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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Potent cytotoxicity against human small cell lung cancer cells of the heptenes from the stem bark of *Xylopia pierrei* Hance

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Abstract Phytochemical investigation of the stem bark of *Xylopia pierrei* Hance led to the isolation of one triterpene, polycarpol (**1**), three heptenes, (7*R*)-acetylmelodorinol (**2**), (7*R*)-melodorinol (**3**), and melodienone (**8**), and four flavonoids, pinocembrin (**4**), isochamanetin (**5**), chrysin (**6**), and dichamanetin (**7**). All compounds were isolated for the first time from this plant species. The structures of the isolated compounds were characterized by spectroscopic techniques and by comparison of the spectroscopic data with the literature values and the stereochemistry at the asymmetric carbon was determined by the modified Mosher's method. Among them, compound **2** displayed potent cytotoxic activity against human small cell lung cancer (NCI-H187) cells with an IC_{50} value of 6.66 μM and it was 2.3-fold higher than that of the reference anticancer drug, ellipticine. In addition, compound **2** was also evaluated against the non-cancerous Vero cells and showed high selectivity index of 8.89, which is 59-fold greater than that of ellipticine. The findings suggest that compound **2** should be further developed as a potential lead molecule for anticancer drug development.

Keywords *Xylopia pierrei* · Heptenes · Cytotoxic activity · Small cell lung cancer

Introduction

The genus *Xylopia* (Annonaceae) comprises about 160 species with occurrence in South and Central America, Africa, and Asia. Approximately five species of *Xylopia* were identified in Thailand (Smitinand 2014). *Xylopia* species are rich source of isoquinoline and tetrahydroberberine-type alkaloids (Nishiyama et al. 2004, 2006, 2010), sequiterpenes (Martins et al. 1998; Moreira et al. 2003, 2005) and diterpenes (Andrade et al. 2004, Tavares et al. 2006). In particular, some species contains dimeric quaianes (Kamperdick et al. 2001, 2003) and dimeric diterpenes (Martins et al. 1999; Moreira et al. 2006). Some of these constituents possess interesting biological activities including antinociceptive (Nishiyama et al. 2010), antifungal (Moreira et al. 2003), and antimicrobial activities (Asekun and Adeniyi 2004). To our knowledge, there have been no reports on phytochemical study and biological activity of *Xylopia pierrei* (*X. pierrei*). In our preliminary investigation on the bioactivities of the stem bark of *X. pierrei*, we found that the crude *n*-hexane and EtOAc extracts showed significant cytotoxic activity against human small cell lung cancer (NCI-H187) cells. We herein report the details on the isolation, structure elucidation and evaluation of anti-NCI-H187 activity of the isolated compounds.

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Materials and methods

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. Infrared (IR) spectra were obtained using a Frontier FT-IR Perkin-Elmer spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 FT-NMR spectrometer, operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). Electrospray mass spectra and electrospray ionization time-of-flight mass spectrometry spectra were measured with a Finnigan LC-Q and a Bruker micrOTOF-II mass spectrometer. Unless otherwise indicated, column chromatography was carried out using Merck silica gel 60 (<0.063 mm) and Pharmacia Sephadex LH-20. For thin layer chromatography (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 stem bark of *X. pierrei* were collected from Sakaerat Environmental Research Station, Nakorn Ratchasima province, Thailand and the plant species was identified by Dr. Piya Chalermglin, Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. The voucher specimen (BKF 073765) is deposited at The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Chatuchak, Bangkok, Thailand.

Extraction and isolation

The air-dried stem bark of *X. pierrei* Hance (1.0 kg) was pulverized and extracted successively with *n*-hexane, EtOAc, and MeOH at room temperature, respectively. The extracted solutions were filtered and evaporated under reduced pressure at temperature 40–45 °C to give 10.74 g from the hexane extract, 39.58 g from the EtOAc extract and 30.35 g from the MeOH extract. The hexane and EtOAc extracts showed significant cytotoxic activities and were therefore investigated for active compounds. The hexane extract (10.0 g) was fractionated by column chromatography, 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 5 groups of eluting fractions were obtained. Group 4 (1.61 g) was further fractionated by column chromatography, using an isocratic solvent system of *n*-hexane-EtOAc (90:10), to give four fractions (fr. 4.1–4.4). Fraction 4.2 (735.3 mg) was separated by column chromatography using by isocratic solvent system of *n*-hexane-EtOAc (75:25) to yield polycarpol (**1**) as colorless crystals (657.5 mg). Fraction 4.3 (310.7 mg) was subjected to column chromatography twice, using *n*-hexane-EtOAc (70:30) to give (7*R*)-acetylmelodorinol (**2**) as white solid (120.5 mg), and (7*R*)-melodorinol (**3**) as white solid (76.6 mg). The absolute stereochemistry at C-7 was determined by the modified Mosher's method (Dale et al. 1969; Ohtani et al. 1991; Suksamrarn et al. 2008). The EtOAc extract (39.0 g) was fractionated by column chromatography, using a gradient solvent system of *n*-hexane, *n*-hexane-EtOAc, EtOAc, EtOAc-MeOH, and MeOH with increasing amounts of the more polar solvent. The eluates were examined by TLC and eight groups of eluting fractions were obtained. Group 2 (3.37 g) was chromatographed three times, using *n*-hexane-EtOAc (85:15), *n*-hexane-EtOAc (80:20), and *n*-hexane-EtOAc (70:30) to give polycarpol (**1**) (285.0 mg) and (7*R*)-acetylmelodorinol (**2**) (324.3 mg). Group 3 (1.62 g) was subjected to column chromatography twice, using *n*-hexane-EtOAc (90:10) as eluting solvent, followed by column chromatography on Sephadex LH-20, eluting with MeOH to yield eight fractions (fr. 3.1–3.8). Fractions 2 and 7 gave pinocembrin (**4**) as white solid (264.6 mg) and dichamanetin (**7**) as white solid (66.2 mg), respectively. Fraction 3 was chromatographed by isocratic elution with *n*-hexane-EtOAc (90:10) to afford isochamanetin (**5**) as white solid (90.2 mg). Fraction 4 was subjected to repeated column chromatography, using *n*-hexane-EtOAc (90:10) as eluent, to furnish chrysin (**6**) as white solid (164.5 mg). Group 5 (1.59 g) was chromatographed on Sephadex LH-20 eluting with MeOH, followed by silica column chromatography eluting with *n*-hexane-EtOAc (90:10) to yield (7*R*)-melodorinol (**3**) (343.4 mg). Group 6 (1.03 g) was further fractionated by column chromatography, using an isocratic solvent system of *n*-hexane-EtOAc (65:30), to give melodienone (**8**) as white amorphous solid (5.5 mg).

*Polycarpol (**1**)*

Colorless crystals (MeOH); mp 149.1 °C; $[\alpha]^{29}_{D+}$ 66 (*c* 0.71, $CHCl_3$); IR (KBr) ν_{max} 3442, 2926, 2884, 1373, 1047, 1034, 987 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz,): $\delta = 5.82$ (1H, d, $J = 5.9$ Hz, H-7), 5.28 (1H, d, $J = 5.5$ Hz, H-11), 5.06 (1H, t, $J = 7.0$ Hz, H-24), 4.25 (1H, dd, $J = 9.4$, 5.2 Hz, H-15), 3.22 (1H, dd, $J = 11.2$, 4.3 Hz, H-3), 2.26 (2H, d, $J = 17.6$ Hz, H-12), 1.98, 1.37 (2H, overlapping signal, H-22), 1.97 (1H, overlapping signal, H-1), 1.92, 1.81 (2H, overlapping signal, H-23), 1.70, 1.62 (2H, overlapping signal, H-2), 1.69 (2H, overlapping signal, H-16), 1.66 (1H, overlapping signal, H-17), 1.66 (3H, s, H-27), 1.56 (3H, s, H-26), 1.41 (1H, *ddd*, $J = 17.2$, 13.1, 3.8 Hz, H-1), 1.33 (1H, m, H-20), 1.07 (1H, dd, $J = 11.8$, 3.5 Hz, H-5), 0.98 (3H, s, H-29), 0.95 (3H, s, H-18), 0.91 (3H, s, H-30), 0.86

(3H, d, H-21), 0.85 (3H, s, H-28), 0.58 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz,): $\delta = 146.0$ (C-9), 140.8 (C-8), 121.2 (C-7), 116.0 (C-11), 78.9 (C-3), 74.7 (C-15), 51.9 (C-14), 48.9 (C-17), 48.8 (C-5), 44.3 (C-13), 40.1 (C-16), 38.6 (C-4), 38.5 (C-12), 37.4 (C-10), 36.2 (C-22), 35.7 (C-1, C-20), 27.7 (C-2), 22.8 (C-6, C-19), 18.3 (C-21), 15.8 (C-18); ESI MS *m/z* 439.3 $[M-H]^-$. The physical and spectral data were in agreement with those reported in the literature (Da Silva et al. 2012).

*(7R)-acetylmelodorinol (**2**)*

White solid; $[\alpha]^{29}_D$- 16 (*c* 0.30, $CHCl_3$); IR (KBr) ν_{max} 2922, 1780, 1743, 1721, 1681, 1601, 1584, 1561, 1451, 1372, 1271, 1226, 1107, 1070, 1026, 940 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz,): $\delta = 8.00$ (2H, d, $J = 7.4$ Hz, H-12, H-16), 7.55 (1H, t, $J = 7.4$ Hz, H-14), 7.42 (2H, t, $J = 7.4$ Hz, H-13, H-15), 7.35 (1H, d, $J = 5.4$ Hz, H-4), 6.25 (1H, d, $J = 5.4$ Hz, H-3), 6.12 (1H, ddd, $J = 8.0$, 6.0, 4.1 Hz, H-7), 5.30 (1H, d, $J = 8.0$ Hz, H-6), 4.55 (1H, dd, $J = 11.7$, 4.1 Hz, H-8α), 4.49 (1H, dd, $J = 11.7$, 6.0 Hz, H-8β), 2.07 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz,): $\delta = 169.7$ (C-17), 168.3 (C-2), 165.9 (C-10), 150.6 (C-5), 143.2 (C-4), 133.2 (C-14), 129.6 (C-12, C-16), 129.4 (C-11), 128.4 (C-13, C-15), 121.5 (C-3), 108.8 (C-6), 67.2 (C-7), 64.5 (C-8), 20.8 (C-18); ESI MS *m/z* 325.0 $[M+Na]^+$. The physical and spectral data were in agreement with those reported in the literature (Lu et al. 1997).

*(7R)-melodorinol (**3**)*

White solid; $[\alpha]^{29}_D$- 11 (*c* 0.30, $CHCl_3$); IR (KBr) ν_{max} 3333, 2947, 2835, 1650, 1449, 1113, 1016 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz,): $\delta = 8.01$(2H, d, $J = 7.4$ Hz, H-12, H-16), 7.54 (1H, t, $J = 7.4$ Hz, H-14), 7.41 (2H, t, $J = 7.4$ Hz, H-13, H-15), 7.36 (1H, d, $J = 5.4$ Hz, H-4), 6.22 (1H, d, $J = 5.4$ Hz, H-3), 5.38 (1H, d, $J = 8.1$ Hz, H-6), 5.15 (1H, ddd, $J = 10.0$, 7.3, 4.2 Hz, H-7), 4.45 (1H, dd, $J = 11.2$, 4.2 Hz, H-8α), 4.43 (1H, dd, $J = 11.2$, 7.3 Hz, H-8β); ^{13}C NMR ($CDCl_3$, 100 MHz,): $\delta = 168.9$ (C-2), 166.6 (C-10), 150.0 (C-5), 143.6 (C-4), 133.3 (C-14), 129.7 (C-12, C-16), 129.4 (C-11), 128.4 (C-13, C-15), 121.0 (C-3), 113.2 (C-6), 67.5 (C-7), 65.7 (C-8); ESI MS *m/z* 283.0 $[M+Na]^+$. The physical and spectral data were in agreement with those reported in the literature (Lu et al. 1997).

*Pinocembrin (**4**)*

White solid; $[\alpha]^{25}_D$- 42 (*c* 0.31, $CHCl_3$); IR (KBr) ν_{max} 3332, 2944, 2833, 1637, 1453, 1342, 1266, 1216, 1162, 1023,734 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 12.1$ (1H, s, 5-OH), 11.0 (1H, s, 7-OH), 7.49 (2H, d, $J = 7.2$ Hz, H-2′, H-6′), 7.39 (3H, m, H-3′, H-4′, H-5′), 5.92 (1H, s, H-8), 5.89 (1H, s, H-6), 5.56 (1H, dd, $J = 12.8$, 3.2 Hz, H-2), 3.23 (1H, dd, $J = 17.2$, 12.8 Hz, H-3b), 2.77 (1H, dd, $J = 17.2$, 3.2 Hz, H-3a); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 196.1$ (C-4), 166.8 (C-7), 163.6 (C-5), 162.9 (C-9), 138.8 (C-1′), 128.8 (C-3′, C-4′, C-5′), 126.8 (C-2′, C-6′), 102.0 (C-10), 96.1 (C-6), 95.2 (C-8), 78.6 (C-2), 42.3 (C-3); ESI MS *m/z* 257.2 $[M+H]^+$. The physical and spectral data were in agreement with those reported in the literature (Tuchinda et al. 1991).

*Isochamanetin (**5**)*

White solid; $[\alpha]^{29}_D$- 11 (*c* 1.05, $CHCl_3$); IR (KBr) ν_{max} 3293, 2942, 2833, 1635, 1488, 1454, 1341, 1297, 1249, 1153, 1021,755 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 12.4$ (1H, s, 5-OH), 11.0 (1H, s, 7-OH), 9.4 (1H, s, 2″-OH), 7.51 (2H, d, $J = 6.8$ Hz, H-2′, H-6′), 7.42 (2H, m, H-3′, H-5′), 7.38 (1H, m, H-4′), 6.93 (1H, ddd, $J = 8.4$, 7.6, 2.8 Hz, H-4″), 6.76 (1H, d, $J = 7.6$ Hz, H-3″), 6.60 (1H, overlapping signal, H-6″), 6.58 (1H, overlapping signal, H-5″), 6.07 (1H, s, H-8), 5.58 (1H, dd, $J = 12.8$, 3.0 Hz, H-2), 3.68 (1H, s, H–a), 3.26 (1H, dd, $J = 17.2$, 12.8Hz, H-3b), 2.77 (1H, dd, $J = 17.2$, 3.0 Hz, H-3a); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 196.3$ (C-4), 165.1 (C-7), 161.5 (C-5), 161.0 (C-9), 155.0 (C-2″), 140.0 (C-1′), 128.8 (C-3′, C-4′, C-5′), 127.7 (C-6″), 126.5 (C-2′, C-6′, C-1″, C-4″), 118.9 (C-5″), 114.6 (C-3″), 106.2 (C-6), 101.8 (C-10), 94.7 (C-8), 78.6 (C-2), 42.4 (C-3), 21.3 (C-a); ESI MS *m/z* 723.5 $[2M-H]^-$. The physical and spectral data were in agreement with those reported in the literature (Achenbach et al. 1997).

*Chrysin (**6**)*

White solid; IR (KBr) ν_{max} 3332, 2943, 2832, 1649, 1613, 1576, 1554, 1497, 1448, 1354, 1167,1023 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 12.8$ (1H, s, 5-OH), 10.9 (1H, s, 7-OH), 8.05 (1H, d, $J = 7.3$, H-2′, H-6′), 7.57 (1H, m, H-3′), 6.95 (1H, s, H-3), 6.51 (1H, s, H-8), 6.21 (1H, s, H-6); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 199.4$ (C-6), 194.5 (C-8), 182.3 (C-4), 164.8 (C-7), 163.6 (C-2), 161.9 (C-5), 157.9 (C-9), 132.4 (C-4′), 131.1 (C-1′), 129.5 (C-3′, C-5′), 126.8 (C-2′, C-6′), 105.6 (C-3), 104.4 (C-10); ESI MS *m/z* 255.2 $[M+H]^+$. The physical and spectral data were in agreement with those reported in the literature (Tuchinda et al. 1991).

*Dichamanetin (**7**)*

White solid; $[\alpha]^{29}_D$- 4 (*c* 0.82, $CHCl_3$); IR (KBr) ν_{max} 3295, 2939, 2833, 1629, 1488, 1455, 1377, 1341, 1286, 1215, 1021,753 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 12.5$ (1H, s, 5-OH), 7.32–7.41 (5H, m, H-2′-H-6′), 6.96 (2H, overlapping signal, H-4″, H-4‴), 6.77 (2H, overlapping

signal, H-3″, H- 3‴), 6.75 (1H, overlapping signal, H-6‴), 6.74 (1H, overlapping signal, H-6″), 6.65 (1H, overlapping signal, H-5‴), 6.63 (1H, overlapping signal, H-5″), 6.07 (1H, s, H-8), 5.57 (1H, dd, $J = 12.8$, 3.0 Hz, H-2), 3.78 (1H, br s, H-b), 3.77 (1H, s, H-a), 3.19 (1H, dd, $J = 17.2$, 12.4 Hz, H-3b), 2.86 (1H, dd, $J = 17.2$, 3.0 Hz, H-3a); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 196.8$ (C-4), 162.7 (C-7), 159.6 (C-5), 158.4 (C-9), 154.5 (C-2″, C-2‴), 139.1 (C-1′), 128.6 (C-2′, C-6′), 128.5 (C-6″, C-6‴), 128.4 (C-1″), 128.3 (C-4″, C-4‴), 126.5 (C-4′), 126.3 (C-3′, C-5′), 119.1 (C-5″, C-5‴), 114.6 (C-3″, C-3‴), 106.6 (C-6), 106.0 (C–8), 102.1 (C-10), 78.1 (C-2), 42.1 (C-3), 22.3 (C-b), 21.7 (C-a); ESI MS *m/z* 467.7 $[M-H]^-$. The physical and spectral data were in agreement with those reported in the literature (Achenbach et al. 1997).

*Melodienone (**8**)*

White solid; IR (KBr) ν_{max} 3345, 2923, 2852, 1723, 1671, 1451, 1270, 1116, 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz,): $\delta = 8.07$ (2H, d, $J = 7.3$ Hz, H-12, H-16), 7.59 (1H, t, $J = 7.4$ Hz, H-14), 7.46 (2H, t, $J = 7.4$ Hz, H-13, H-15), 7.36 (1H, d, $J = 15.7$ Hz, H-4), 7.07 (1H, dt, $J = 15.9$, 4.2 Hz, H-7), 6.75 (1H, d, $J = 15.7$ Hz, H-3), 6.59 (1H, dt, $J = 15.9$, 1.7 Hz, H-6), 5.06 (1H, br dd, $J = 4.2$, 1.7 Hz, H-8β), 5.05 (1H, br dd, $J = 4.2$, 1.7 Hz, H-8α), 3.80 (3H, s, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz,): $\delta = 187.8$ (C-5), 165.8 (C-2,10), 142.3 (C-7), 137.7 (C-4), 133.4 (C-14), 131.4 (C-3), 129.6 (C-11),129.3 (C-12, C-16), 128.6 (C-6), 128.5 (C-13, C-15), 63.0 (C-8), 52.3 (C-1); ESI MS *m/z* 297.0 $[M+Na]^+$. The physical and spectral data were in agreement with those reported in the literature (Jung et al. 1990).

Determination of the stereochemistry at the asymmetric carbon of compound 3

In order to determine the stereochemistry at the asymmetric carbon of compound **3**, the modified Mosher's method was performed. Briefly, a solution of the compound **3** (2.1 mg) in dry pyridine (100 µL) was added (*R*)-(−)-MTPA chloride (15 µL) at 10 °C and the mixture was stirred for 5 min. Stirring continued at ambient temperature and the completion of reaction was monitored by TLC. Two milliliters of *n*-hexane was added to the reaction mixture and the hexane-soluble part was subjected to flash column chromatography using *n*-hexane and 15% EtOAc/*n*-hexane as eluting solvent to give the (*S*)-MTPA ester **3x** (3.2 mg). The procedure was repeated, but using (*S*)-(+)-MTPA chloride in place of (*R*)-(−)-MTPA chloride, to yield the (*R*)-MTPA ester **3y** (3.5 mg). The ^{1}H NMR spectra of **3x** and **3y** were recorded in $CDCl_3$; the chemical shift differences of the proton resonances between the (*S*)-MTPA ester **3x** and the (*R*)-MTPA ester **3y** were calculated and the results are summarized in Fig. 1.

Cytotoxic activity

The cytotoxicity against human small cell lung cancer (NCI-H187) cells was evaluated by resazurin microplate assay (O'Brien et al. 2000). Briefly, cells at a logarithmic growth phase were harvested and diluted to 6.6×10^4 cells/ml in fresh medium. Successively, 5 µl of test compounds, diluted in 5% dimethyl sulfoxide (DMSO) and 45 µl of cells suspension were added to 384-well plates. The plates were incubated in 5% CO_2 incubator at 37 °C. After incubation for 5 days, 12.5 µl of 62.5 µg/ml resazurin solution was added to each well and the plates were then incubated at 37 °C for 4 h. Fluorescence signal was measured using SpectraMax M5 multi-detection microplate reader at the excitation and emission wavelengths of 530 and 590 nm, respectively. IC_{50} values were calculated from dose response curves, using six concentrations of three-fold serially diluted test compounds, by the SOFTMax Pro software. Ellipticine and 0.5% DMSO were used as positive and negative controls, respectively.

The cytotoxicity against African green monkey kidney (Vero) cell line was evaluated by the green fluorescent protein detection (Hunt et al. 1999). Briefly, 45 µl of cell suspension at 3.3×10^4 cells/ml were added to each well of 384-well plates containing 5 µl of test compounds previously diluted in 0.5% DMSO. The plates were incubated with 5% CO_2 in 37 °C incubator for 4 days. Fluorescence signals were measured using SpectraMax M5 multi-detection microplate reader in the bottom-reading mode with excitation and emission wavelengths of 485 and 535 nm. The signal on day 4 was subtracted by the signal on day 0, as background signal. IC_{50} values were calculated from dose response curves. Ellipticine and 0.5% DMSO were used as positive and negative controls, respectively.

Results and discussion

From bioassay-guided cytotoxicity evaluations against human small cell lung cancer (NCI-H187) cells, column chromatography of the active *n*-hexane and EtOAc extracts from stem bark of *X. pierrei* has led to the isolation of one triterpene, polycarpol (**1**), three heptenes, (7*R*)-acetylmelodorinol (**2**), (7*R*)-melodorinol (**3**), and melodienone (**8**) and four flavonoids, pinocembrin (**4**), isochamanetin (**5**), chrysin (**6**), and dichamanetin (**7**). These compounds were isolated for the first time from this plant species (Fig. 1). The structures of the isolated compounds were identified by comparison of the spectroscopic and physical data with the literature values. The cytotoxic activities of the isolated

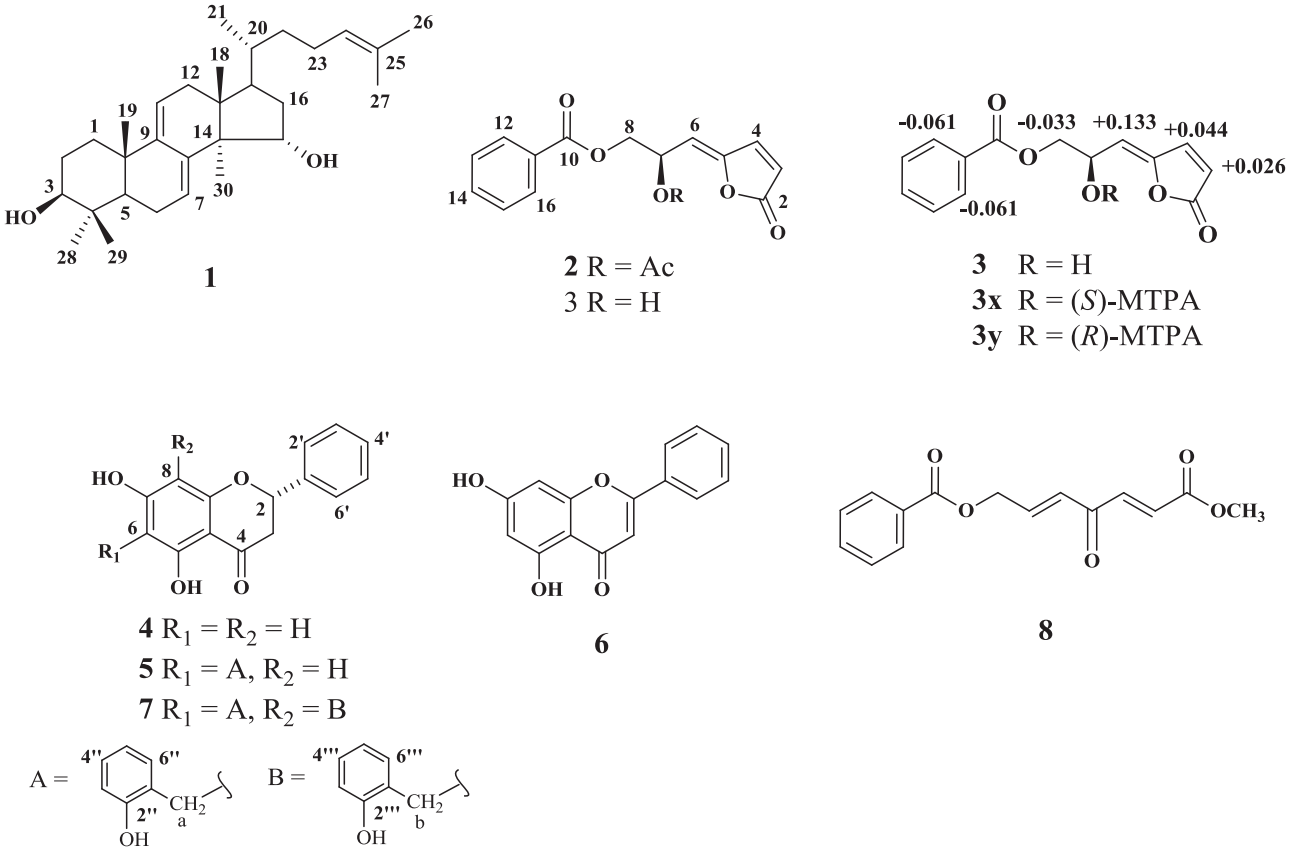

Fig. 1 Structures of compounds **1–8** isolated from stem bark of *X. pierrei* Hance

Table 1 Cytotoxic activities of compounds **1–8** isolated from stem bark of *X. pierrei* Hance

Compounds	Cytotoxicity (IC_{50}, μM)		
	NCI-H187	Vero	SI[a]
Polycarpol (**1**)	45.46	12.53	0.28
(7R)-Acetylmelodorinol (**2**)	6.66	59.14	8.89
(7R)-Melodorinol (**3**)	inactive[b]	7.27	–
Pinocembrin (**4**)	inactive[b]	inactive[b]	–
Isochamanetin (**5**)	40.28	19.31	0.48
Chrysin (**6**)	inactive[b]	inactive[b]	–
Dichamanetin (**7**)	17.26	22.29	1.29
Melodienone (**8**)	13.65	5.62	0.41
Ellipticine[c]	15.47	2.28	0.15

[a] Selectivity index = cytotoxicity to Vero cells/NCI-H187 cells

[b] Inactive at 50 μg/ml

[c] Ellipticine was used as a positive control

compounds against the NCI-H187 and Vero cells are summarized in Table 1. The results indicated that compound **2** presented high cytotoxic activity against the NCI-H187 cell with an IC_{50} value of 6.66 μM, followed by compounds **8** (IC_{50} 13.65 μM) and **7** (IC_{50} 17.26 μM). Compounds **5** and **1** exhibited weak activities with IC_{50} values of 40.28 and 45.46 μM, respectively, whereas compounds **3**, **4**, and **6** were inactive. The cytotoxic activity of compounds **2** was 2.3-fold higher than that of the reference anticancer drug, ellipticine (IC_{50} 15.47 μM) whereas that of compound **8** was slightly more active than the reference drug. Compound **8** was very toxic to Vero cells (IC_{50} 5.62 μM), whereas compound **2** was much less cytotoxic (IC_{50} 59.14 μM). The heptene **2** thus showed high selectivity index of 8.89, which is 59-fold greater than that of ellipticine. On comparing the activity of compounds **2** and **3**, it can be suggested that the acetyl group of compound **2** at 7-position should play important role in mediating cytotoxic activity whereas the presence of a free hydroxyl group seemed to decrease cytotoxic activity of compound **3**. The sharp decrease in cytotoxic activity of compound **3** indicated that the 7-position is very sensitive to change in bioactivity. These results suggest that compound **2** may be used as a potential lead molecule for anticancer therapeutic development. Furthermore, in comparing the activity of compounds **2**, **3** with that of compound **8**, it can be concluded that the lactone ring exerted cytotoxic effect of the compound. The absence of a lactone ring seemed to decrease cytotoxic activity of compound **8**. In addition, to

the best of our knowledge, this is the first report of phytochemical investigation on *X. pierrei*.

Conclusion

In this study, we have reported the first phytochemical investigation of the stem bark of *X. pierrei* Hance. The isolated compounds were one triterpene, polycarpol (**1**), three heptenes, (7*R*)-acetylmelodorinol (**2**), (7*R*)-melodorinol (**3**), and melodienone (**8**), and four flavonoids, pinocembrin (**4**), isochamanetin (**5**), chrysin (**6**), and dichamanetin (**7**). The structures of all compounds were characterized by spectroscopic techniques and by comparison with the literature values. Compound **2** displayed potent cytotoxic activity against NCI-H187 cells with an IC_{50} value of 6.66 μM and showed high selectivity index of 8.89, which is 59-fold greater than cytotoxic activity of ellipticine. Compound **2** should be selected as a potential lead molecule for anticancer drug development.

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Conflict of interest The authors declare that they have no competing interests.

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