Cite this: *RSC Adv.*, 2021, **11**, 37643

Dalpulapans A–E from the roots of *Dalbergia stipulacea*†

Priyapan Posri,^a Thurdpong Sribuhom,^a Sookkawath Walunchapruk,^b Thanaset Senawong,^b Sarawut Tontapha,^c Vittaya Amornkitbamrung^c and Chavi Yenjai^{*a}

Five new compounds, dalpulapans A–E (1–5), were isolated from the hexane extract of the roots of *Dalbergia stipulacea* Roxb. Five new compounds, dalpulapans A–E (1–5), were isolated from the hexane extract of the roots of *Dalbergia stipulacea* Roxb. An evaluation of cytotoxic activity against HeLa, A549 and normal cell lines using MTT assay was performed. The results showed that *R,R*-velucarpin A (6) was the most active against HeLa cells with an IC₅₀ value of 10.9 ± 0.42 μM, while fortunately this compound exhibited weak cytotoxicity against normal cells (29.20 ± 1.16 μM). Structures of all isolates were identified from their 1D and 2D NMR spectroscopic data and MS analysis. Experimental and calculated ECD spectra were studied to define the absolute configurations.

Received 20th September 2021
Accepted 15th November 2021

DOI: 10.1039/d1ra07041j

rsc.li/rsc-advances

1. Introduction

Dalbergia stipulacea Roxb., which belongs to the family Fabaceae, is found throughout southern China, eastern India, Myanmar, Thailand, Vietnam and Laos and is known as “Ma Kham Tao” in Thai. This plant has been used as an emmenagogue and for abortion when taken in moderate amounts. It is believed that this plant can be used for gonorrhea, syphilis and mouth ulcers.¹ Moreover, the roots of this plant are poisonous to fish.² The chemical constituents from this plant which include isoflavonoids, chalcone, pterocarpin and phenylpropene have been reported.^{2,3} Antifungal activity against *Pythium insidiosum*, a fungus-like microorganism for which at present there is no effective agent for treatment, was evaluated and shows interesting results.⁴ Further investigation of compounds from the roots of this plant and testing for cytotoxicity against A549 (lung cancer cells), HeLa (cervical cancer cells) and Vero cells (kidney of African green monkey cells; normal cells) was made. In this work, five new compounds (dalpulapans 1–5) and seven known compounds were reported. The absolute configurations of chiral carbons were determined using experimental electronic circular dichroism (ECD) analysis and comparing the specific rotations with those previously reported.

2. Discussion

The extraction and isolation of hexane extract of the roots of *D. stipulacea* by chromatographic methods led to five new compounds named dalpulapans A–E (1–5) (Fig. 1) and seven known compounds (6–12) including *R,R*-velucarpin A (6),⁵ *R,R*-velucarpin C (7),^{5,6} taepeenin A (8),⁷ taepeenin E (9),⁷ pteroloterin A (10),⁸ nortaepeenin A (11)⁸ and 2-allyl-1,4-dimethoxybenzene (12) (Fig. 1). All chemical structures were identified by spectroscopic methods, HRESIMS and ECD data.

Compound 1 showed the molecular formula C₁₈H₁₂O₅ identified from its HRESIMS ion at *m/z* 331.0580 [M + Na]⁺ (calcd 331.0582). It contains five oxygenated aromatic carbons at δ_C 156.4 (C-3), 154.4 (C-10a), 149.6 (C-4a), 148.3 (C-9) and 141.9 (C-8) from the ¹³C NMR data (Table 1). The ¹H NMR displayed two doublet signals (*J* = 8.4 Hz) at δ_H 7.42 and δ_H 7.22 of aromatic protons H-1 and H-2, respectively. This molecule contains a furan moiety, shown by proton signals at δ_H 6.85 (*J* = 2.0 Hz, H-1') and δ_H 7.56 (*J* = 2.0 Hz, H-2'); in addition, these protons were linked to carbons at δ_C 144.5 and δ_C 104.2, respectively. The HMBC correlations between H-1 (δ_H 7.42) and C-3 (δ_C 156.4), C-4a (δ_C 149.6) and C-11a (δ_C 78.8), and between H-2 (δ_H 7.22) and C-4 (δ_C 117.4) and C-11b (δ_C 113.1), and between H-2' (δ_H 7.56) and C-3 (δ_C 156.4) and C-4 (δ_C 117.4) confirmed the connectivity of a furan moiety at the C-3 and C-4 positions (Fig. 2). Two singlet signals of aromatic protons H-7 (δ_H 6.86) and H-10 (δ_H 6.46) were evident. Methylenedioxy protons were observed at δ_H 5.90 and δ_H 5.92 in the ¹H NMR spectrum and connected to the same carbon at δ_C 101.6. A doublet of doublets signal at δ_H 4.37 (*J* = 10.8, 4.8 Hz) was assigned to H-6α, while a triplet signal at δ_H 3.76 (*J* = 10.8 Hz) was given as H-6β. The coupling constant of oxymethine proton

^aNatural Products Research Unit, Center of Excellence for Innovation in Chemistry, Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand. E-mail: chayen@kku.ac.th; Tel: +66-4320-2222-41 ext. 12243

^bNatural Products Research Unit, Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

^cIntegrated Nanotechnology Research Centre, Department of Physics, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1ra07041j

Fig. 1 Structures of all isolated compounds 1–12.

H-11a was 7.2 Hz, confirming the cofacial orientation to H-6a.⁹ Long-range couplings between H-7 (δ_{H} 6.86) and C-6a (δ_{C} 40.3) and C-10a (δ_{C} 154.4), and between H-10 (δ_{H} 6.46) and C-6b (δ_{C} 118.1) and C-10a (δ_{C} 154.4) were detected in the HMBC spectrum (Fig. 2). The absolute configuration was confirmed by comparison with the ECD spectrum and specific rotation with pterocarpan (Chem. Rev. 2013).¹⁰ The compound **1** showed a negative optical rotation value ($[\alpha]_{\text{D}}^{20}$ –104.7) and displayed negative Cotton effect at 238 nm ($\Delta\epsilon$ –41.14). Kaennakam and coworkers reported the specific rotation of velucarpin A as $[\alpha]_{\text{D}}^{20}$ –83.8 and also showed negative Cotton effect at 247 ($\Delta\epsilon$ –8.71).⁵ Thus, the absolute configuration of compound **1** was 6aR,11aR and it was named dalpulapan A.

Isoflavone derivative **2**, dalpulapan B, showed the molecular formula of $\text{C}_{26}\text{H}_{26}\text{O}_5$ identified from its HRESIMS ion at m/z 419.1858 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{26}\text{H}_{27}\text{O}_5$, 419.1858). The singlet signal at δ_{H} 7.93 was located at oxygenated carbon C-2 (δ_{C} 152.0); in addition, this proton correlated with C-3 (δ_{C} 124.8), C-4 (δ_{C} 175.9) and C-9 (δ_{C} 152.4) in the HMBC spectrum, indicating the isoflavone core structure. The protons H-5 (δ_{H} 8.05) and H-6 (δ_{H} 6.84) showed two doublet signals with a coupling constant of 9.0 Hz (Table 1). The HMBC correlations were found between H-5 (δ_{H} 8.05) and C-4 (δ_{C} 175.9), C-7 (δ_{C} 157.7) and C-9 (152.4). The protons on the B-ring displayed an ABX system at δ_{H} 7.11 (d, J = 8.0 Hz, H-6'), δ_{H} 6.92 (d, J = 8.0 Hz, H-5') and δ_{H} 7.11 (s, H-2'). Cross-peaks between H-5' (δ_{H} 6.92) to C-1' (δ_{C} 125.3) and C-3' (δ_{C} 145.7) and between methoxy proton (δ_{H} 3.92) and C-4' (δ_{C} 146.7) were observed in the HMBC data. The presence of a pyran ring and prenyl group were detected in the ^1H and ^{13}C NMR spectra. The olefinic protons in the pyran ring

resonated at $\delta_{\text{H}}/\delta_{\text{C}}$ 6.84/115.3 of 1'' position and at $\delta_{\text{H}}/\delta_{\text{C}}$ 5.66/129.4 of 2'' position, in addition, an oxygenated quaternary carbon, C-3'' exhibited at δ_{C} 80.3. The ^1H NMR data displayed a triplet signal of an olefinic proton H-6'' (δ_{H} 5.10, t, J = 6.8 Hz) which coupled with H-5'' (δ_{H} 2.12, m). Cross-peaks between CH_3 -8'' and CH_3 -9'' and C-6'' were observed in the HMBC data. The experimental ECD spectrum displayed negative Cotton effects at 233 nm ($\Delta\epsilon$ –11.50) and positive Cotton effects at 265 nm ($\Delta\epsilon$ +7.89) which was similar to the calculated spectrum for the (3'R) configuration confirming the structure of **2** as shown in Fig. 1.

The ^1H and ^{13}C NMR data from dalpulapan C (**3**) were similar to **2**, except for the presence of a hydroxy group at the C-5 position in compound **3**. The molecular formula, $\text{C}_{26}\text{H}_{26}\text{O}_6$, confirmed the additional oxygen atom compared to **2**. An aromatic proton H-6 (δ_{H} 6.28, s) showed long-range coupling with C-5 (δ_{C} 162.4), C-7 (δ_{C} 160.0), C-8 (δ_{C} 101.0) and C-10 (δ_{C} 106.0) in the HMBC experiment (Table 1). Intramolecular H-bonding was detected at δ_{H} 12.95, confirming the presence of a hydroxy group at the C-5 position, in addition, this hydroxy proton showed long-range coupling with C-5, C-6 and C-10 in the HMBC data. The specific rotation of compound **3**, $[\alpha]_{\text{D}}^{28}$ +142.5, was the same as compound **2** and the experimental ECD was match to calculated ECD of 3R' configuration. Thus the structure of compound **3** was identified as shown in Fig. 1.

Dalpulan D (**4**) possessed a protonated adduct ion at m/z 421.2006 corresponding to the molecular formula $\text{C}_{26}\text{H}_{28}\text{O}_5$. This molecule was an isoflavan derivative and contained a pyran moiety, as characterized from 1D and 2D NMR data. An oxygenated methylene proton at δ_{H} 4.33 (dd, J = 10.4, 1.4 Hz, H-

Table 1 ^1H and ^{13}C NMR data for compounds 1–5 in CDCl_3 (δ in ppm)

1			2			3			4			5		
Position	δ_{H} (J in Hz)	δ_{C}	Position	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}		δ_{H} (J in Hz)	δ_{C}		δ_{H} (J in Hz)	δ_{C}	
1	7.42, d (8.4)	126.9	1	—	—	—	—	—	—	—	—	—	—	—
2	7.22, d (8.4)	105.9	2	7.93, s	152.0	7.87, s	152.6	4.33, dd (10.4, 1.4)	70.1	6.33, s	110.3	—	—	—
3	—	156.4	—	—	—	—	—	3.99, t (10.4)	—	—	—	—	—	—
4	—	117.4	3	—	124.8	—	123.6	3.51, m	32.0	—	80.4	—	—	—
4a	—	149.6	4	—	175.9	—	180.9	2.92, dd (15.7, 10.3)	30.8	—	189.4	—	—	—
6 α	4.37, dd (10.8, 4.8)	66.7	—	—	—	—	—	2.83, dd (15.7, 5.2)	—	—	—	—	—	—
6 β	3.76, t (10.8)	—	5	8.05, d (9.0)	126.9	—	162.4	6.81, d (8.2)	129.3	7.62, d (8.8)	127.1	—	—	—
6a	3.55, m	40.3	6	6.84, d (9.0)	115.1	6.28, s	100.2	6.36, d (8.2)	108.8	6.55, d (8.8)	112.2	—	—	—
6b	—	118.1	7	—	157.7	—	160.0	—	152.3	—	162.9	—	—	—
7	6.86, s	104.9	8	—	109.1	—	101.0	—	109.9	—	117.9	—	—	—
8	—	141.9	9	—	152.4	—	152.2	—	149.8	—	158.1	—	—	—
9	—	148.3	10	—	118.4	—	106.0	—	114.0	—	115.3	—	—	—
10	6.46, s	94.0	1'	—	125.3	—	124.0	—	128.1	—	110.3	—	—	—
10a	—	154.4	2'	7.11, s	115.6	7.07, d (2.0)	115.3	—	148.0	—	155.6	—	—	—
11a	5.61, d (7.2)	78.8	3'	—	145.7	—	145.8	6.37, s	98.5	6.48, s	94.2	—	—	—
11b	—	113.1	4'	—	146.7	—	147.0	—	146.6	—	150.5	—	—	—
1'	6.85, d (2.0)	144.5	5'	6.92, d (8.0)	110.8	6.91, d (8.0)	110.9	—	142.0	—	143.4	—	—	—
2'	7.56, d (2.0)	104.2	6'	7.11, d (8.0)	121.3	7.05, dd (2.0, 8.0)	121.1	6.60, s	107.1	6.66, s	103.7	—	—	—
OCH ₂ O	5.92, d (1.2)	101.6	1''	6.84, d (10.0)	115.3	6.71, d (10.2)	115.2	6.67, d (10.0)	117.4	3.46, d (6.8)	22.3	—	—	—
	5.90, d (1.2)	—	2''	5.66, d (10.0)	129.4	5.53, d (10.2)	126.4	5.51, d (10.0)	128.1	5.23, t (6.8)	120.3	—	—	—
	—	—	3''	—	80.3	—	80.7	—	78.1	—	140.3	—	—	—
	—	—	4''	1.77, m	41.6	1.73, m	41.7	1.71, m	41.0	2.08, m	39.8	—	—	—
	—	—	5''	2.12, m	22.8	2.10, m	22.7	2.10, m	22.8	2.08, m	26.4	—	—	—
	—	—	6''	5.10, t (6.8)	123.8	5.09, t (7.2)	123.8	5.09, t (7.0)	124.4	5.04, t (6.8)	123.7	—	—	—
	—	—	7''	—	132.2	—	132.1	—	131.7	—	132.3	—	—	—
	—	—	8''	1.66, s	25.8	1.66, s	25.8	1.66, s	25.8	1.66, s	25.8	—	—	—
	—	—	9''	1.57, s	17.8	1.58, s	17.8	1.58, s	17.8	1.59, s	17.9	—	—	—
	—	—	10''	1.46, s	27.0	1.44, s	27.1	1.39, s	26.3	1.81	16.4	—	—	—
	—	—	OCH ₂ O	—	—	—	—	5.89, d (1.2)	101.3	5.92, d (1.2),	102.0	—	—	—
	—	—	—OH	5.65, s	—	5.71, s	—	5.88, d (1.2)	—	5.89, d (1.2)	—	—	—	—
	—	—	—OCH ₃	3.92, s	56.2	3.91, s	56.2	4.74, s	—	4.16, s	—	—	—	—
	—	—	—	—	—	12.95, s	—	—	—	6.16, s	—	—	—	—

Fig. 2 Key HMBC correlations of compounds 1–5.

2a) and δ_{H} 3.99 (t, $J = 10.4$ Hz, H-2b) correlated with carbon at δ_{C} 70.1 in the HMQC data. From the multiplicity and coupling constant, it can be identified that H-2b was located at the axial position. Two doublet of doublet signals at δ_{H} 2.92 ($J = 15.7$, 10.3 Hz, H-4a) and δ_{H} 2.83 ($J = 15.7$, 5.2 Hz, H-4b) were located on the carbon at δ_{C} 30.8. The multiplet signal of a methine proton H-3 showed at δ_{H} 3.51. The connection of the H-2/H-3/H-4 system was observed in the ^1H - ^1H COSY data. Two singlet signals at δ_{H} 6.37 and δ_{H} 6.60 were assigned as H-3' and H-6', respectively. This compound showed a methylenedioxy group at δ_{H} 5.89 and δ_{H} 5.88 and located on the same carbon at δ_{C} 101.3. Cross-peaks between H-6' (δ_{H} 6.60) and C-3 (δ_{C} 32.0) and between H-3' (δ_{H} 6.37) and C-1' (δ_{C} 128.1) were observed in the HMBC spectrum. The ^1H and ^{13}C NMR spectra displayed the containing of pyran ring and prenyl side chain as compounds 2 and 3. Compound 4 showed positive Cotton effect at 218 nm ($\Delta\epsilon +2.28$) and 284 nm ($\Delta\epsilon +5.75$) and a negative positive Cotton effect at 243 nm ($\Delta\epsilon -3.32$), which corresponded with the calculated ECD spectrum of 3*R*,3''*R* configuration.^{11,12} It should be note that the absolute configuration at C-3'' was the same as compounds 2 and 3. All data concluded that the structure of 4 was shown in Fig. 1.

Dalpulapan E (5) was given a molecular formula $\text{C}_{26}\text{H}_{26}\text{O}_7$ characterized from the negative molecular ion peak $[\text{M} - \text{H}]^+$ at m/z 449.1598. The signals of the aromatic protons H-5 and H-6 displayed as doublets ($J = 8.8$ Hz) at δ_{H} 7.62 and δ_{H} 6.55 and correlated with carbons at δ_{C} 127.1 and δ_{C} 112.2, respectively, in the HMQC experiment. Two singlet signals on the aromatic B-ring were evident (δ_{H} 6.48, H-3' and δ_{H} 6.66, H-6'). Methylenedioxy protons showed two doublets ($J = 1.2$ Hz) at δ_{H} 5.92 and δ_{H} 5.89 and were located at carbon δ_{C} 102.0. This compound exhibited a geranyl group by showing two olefinic protons at δ_{H} 5.23 (t, $J = 6.8$ Hz, H-2'') and δ_{H} 5.04 (t, $J = 6.8$ Hz, H-6''), three methylene protons and three methyl protons. The HMBC cross-peaks showed correlation between H-1'' (δ_{H} 3.46) and oxygenated carbons C-7 (δ_{C} 162.9) and C-9 (δ_{C} 158.1), which confirmed the hydroxy group at the C-7 position. A singlet signal proton at δ_{H} 6.33 (H-2) was located at C-2 (δ_{C} 110.3), which bears two oxygen atoms. This proton correlated with carbons C-9 (δ_{C} 158.1) and C-2' (δ_{C} 155.6) in the HMBC spectrum, maintaining the presence of an acetal group. The broad singlet signal at δ_{H} 4.16 was assigned as a hydroxy proton, OH-3, and in addition the ^{13}C NMR signal of the oxygenated quaternary carbon C-3 exhibited at δ_{C} 80.4. The experimental ECD data, shows a negative Cotton effect at 239 nm ($\Delta\epsilon -32.31$) and a positive Cotton effect at 315 nm ($\Delta\epsilon +13.47$) and possessed positive specific rotation at $[\alpha]_{\text{D}}^{27.5} +141.3$. These information corresponded with the calculated ECD spectrum of 2*S*,3*R* configuration. In addition, both ECD data and the specific rotation of 5 are opposite to previous report, (2*R*,3*S*)-3,7,4'-trihydroxy-5-methoxycoumaronochromone.¹³ That compound showed negative specific rotation at $[\alpha]_{\text{D}}^{22} -184.6$; and positive and negative Cotton effects at 211 nm ($\Delta\epsilon +19.9$) and 292 nm ($\Delta\epsilon -19.9$), respectively. Thus the absolute configuration at C-2 and C-3 were confirmed as 2*S*,3*R* as shown in Fig. 1.

All isolated compounds, except 12, were evaluated for cytotoxicity against A549 (lung cancer cells), HeLa (cervical cancer

cells) and Vero cells using the MTT assay. The cytotoxicity results showed the most active compound was 6, which exhibited an IC_{50} value of 10.9 ± 0.42 μM against HeLa cells. In addition, IC_{50} values against A549 and Vero cells were 14.6 ± 1.31 and 29.2 ± 1.16 μM , respectively. The remaining compounds showed inactive ($\text{IC}_{50} > 15$ μM) to the test.

3. Experimental section

3.1. General experimental procedures

A Sanyo Gallenkamp (UK) melting point apparatus was used to find the melting points. Optical rotations were measured on a JASCO P-1020 digital polarimeter (Japan). The UV spectra were obtained on an Agilent 8453 UV-visible spectrophotometer (Germany). A PerkinElmer Spectrum One FT-IR spectrophotometer (USA) was used to acquire the IR spectra. NMR spectra were collected at 400 MHz (^1H) and at 100 MHz (^{13}C) using a Varian Mercury Plus spectrometer (USA). HRESIMS was performed on a Micromass Q-TOF 2 hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer (Micromass, UK). Analytical thin-layer chromatography (TLC) was accomplished on Merck silica gel 60 F_{254} . Column chromatography separations were carried out on silica gel less than 0.063 mm, 0.063–0.200 mm or RP-18.

3.2. Plant material

The roots of *D. stipulacea* were collected in February 2018 at Ban Na Phaeng Village, Wiang Kao District, Khon Kaen Province, Thailand ($16^\circ 41' 53.5''\text{N}$, $102^\circ 20' 24.1''\text{E}$). Plant material (voucher specimen KKU012018) was identified by Assoc. Prof. Suppachai Tiaworanant, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The dried powdered roots (12 kg) of *D. stipulacea* were extracted with hexanes (15 L $\times$ 3), EtOAc (10 L $\times$ 3) and MeOH (10 L $\times$ 3) at ambient temperature. The solutions were concentrated *in vacuo* to give the crude hexane (162 g, 1.35%), EtOAc (368 g, 3.07%) and crude MeOH (649 g, 5.41%) extracts. The crude hexane extract (162 g) was subjected to column chromatography (silica gel 60) and obtained five fractions, F1–F5. Fraction F2 was purified by FCC (hexane : EtOAc) to give 7 (10.35 g) and 8 (0.12 g). Fraction F3 was further purified and gave four fractions, F3.1–F3.4. Compound 1 (7.5 mg) was obtained from F3.1 while compounds 4 (5.7 mg) and 12 (3.2 mg) were found from F3.2. Subfraction F3.4 was chromatographed on a silica gel column (90 : 10 hexane : EtOAc) to obtain two subfractions, F3.4.1 and F3.4.2. Compounds 9 (5.5 mg) and 10 (9.9 mg) were found from F3.4.2. Fraction F4 was chromatographed out on silica gel FCC, eluting with hexane : EtOAc (70 : 30) to obtain four subfractions, F4.1–F4.4. Compounds 3 (59.1 mg), 6 (0.40 g) and 11 (36.6 mg) were obtained from subfraction F4.1. Compound 5 (7.0 mg) was obtained from F4.2. Subfraction F4.4 was purified and gave 2 (3.2 mg).

Dalpulapan A (1). A brown solid; mp 191–194 $^\circ\text{C}$; $[\alpha]_{\text{D}}^{27} -104.7$ (c 0.1, CHCl_3); UV (CH_3OH) λ_{max} (log ϵ) 249 (4.39), 311 (4.13) nm; ECD 205 nm ($\Delta\epsilon +37.91$), 215 nm ($\Delta\epsilon -102.05$); IR (neat) ν_{max} 2884, 1602, 1476, 1372, 1142, 1057, 935, 766 cm^{-1} ;

^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data (CDCl_3), see Table 1; HRESIMS m/z 331.0580 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{18}\text{H}_{12}\text{O}_5\text{Na}$, 331.0582).

Dalpulapan B (2). A yellowish oil; $[\alpha]_{\text{D}}^{28} +144.0$ (c 0.1, CHCl_3); UV (CH_3OH) λ_{max} ($\log \epsilon$) 265 (4.80) nm; IR (neat) ν_{max} 3367, 2929, 1626, 1576, 1511, 1438, 1395, 1274, 1196, 1134, 1080, 906, 807, 762 cm^{-1} ; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data (CDCl_3), see Table 1; HRESIMS m/z 419.1858 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{26}\text{H}_{27}\text{O}_5$, 419.1858).

Dalpulapan C (3). Yellowish solid; mp 121–124 $^\circ\text{C}$; $[\alpha]_{\text{D}}^{28} +142.5$ (c 0.1, CHCl_3); UV (CH_3OH) λ_{max} ($\log \epsilon$) 274 (4.38) nm; IR (neat) ν_{max} 3442, 2926, 1615, 1574, 1512, 1429, 1376, 1274, 1174, 1081, 1033, 954, 913, 819, 764 cm^{-1} ; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data (CDCl_3), see Table 1; HRESIMS m/z 435.1808 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{26}\text{H}_{27}\text{O}_6$, 435.1808).

Dalpulapan D (4). An brown oil; $[\alpha]_{\text{D}}^{28} +1.9$ (c 0.1, CHCl_3); UV (CH_3OH) λ_{max} ($\log \epsilon$) 209 (4.05), 282 (3.51) nm; IR (neat) ν_{max} 3728, 3404, 2921, 1634, 1610, 1586, 1479, 1440, 1378, 1280, 1213, 1168, 1096, 1064, 1038, 935, 863, 772, 720 cm^{-1} ; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data (CDCl_3), see Table 1; HRESIMS m/z 421.2006 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{26}\text{H}_{29}\text{O}_5$, 421.2015).

Dalpulapan E (5). A yellowish oil; $[\alpha]_{\text{D}}^{27.5} +141.3$ (c 0.1, CHCl_3); UV (CH_3OH) λ_{max} ($\log \epsilon$) 204 (4.58), 298 (4.06) nm; IR (neat) ν_{max} 3352, 2923, 1663, 1598, 1466, 1445, 1280, 1216, 1148, 1034, 992, 938, 826 cm^{-1} ; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data (CDCl_3), see Table 1; HRESIMS m/z 449.1598 $[\text{M} - \text{H}]^+$ (calcd for $\text{C}_{26}\text{H}_{25}\text{O}_7$, 449.1600).

3.4. Cytotoxic activity assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to evaluate the cytotoxic effect of the compounds. The procedure has been explained in a previous report.¹⁴ In brief, all cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U mL^{-1}) and streptomycin (100 $\mu\text{g mL}^{-1}$) (Gibco-BRL, USA) and incubated at 37 $^\circ\text{C}$ in a humidified atmosphere of 5% CO_2 . For preliminary testing, cells were exposed to the selected compounds at a concentration of 100 $\mu\text{g mL}^{-1}$ for 24, 48 and 72 hours. The compounds that caused less than 50% cell viability were further evaluated for their half maximal inhibitory concentration (IC_{50}) values. Control groups were treated with a solvent (a mixture of DMSO and ethanol; 1 : 1). After incubation, the medium was replaced with 110 μL of fresh medium containing MTT (0.5 mg mL^{-1} in PBS) (Sigma Chemical Co., St. Louis, MO, USA) and incubated for 2 h. The formazan formed after conversion of MTT was dissolved in DMSO. The absorbance of formazan was measured with a microplate reader (Bio-Rad Laboratories, USA) at the wavelength of 550 nm using 655 nm as a reference wavelength. Each assay was replicated four times. The percentage of viable cells which corresponds to the production of formazan was calculated as previously described (Kumnerdkhonkaen *et al.* 2018).

$$\% \text{ Cell viability} = [\text{sample (A550)} - \text{A655}] / [\text{control (A550)} - \text{A655}] \times 100$$

3.5. Calculation

The preliminary conformational analyses were evaluated using HyperChem software. These dominant conformers were further optimized at the B3LYP/6-311g (d,p) basis set by density functional theory.¹⁵ The GAUSSIAN 09 program was used to calculate the ECD spectra.¹⁶ The single point energy calculations were computed using time-dependent density functional theory (TD-DFT)¹⁷ at the CAM-B3LYP/6-311++g (d,p) level of theory.¹⁸ The CPCM polarizable conductor calculation model was used for bulk solvent effects.¹⁹

4. Conclusions

In this study, twelve compounds were isolated from hexane extract of the roots of *Dalbergia stipulacea* Roxb. They were five new compounds, dalpulapans A–E (1–5), and seven known compounds. The cytotoxicity evaluation against HeLa, A549 and normal cell lines using MTT assay was examined. It was found that **6** was the most active against HeLa cells with IC_{50} value of $10.9 \pm 0.42 \mu\text{M}$, and showed weak cytotoxicity against normal cells ($29.20 \pm 1.16 \mu\text{M}$). The structures of all compounds were determined by 1D and 2D NMR spectroscopic studies. MS analysis and experimental and calculated ECD spectra were also studied to define the absolute configurations of new compounds.

Conflicts of interest

The authors declare no competing financial interests.

Acknowledgements

We are indebted the Thailand Research Fund (Grant No. RSA6280050) and Khon Kaen University for financial support. T. Sribuham acknowledges The Post-Doctoral Training Program from the Research Affairs and Graduate School, Khon Kaen University (Grant No. 591471). We also thank The Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education, Thailand.

References

- 1 R. Nongmaithem, S. D. Yumkham, N. P. Devi, S. Salam, A. K. Das and P. K. Singh, *Indian J. Tradit. Knowl.*, 2018, 17(4), 754–762.
- 2 P. Bhatt and R. Dayal, *Phytochemistry*, 1992, 31(2), 719–721.
- 3 P. Borai and R. Dayal, *Phytochemistry*, 1993, 33(3), 731–732.
- 4 P. Posri, J. Suthiwong, Y. Thongsri and C. Yenjai, *Nat. Prod. Res.*, 2019, DOI: 10.1080/14786419.2019.1672068.
- 5 S. Kaennakam, P. Siripong and S. Tip-pyang, *Fitoterapia*, 2015, 105, 165–168.
- 6 A. Vanangamudi, R. Gandhidasan and P. V. Raman, *Fitoterapia*, 1998, 69(2), 180.

- 7 S. Cheenpracha, R. Srisuwan, C. Karalai, C. Ponglimanont, S. Chantrapromma, K. Chantrapromma, H. K. Fun, S. Anjum and R. Atta, *Tetrahedron*, 2005, **61**(36), 8656–8662.
- 8 J. Suthiwong, S. Pitchuanom, W. Wattanawongdon, C. Hahnvajjanawong and C. Yenjai, *J. Nat. Prod.*, 2014, **77**(11), 2432–2437.
- 9 L. V. Puyvelde, N. D. Kimpe, J. P. Mudaheranwa, A. Gasiga, N. Schamp, J. P. Declercq and M. V. Meerssche, *J. Nat. Prod.*, 1987, **50**(3), 349–356.
- 10 A. Goel, A. Kumar and A. Raghuvanshi, *Chem. Rev.*, 2013, **113**(3), 1614–1640.
- 11 D. Slade, D. Ferreira and J. Marais, *Phytochemistry*, 2005, **66**(18), 2177–2215.
- 12 S. Cheenpracha, C. Karalai, C. Ponglimanont and A. Kanjana-Opas, *J. Nat. Prod.*, 2009, **72**(8), 1395–1398.
- 13 Y. H. Seo, J. H. Jeon, M. Jeong, S. M. Ryu, W. K. Jeon, D. S. Jang, S. H. Shim, D. Lee, J. H. Choi and J. Lee, *J. Nat. Prod.*, 2018, **81**(7), 1598–1603.
- 14 P. Kumnerdkhonkaen, S. Saenglee, M. Asgar, G. Senawong, K. Khongsukkiwat and T. Senawong, *BMC Complementary Altern. Med.*, 2018, **18**, 130.
- 15 A. D. Becke, *J. Chem. Phys.*, 1993, **98**(7), 5648–5652.
- 16 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09, Revision B.01*, Gaussian, Inc., Wallingford, CT, 2010.
- 17 G. Pescitelli and T. Bruhn, *Chirality*, 2016, **28**(6), 466–474.
- 18 T. Yanai, D. P. Tew and N. C. Handy, *Chem. Phys. Lett.*, 2004, **393**(1–3), 51–57.
- 19 M. Cossi, N. Rega, G. Scalmani and V. Barone, *J. Comput. Chem.*, 2003, **24**(6), 669–681.