Figure 20) which showed interactions of 3-OMe with H-2 and H-4, and 3′-OMe with H-2′. Therefore, these methoxyl groups were connected to the C-3 and C-3′, respectively.

The ^{13}C NMR and DEPT 135 spectra (Figure 19, 21 and Table 4) showed sixteen carbon signals, including, six aromatic quarternary carbon signals, which supported the presence of four substituents on the bibenzyl skeleton, six methine carbon signals, two methylene carbon signals and two methyl carbon signals.

From the above data and through comparison of its ^{1}H NMR, ^{13}C NMR UV and MS spectra with the previous reported data (Juneja, Sharma, and Tandon, 1985; Chen *et al.*, 2008d), DB3 was identified as gigantol [50].

This compound was originally discovered in 1985 from the orchid *Cymbidium giganteum* (Juneja *et al.*, 1985). Later, it was also isolated from other orchids such as *D. aphyllum*, *D. aurantiacum* var. *denneanum*, *D. candidum*, *D. capillipes*, *D. cariniferum*, *D. chrysanthum*, *D. chrysotoxum*, *D. densiflorum*, *D. draconis*, *D. gratiosissimum*, *D. loddigesii*, *D. longicornu*, *D. nobile*, *D. polyanthum* and *D. trigonopus* (Chen *et al.*, 2008b; Liu *et al.*, 2009a; Li *et al.*, 2008; Phechrmeekha *et al.*, 2012; Chen *et al.*, 2008c; Yang *et al.*, 2006b; Li *et al.*, 2009c; Fan *et al.*, 2001; Sritularak *et al.*, 2011a; Zhang *et al.*, 2008a; Ito *et al.*, 2010; Hu *et al.*, 2008a; Zhang *et al.*, 2007a; Hu *et al.*, 2009; Hu *et al.*, 2008b).



Gigantol [**50**]

Table 4 NMR spectral data of compound DB3 (in acetone-d_6) and gigantol (in acetone-d_6)

Position	Compound DB3		Gigantol[a]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	-	145.4	-	144.5
2	6.22 (t, 2.0)	108.8	6.26 (t, 2.0)	107.9
3	-	159.1	-	158.2
4	6.28 (t, 2.0)	99.6	6.30 (t, 2.0)	98.7
5	-	161.7	-	160.8
6	6.30 (t, 2.0)	106.2	6.33 (t, 2.0)	105.3
α	2.78 (m)	39.0	2.78 (s)	37.9
α′	2.78 (m)	37.9	2.79 (s)	36.9
1′	-	134.0	-	133.1
2′	6.79 (d, 1.5)	115.4	6.80 (d, 2.0)	114.6
3′	-	147.9	-	147.0
4′	-	145.1	-	144.2
5′	6.70 (d, 8.0)	112.8	6.74 (d, 8.0)	111.9
6′	6.64 (dd, 8.0, 1.5)	121.5	6.66 (dd, 2.0, 8.0)	120.6
3-OMe	3.69 (s)	55.2	3.69 (s)	54.3
3′-OMe	3.78 (s)	56.0	3.78 (s)	55.2

[a]Chen *et al.*, 2008d

1.4 Structure determination of compound DB4

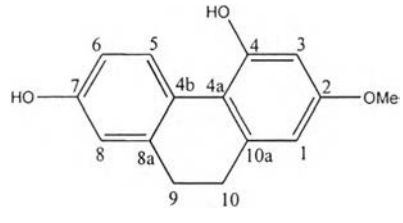
Compound DB4 was obtained as a brown amorphous solid. The HRESIMS of this compound (Figure 22) showed a sodium-adduct molecular ion $[M+Na]^+$ at *m/z* 265.0852 (calcd. for $C_{15}H_{14}O_3Na$, 265.0840), suggesting the molecular formula $C_{15}H_{14}O_3$. The IR spectrum (Figure 23) showed absorption bands at 3367 (hydroxyl), 3004, 1611 and 1454 (aromatic). The UV spectrum (Figure 24) showed absorption bands at 220 and 277 nm, suggestive of a 9,10-dihydrophenanthrene derivative (Majumder and Lahiri., 1990b).

The ^1H NMR spectrum (Figure 25 and table 5) showed signals for an aromatic methoxyl group at δ_H 3.72 (3H, s), two pairs of methylene protons at δ_H 2.67 (H_2-9 and H_2-10) and five aromatic protons at δ_H 6.36 (1H, d, *J* = 1.5 Hz, H-1), 6.42 (1H, d, *J* = 1.5 Hz, H-3), 6.68 (1H, dd, *J* = 9.0, 2.5 Hz, H-6), 6.69 (1H, d, *J* = 2.5 Hz, H-8) and 8.22 (1H, d, *J* = 9.0 Hz, H-5).

One set of ABX system aromatic protons at δ_H 8.22, 6.69, and 6.68, together with the *ortho*-coupled doublet of H-5, indicated that C-6 of this compound must be unsubstituted. The signals of H-6 and H-8, which merged to give a two-proton ill-resolved multiplet, suggested the presence of another oxygen functionality at C-7. The remaining two aromatic proton signals at δ_H 6.36 and 6.42, appeared as clear *meta*-coupled doublets, which could then be assigned to their respective H-1 and H-3 positions. The assignment of H-1 was based on its NOESY interaction with H_2-10. The methoxyl group was placed at C-2 according to its NOESY correlation peaks with H-1 and H-3 (Figure 26).

The ^{13}C NMR spectrum (Figure 27 and Table 5) exhibited fifteen carbon signals, including, one methyl carbon signal, two methylene carbon signals, five methine carbon signals and seven quarternary carbon signals.

Based on the above spectral evidence and through comparison with its previous reported data (Majumder and Lahiri, 1990b; Guo et al., 2007), it was identified as lusianthridin (4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene) [**185**]. This compound was firstly isolated from the orchid *Lusia indivisa* and was later found in *D. aphyllum*, *D. loddigesii*, *D. nobile*, *D. plicatile* and *Pholidota yunanensis* (Chen *et al.*, 2008b; Ito *et al.*, 2010; Yang *et al.*, 2007; Hwang *et al.*, 2010; Yamaki and Honda, 1996; Guo *et al.*, 2007).



Lusianthridin [**185**]

Table 5 NMR spectral data of compound DB4 (in acetone-d_6) and lusianthridin (in acetone-d_6)

Position	Compound DB4		Lusianthridin[a]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	6.36 (d, 1.5)	105.8	6.37 (d, 2.6)	106.0
2	-	159.2	-	159.3
3	6.42 (d, 1.5)	101.5	6.44 (d, 2.6)	101.6
4	-	155.8	-	155.9
4a	-	115.7	-	115.9
4b	-	125.8	-	125.9
5	8.22 (d, 9.0)	129.8	8.22 (d, 7.5)	129.9
6	6.68 (dd, 9.0, 2.5)	113.4	6.68 (dd, 7.5, 2.7)	113.5
7	-	155.9	-	156.1
8	6.69 (d, 2.5)	114.9	6.69 (m)	115.0
8a	-	139.7	-	139.8
9	2.66	30.6	2.67	30.8
10	2.66	31.4	2.67	31.5
10a	-	141.3	-	141.4
2-OMe	3.72 (s)	55.2	3.74 (s)	55.3

[a]Guo *et al.*, 2007

1.5 Structure determination of compound DB5

Compound DB5 was obtained as a red amorphous solid. Its HRESIMS (Figure 28) showed a sodium-adduct molecular ion $[M+Na]^+$ at *m/z* 265.0479 (calcd. for $C_{14}H_{10}O_4Na$, 265.0476), suggesting the molecular formula $C_{14}H_{10}O_4$. The IR spectrum (Figure 29) showed absorption bands at 3288 (hydroxyl), 1699 (carbonyl) and 3030, 1608, 1448 (aromatic) cm^{-1}. The UV spectrum (Figure 30) showed absorption maxima at 274 nm, characteristic of a fluorenone structure (Zhang *et al.*, 2007a).

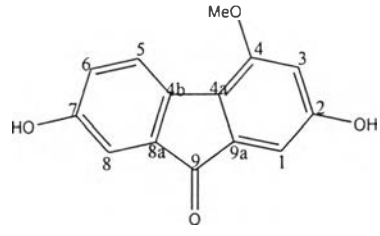
The ^1H NMR spectrum of this compound (Figure 31 and Table 6) exhibited signals for one methoxyl group at δ_H 4.13 (3H, s) and five aromatic protons, appearing as a pair of meta-coupled doublets at δ_H 6.78 (1H, d, *J* = 2.0 Hz) and 6.80 (1H, d, *J* = 2.0 Hz) and an ABX splitting system at 6.93 (1H, dd, *J* = 7.5, 1.5 Hz), 7.10 (1H, d, *J* = 1.5 Hz) and 7.12 (1H, d, *J* = 7.5 Hz).

The ^{13}C NMR and HSQC spectra (Figure 32, 33 and Table 6) displayed signals for one methoxyl carbon, five aromatic methine carbons, seven aromatic quarternary carbons (three oxygenated), and one carbonyl carbon.

In the HMBC spectrum (Figure 34), the aromatic protons at δ_H 6.80 and 7.10 were assigned as H-1 and H-8, respectively, due to their HMBC correlation peaks with the carbonyl carbon at δ_C 193.2. The aromatic protons at δ_H 6.78, 7.12 and 6.93 should be assigned to H-3, H-5 and H-6, respectively. Moreover, the methoxyl proton at δ_H 4.13 and H-3 showed HMBC correlation with C-4, indicating the position of the methoxyl group at C-4. This was confirmed by the NOESY interaction (Figure 35) of 4-OMe with H-3.

Through comparison of its ^1H,^{13}C NMR, MS, IR and UV data with reported values (Zhang *et al.*, 2007a), DB5 was identified as nobilone (2,7-dihydroxy-4-

methoxy-9-fluorenone) [**112**]. This compound was firstly isolated from *D. nobile* in 2007 (Zhang *et al.*, 2007a).



Nobilone [**112**]

Table 6 NMR spectral data of compound DB5 (in acetone-d_6) and nobilone (in acetone-d_6)

Position	Compound DB5		HMBC	Nobilone[a]	
	δ_H (mult., J in Hz)	δ_C	(correlation with ^{1}H)	δ_H (mult., J in Hz)	δ_C
1	6.80 (d, 2.0)	105.9	H-3	6.82 (d, 2.0)	106.1
2	-	160.9	H-1, H-3	-	161.2
3	6.78 (d, 2.0)	106.2	H-1	6.80 (d, 2.0)	106.3
4	-	153.5	H-3, 4-OMe	-	153.6
4a	-	122.6	H-1, H-3	-	122.5
4b	-	128.0	H-8, H-6	-	128.0
5	7.12 (d, 7.5)	130.2	H-6	7.12 (d, 7.2)	130.2
6	6.93 (dd, 7.5, 1.5)	125.0	H-5, H-8	6.94 (dd, 7.3, 1.9)	125.0
7	-	151.6	H-5	-	151.6
8	7.10 (d, 1.5)	116.7	H-6	7.11 (d, 1.9)	116.8
8a	-	135.8	H-5	-	135.9
9	-	193.2	H-1, H-8	-	193.4
9a	-	137.2	-	-	137.2
4-OMe	4.13 (s)	57.5	-	4.13 (s)	57.6

[a]Zhang *et al.*, 2007a

1.6 Structure determination of compound DB6

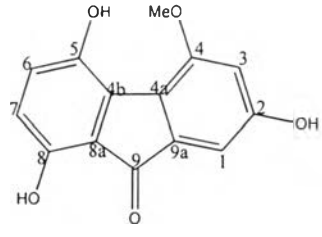
Compound DB6 was obtained as a red amorphous solid. The HRESIMS of this compound (Figure 36) showed a sodium-adduct molecular ion $[M+Na]^+$ at *m/z* 281.0445 (calcd. for $C_{14}H_{10}O_5Na$, 281.0425), indicating the molecular formula $C_{14}H_{10}O_5$. The IR spectrum (Figure 37) displayed hydroxyl group (3276 cm^{-1}), carbonyl group (1682 cm^{-1}) and aromatic rings (3166, 1608, 1494 cm^{-1}). The UV spectrum (Figure 38) showed absorption band at 258 nm, suggesting a fluorenone (Fan *et al.*, 2001).

The ^1H NMR (Figure 39 and Table 7) exhibited signals for one methoxyl group at δ_H 4.10 (3H, s) and aromatic protons at δ_H 6.59 (1H, d, *J* = 9.0 Hz, H-7), 6.76 (1H, d, *J* = 1.5 Hz, H-3), 6.79 (1H, d, *J* = 1.6 Hz, H-1) and 6.87 (1H, d, *J* = 9.0 Hz, H-6). These indicated the presence of two *ortho*-coupled protons and two *meta*-coupled protons.

A NOESY spectrum (Figure 40) showed correlation between the methoxyl protons at δ_H 4.10 and the proton at δ_H 6.76 (H-3), confirming that a methoxyl group was linked to C-4.

In the ^{13}C NMR spectrum (Figure 41 and Table 7), fourteen carbon signals were observed as one methyl, four methines and nine quarternary carbons, two of which should be a carbonyl carbons (δ_C 195.3; C-9) and methoxyl carbon (δ_C 57.4; 4-OMe).

Based on the above spectral evidence and comparison of previous reported data (Chen *et al.*, 2008c), DB6 was identified as dendroflorin [**110**]. This compound was originally isolated from *D. densiflorum* (Talapatra *et al.*, 1984). It was also found in *D. aurantiacum* var. *denneanum*, D. *chrysotoxum* and *D. nobile* (Yang *et al.*, 2006a; Chen *et al.*, 2008c; Zhang *et al.*, 2007b)



Dendroflorin [**110**]

Table 7 NMR spectral data of compound DB6 (in acetone-d_6) and dendroflorin (in acetone-d_6)

Position	Compound DB6		Dendroflorin[a]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	6.79 (d, 1.6)	105.6	6.78 (s)	104.3
2	-	160.9	-	160.0
3	6.76 (d, 1.6)	106.1	6.74 (s)	104.8
4	-	154.1	-	152.8
4a	-	122.4	-	121.0
4b	-	124.3	-	123.1
5	-	145.1	-	143.8
6	6.87 (d, 9.0)	128.9	6.86 (d, 8.8)	128.3
7	6.59 (d, 9.0)	119.7	6.60 (d, 8.8)	118.4
8	-	152.8	-	151.6
8a	-	117.4	-	116.2
9	-	195.3	-	194.0
9a	-	137.4	-	136.1
4-OMe	4.10 (s)	57.4	4.10 (s)	56.1

[a]Chen et al., 2008c

1.7 Structure determination of compound DB7

Compound DB7 was obtained as a red amorphous solid. Its HRESIMS (Figure 42) showed a sodium-adduct ion [M+Na^+] at *m/z* 267.0634 (calcd. For $C_{14}H_{12}O_4Na$, 267.0633), suggesting the molecular formula $C_{14}H_{12}O_4$. The IR spectrum (Figure 43) demonstrated peaks at 3330 (hydroxyl), 3005, 1601 and 1448 (aromatic ring) cm^{-1}. The UV spectrum (Figure 44) showed absorption maxima at 276 and 220 nm, similar to those of fluorenone derivatives (Yang *et al.*, 2004).

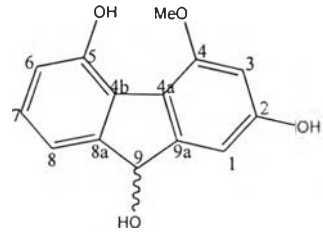
The ^1H NMR spectrum (Figure 45 and Table 8) showed five aromatic protons, which were at δ_H 6.59 (1H, brs, H-3), 6.73 (1H, dd, *J* = 8.1, 1.5 Hz, H-6), 6.83 (1H, brs, H-1), 7.03 (1H, m, H-8) and 7.07 (1H, m, H-7), and two resonances at δ_H 4.06 (3H, s, 4-OMe) and 5.37 (1H, brs, H-9). From the spectrum, two signals at δ_H 6.59 and 6.83 indicated the presence of one pair of *meta*-coupled aromatic protons. The additional signals at δ_H 6.73, 7.03 and 7.07 revealed another aromatic ring with 1,2,3-substitution pattern.

The ^{13}C NMR spectrum (Figure 46 and Table 8) showed 14 carbon signals, including one methyl (as methoxyl at δ_C 57.0), six methine (one oxygenated at δ_C 74.5) and seven quarternary carbons (three oxygenated).

The assignment was further confirmed by HSQC and HMBC experiments (Figure 47 and 48). In the HSQC spectrum, the proton at δ_H 5.37 (H-9) exhibited correlation with the tertiary carbon at δ_C 75.4, which was C-9. In the HMBC spectrum, the protons at δ_H 6.83 and 7.03 showed correlations with C-9, thus corresponding to H-1 and H-8, respectively. Moreover, the proton signal at δ_H 6.59 was assigned to H-3 (meta to H-1), and those at δ_H 6.73 and 7.07 were assigned to H-6 and H-7, respectively, according to their cross peaks with C-1, C-4a, C-4 and C-2

(for H-3), with C-8 and C-4b (for H-6) and with C-8a and C-5 (for H-7). These observations were further confirmed by the HSQC correlations. In addition, the HMBC spectrum also revealed that the signal of methoxyl protons at δ_H 4.06 correlated with the C-4 signal at δ_C 153.0. Therefore, the methoxyl group was placed at C-4.

Based on these observations and through comparison of its ^{1}H NMR, ^{13}C NMR, UV and MS data with those previously reported in the literature (Yang *et al.*, 2004), DB7 was identified as denchrysan B (2,5,9-trihydroxy-4-methoxy-9H-fluorenone) [**109**]. This compound was firstly isolated from *Dendrobium chrysanthum* and also found in *D. chrysotoxum* (Li *et al.*, 2009).



Denchrysan B [**109**]

Table 8 NMR spectral data of compound DB7 (in acetone-d_6) and denchrysan B (in acetone-d_6)

Position	Compound DB7		HMBC	Denchrysan B[a]	
	δ_H (mult., *J* in Hz)	δ_C	(correlation with ^{1}H)	δ_H (mult., *J* in Hz)	δ_C
1	6.83 (brs)	107.2	H-3	6.85 (dd, 1.8, 0.8)	106.0
2	-	159.6	H-3, H-1	-	159.1
3	6.59 (brs)	100.3	H-1	6.58 (d, 1.8)	99.6
4	-	153.0	H-3	-	152.3
4a	-	118.9	H-1, H-3	-	118.0
4b	-	124.7	H-8, H-6	-	124.0
5	-	151.4	5-OH	-	150.5
6	6.73 (dd, 8.1, 1.5)	116.9	5-OH, H-8	6.74 (dd, 7.7, 1.9)	116.2
7	7.07 (m)	128.4	-	7.08 (m)	127.8
8	7.03 (m)	117.0	H-6	7.06 (m)	116.3
8a	-	148.5	H-8, H-9	-	147.8
9	5.37 (brs)	75.4	H-8, H-1	5.40 (s)	74.5
9a	-	150.8	H-9	-	149.9
4-OMe	4.06 (s)	57.0	-	4.02 (s)	56.4
5-OH	9.07 (s)	-	-	-	-

[a]Ye, Zhao and Qin., 2002b

1.8 Structure determination of compound DB8

Compound DB8 was obtained as a brown amorphous solid. The HRESIMS of this compound (Figure 49) showed a sodium-adduct molecular ion $[M+Na]^+$ at *m/z* 283.0943 (calcd. For $C_{15}H_{16}O_4Na$, 283.0946), suggesting the molecular formula $C_{15}H_{16}O_4$. The IR spectrum (Figure 50) showed absorption bands at 3419, 3003, 1607 and 1463 cm^{-1}, indicating the presence of hydroxyl group and aromatic ring. The UV spectrum (Figure 51) showed absorption bands at 220 and 277 nm, characteristic of a bibenzyl derivative (Majumder and Pal., 1993).

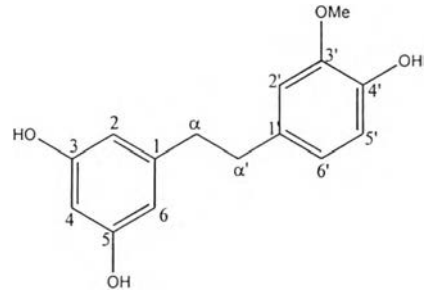
The ^1H NMR spectrum (Figure 52 and Table 9) of this compound showed signals for one aromatic methoxyl at δ_H 3.78 (1H, s, 3′-OMe), six aromatic protons at δ_H 6.17 (1H, d, *J* = 1.8 Hz, H-4), 6.20 (2H, d, *J* = 1.8 Hz, H-2, H-6), 6.65 (1H, dd, *J* = 1.5, 7.8 Hz, H-6′), 6.69 (1H, d, *J* = 7.8 Hz, H-5′), and 6.79 (1H, d, *J* = 1.5 Hz, H-2′) and four benzylic methylene protons at δ_H 2.74 (4H, m, H_2-α, H_2-α′). The last signal at δ_H 2.74 was typical of the four benzylic protons of a bibenzyl derivative. The splitting patterns of three aromatic protons resonating at δ_H 6.17 and 6.20, indicating the presence of *meta*-substitution on the aromatic ring. The remaining three aromatic protons corresponding to the signals at δ_H 6.65, 6.69 and 6.79, exhibited an ABX splitting pattern which indicated a 3′-4′-dioxygenated benzyl moiety, similar to those of gigantol [**50**] and moscatilin [**59**].

The ^{13}C NMR spectrum (Figure 53 and Table 9) displayed thirteen carbon signals representing fifteen carbons, including one signal for methyl carbon of the 3′-OMe, two signals for methylene carbons, five signals for methine carbons and five signals for quarternary carbons.

The HMBC spectrum (Figure 54) showed a cross-peak between 3′-OMe and C-3′, which was in agreement with the NOESY correlation of 3′-OMe with H-2′ (Figure 55). Based on these observations, the position of 3′-OMe could be confirmed to link with

C-3′.

Through comparison of these spectroscopic data with reported values, compound DB8 was identified as tristin (3,4′,5-trihydroxy-3′-methoxy bibenzyl) [70]. This compound was originally isolated from *Dendrobium cumulatum* and *Bulbophyllum triste*. Later, it was also found in *B. odoratissimum* (Majumder and Pal., 1993; Chen *et al.*, 2008d)



Tristin [70]

Table 9 NMR spectral data of compound DB8 (in acetone-d_6) and tristin (in acetone-d_6)

Position	Compound DB8		HMBC	Tristin[a]	
	δ_H (mult., *J* in Hz)	δ_C	(correlation with ^{1}H)	δ_H (mult., *J* in Hz)	δ_C
1	-	145.5	H_2-α, H_2-α′	-	145.7
2	6.20 (d, 1.8)	107.8	H-4, H-α	6.28 (d, 2.1)	108.5
3	-	159.2	H-2, H-4	-	159.6
4	6.17 (d, 1.8)	101.0	H-2, H-6	6.26 (t, 2.1)	101.7
5	-	159.2	H-6, H-4	-	159.6
6	6.20 (d, 1.8)	107.8	H-4, H-α	6.28 (d, 2.1)	108.5
α	2.74 (m)	38.9	H-2, H-6	2.81 (m)	39.3
α′	2.74 (m)	37.9	H-2′, H-6′	2.88 (m)	38.3
1′	-	134.1	H-5′, H_2-α, H_2-α′	-	134.8
2′	6.79 (d, 1.5)	112.8	H-6′	6.80 (d, 1.9)	113.4
3′	-	148.0	H-5′, 3′-OMe	-	148.5
4′	-	145.1	H-2′, H-6′	-	145.8
5′	6.69 (d, 7.8)	115.5	-	6.76 (d, 8.0)	116.1
6′	6.65 (dd, 7.8, 1.5)	121.5	H-2′	6.67 (dd, 8.0,	122.1
3′-OMe	3.78 (s)	56.1	-	1.9)	56.7
				3.79 (s)	

[a]Chen *et al.*, 2008d

2. Cytotoxic activity

All of the isolated compounds were evaluated for their cytotoxic activity against human cancer cell lines. In this study, the cytotoxic assays against KB (oral human epidermal carcinoma cell) and H460 (non-small lung cancer cells) were conducted by the bioassay laboratory of National Center of Genetic Engineering and Biotechnology (BIOTEC) and Department of Pharmacology and Physiology, Faculty of Pharmaceutical Science, Chulalongkorn University, respectively. The results are summarized in Table 10 and Figures 56-60.

Table 10 IC_{50} values (µg/mL and µM) for cytotoxicity of isolated compounds and positive controls.

Compound	IC_{50} values against KB cells		IC_{50} values against H460 cell	
	µg/mL	µM	µg/mL	µM
Moscatilin [DB1]	0.795	2.62	196.7	674.04
Flavanthrinin [DB2]	19.12	79.67	Inactive[b]	Inactive[b]
Gigantol [DB3]	inactive[a]	inactive[a]	23.4	85.40
Lusianthridin [DB4]	10.68	44.13	65.0	268.60
Nobilone [DB5]	inactive[a]	inactive[a]	Inactive[b]	Inactive[b]
Dendroflorin [DB6]	inactive[a]	inactive[a]	125.8	487.60
Denchrysan B [DB7]	41.00	158.91	Inactive[b]	Inactive[b]
Tristin [DB8]	42.48	163.83	Inactive[b]	Inactive[b]
Ellipticine	1.23	5.00	-	-
Doxorubicin	0.832	1.44	-	-

[a]Less than 50% inhibition at concentration of 50 µg/mL

[b]More than 50% cell viability at concentration of 200 µg/mL

For cytotoxicity against KB oral cavity cancer cell line evaluation, moscatilin [**DB1**] exhibited the strongest cytotoxic effect with an IC_{50} value of 2.62 μM, whereas flavanthrinin [**DB2**] and lusianthridin [**DB4**] showed moderate activity (IC_{50} 79.67 and 44.13 μM, respectively), followed by denchrysan B [**DB7**] and Tristin [**DB8**] (IC_{50} 158.91 and 163.83 μM, respectively). Ellipticine (IC_{50} 5.00 μM) and Doxorubicin (IC_{50} 1.44 μM) were used as a positive control.

Following the cytotoxicity against H460 cells, the active compounds, including moscatilin [**DB1**], gigantol [**DB3**], lusianthridin [**DB4**], and dendroflorin [**DB6**] were subjected to the wound-healing assay to investigate their anti-migration activity. The compounds were evaluated only at their non-cytotoxic concentrations (0.1 μg/mL). H460 cells were allowed to migrate in the presence or absence of the tested compounds for 0, 6, 12, 24, and 48 h, and the migratory activity was measured. Results in figures 56-59 indicated that all tested compounds exhibited significant anti-migration activity in comparison to that of their untreated controls. Figure 60 shows that these tested compounds inhibited the migration of the cells across the wound space in a time-dependent manner. At 12 h and 24 h, dendroflorin [**DB6**] exhibited the strongest anti-migration activity. At 48 h, moscatilin [**DB1**] showed the strongest anti-migration effect because its ability to inhibit cell migration rapidly increased. In this study, gigantol [**DB3**], which showed the strongest cytotoxicity, has less activity in terms of migration in comparison to that of dendroflorin [**DB6**]. These results indicated that the effect of compound in inhibition of cancer migration may not correlate with its cytotoxic effect.

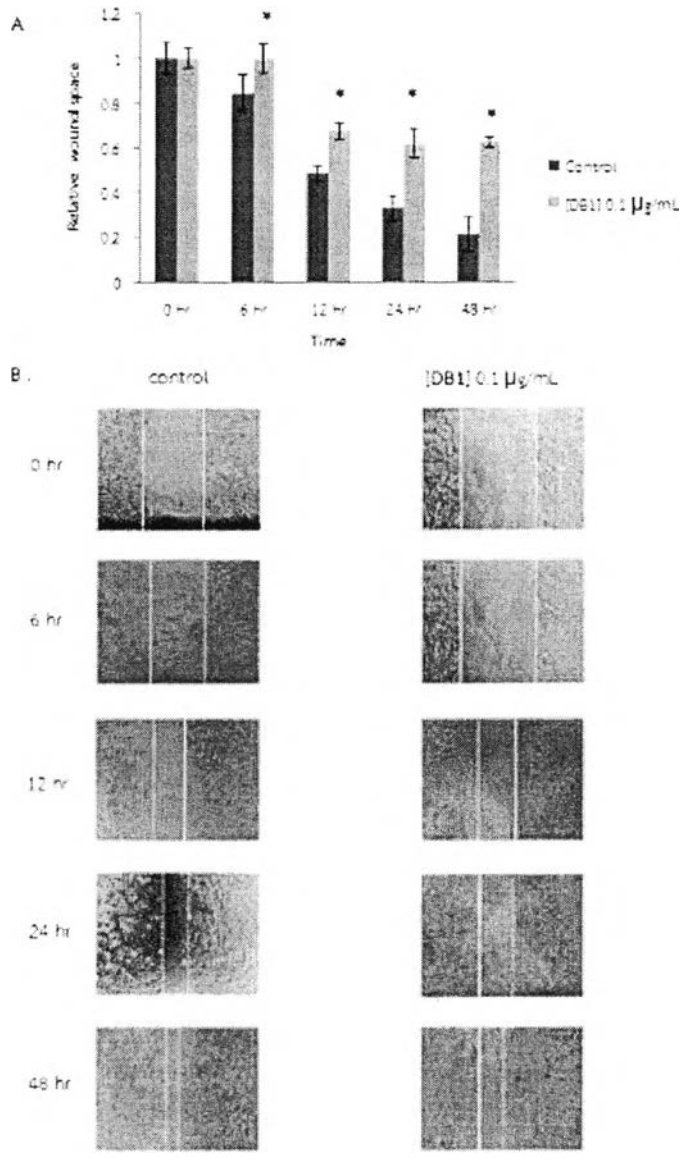


Figure 56 Effect of moscatilin [DB1] on H460 cell migration. (A) Confluent monolayer of H460 cells was wounded using a 1 mm width tip and with moscatilin [DB1] at 0.1 µg/ml or without for various times (0-48 h). Wound space was analyzed and represented as migration level relatively to the change of those in untreated cells. Data represent the mean ± SD (n =3). **P* < 0.05 versus untreated control cells. (B) Wound space was visualized under a phase-contrast microscope at the indicated times.

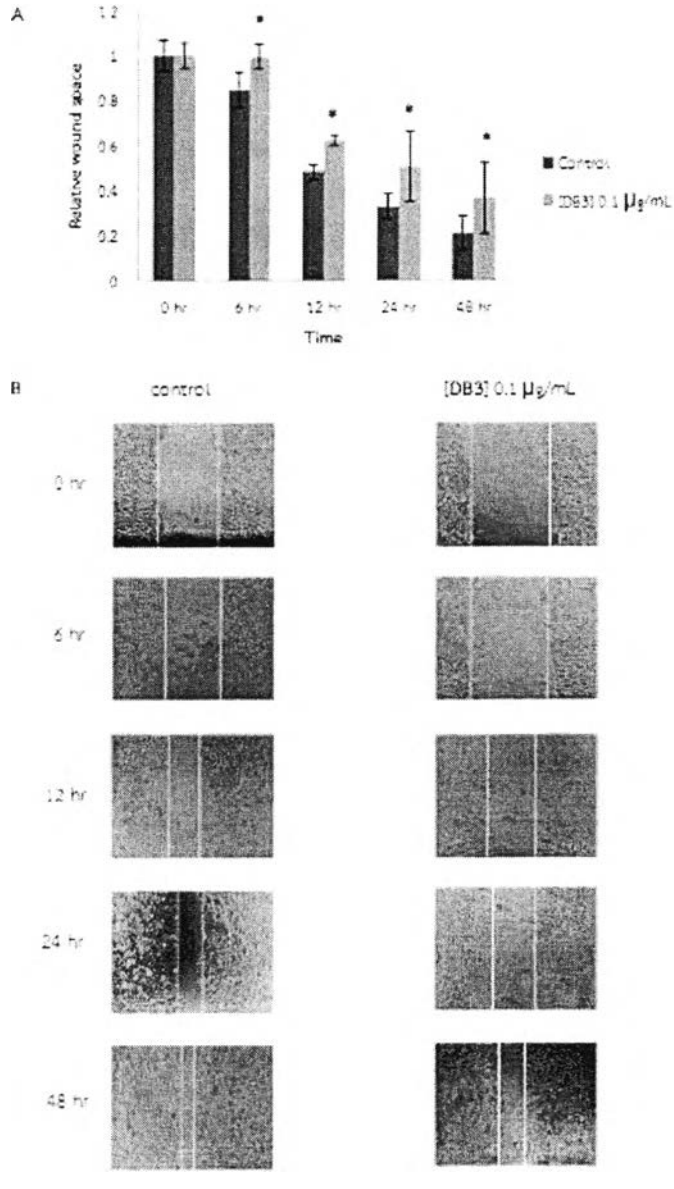


Figure 57 Effect of gigantol [**DB3**] on H460 cell migration. (A) Confluent monolayer of H460 cells was wounded using a 1 mm width tip and with gigantol [**DB3**] at 0.1 µg/ml or without for various times (0-48 h). Wound space was analyzed and represented as migration level relatively to the change of those in untreated cells. Data represent the mean ± SD (n =3). **P* < 0.05 versus untreated control cells. (B) Wound space was visualized under a phase-contrast microscope at the indicated times.

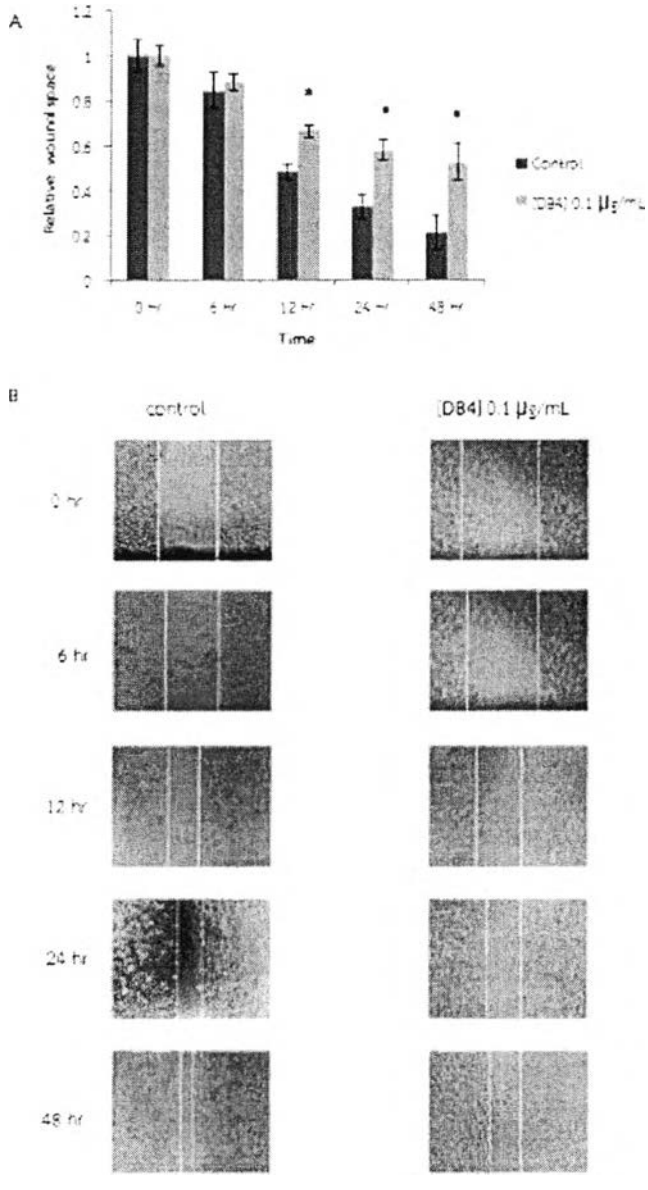


Figure 58 Effect of lusianthridin [DB4] on H460 cell migration. (A) Confluent monolayer of H460 cells was wounded using a 1 mm width tip and with lusianthridin [DB4] at 0.1 µg/ml or without for various times (0-48 h). Wound space was analyzed and represented as migration level relatively to the change of those in untreated cells. Data represent the mean ± SD (n =3). *P < 0.05 versus untreated control cells. (B) Wound space was visualized under a phase-contrast microscope at the indicated times.

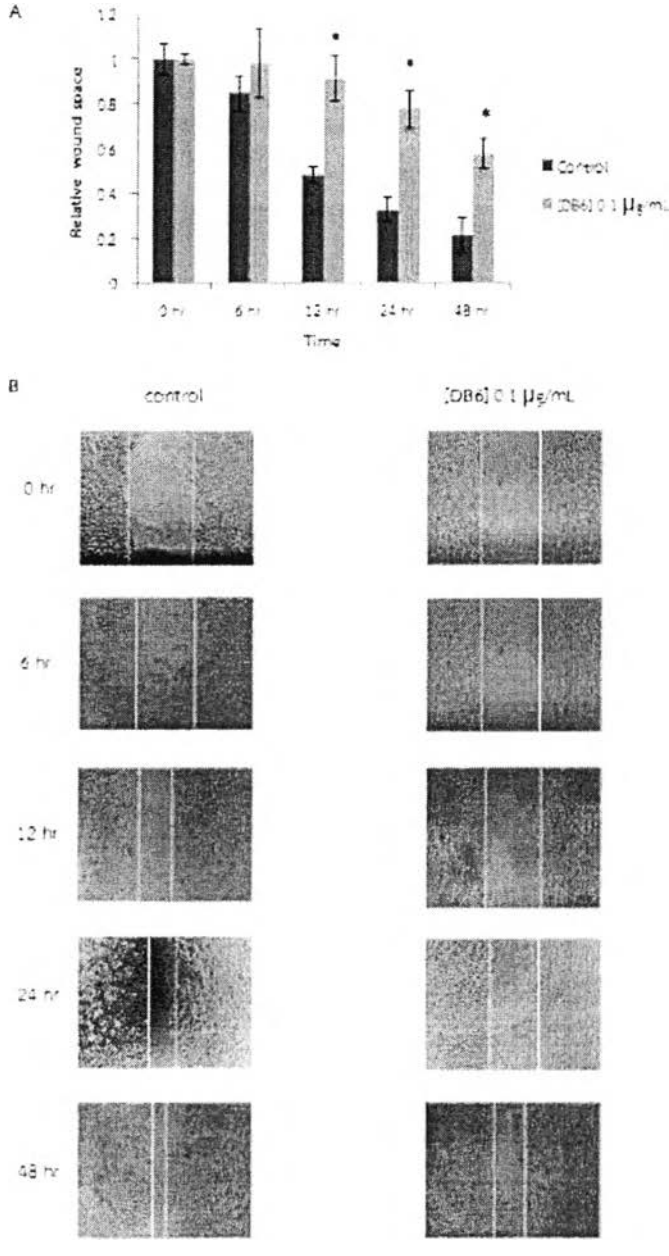


Figure 59 Effect of dendroflorin [DB6] on H460 cell migration. (A) Confluent monolayer of H460 cells was wounded using a 1 mm width tip and with dendroflorin [DB6] at 0.1 µg/ml or without for various times (0-48 h). Wound space was analyzed and represented as migration level relatively to the change of those in untreated cells. Data represent the mean ± SD (n =3). **P* < 0.05 versus untreated control cells. (B) Wound space was visualized under a phase-contrast microscope at the indicated times.

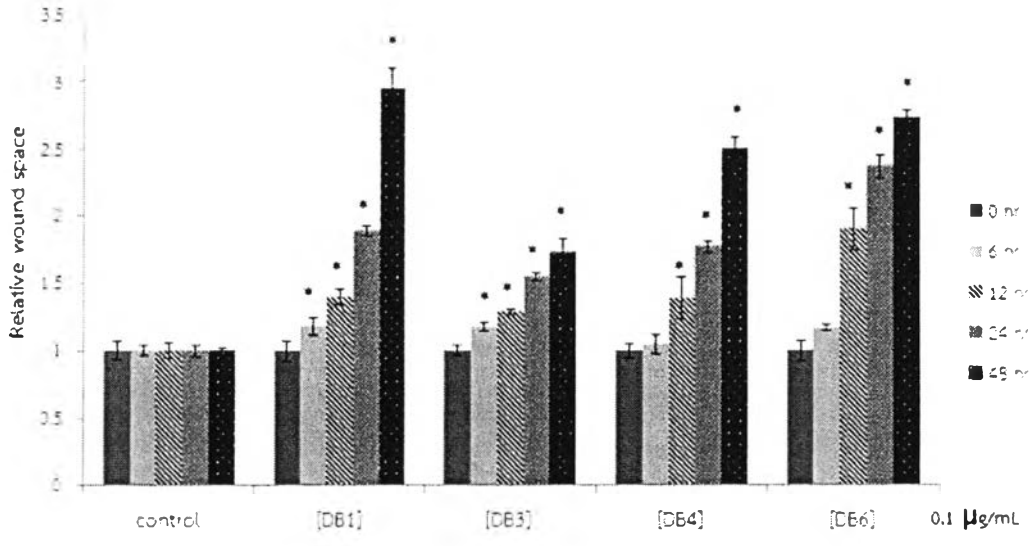


Figure 60 The relative wound space was analyzed by comparison of the relative change in wound space of the treated groups over that of the untreated control. Data represent the mean ± SD (n =3). *P < 0.05 versus untreated control cells.