Synthesis of combretastatin A-4 and erianin

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A concise route to two anti-tubulin natural products combretastatin A-4 and erianin has been developed. Combretastatin A-4 was obtained by a Perkin reaction between 3-bromo-4-methoxyphenyl acetic acid and 3,4,5-trimethoxybenzaldehyde, hydroxyl transformation, decarboxylation with a high level of *cis*-selectivity (*cis/trans* = 95/5), and erianin was obtained by subsequent hydrogenation. The overall yields of combretastatin A-4 and erianin were 37.5 and 30.8%, respectively.

Keywords: Perkin reaction, combretastatin A-4, erianin

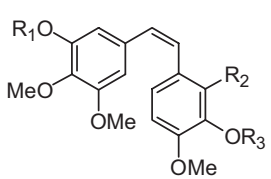
Combretastatin A-4 is a polyphenolic *cis*-stilbene, which was first isolated from the South African tree *Combretum caffrum* in 1989 by Pettit *et al*.[1] Since then, a series of combretastatins have been isolated from this plant (Fig.1) and many of them were found to be anti-tubulin and anti-vascular lead compounds. Combretastatin A-4, which binds to the same binding site of tubulin as colchicine and exhibited excellent activity by inhibiting mitosis and microtubule assembly,[2] is one of the most potent compound and has attracted considerable interest in recent years.[3,4] A prodrug of combretastatin A-4 (CA-4P), the water soluble disodium phosphate, is regarded as the flagship compound of the vascular disrupting agents (VDA),[5] and is in phase III clinical trials in the USA. However, the *trans*-isomer showed a dramatic decrease in its inhibitory effects on cancer cell growth and tubulin polymerisation. Structure–activity relationship studies[6] strongly indicate that a (*Z*)-stilbene configuration is essential for the cytotoxicity of the combretastatins. Consequently, the stereo-selective synthesis of combretastatin A-4 has received considerable attention in the last few years, but a large-scale preparation has not been reported.

Erianin **2**, 3-hydroxy-3',4',4,5'-tetramethoxylbibenzyl, was first isolated from the Orchid *Eria carinata* in 1984[7] and was subsequently isolated from a traditional Chinese herb, the *Dendrobium chrysotoxum Lindl*, by Chinese chemists in the 1990s. Since then the biological activity of erianin has received more attention.[8-10] Structurally, erianin can be regarded as a dihydro analogue of combretastatin A-4. Although they are closely related to each other, they do not occur in the same plants. Studies showed that erianin significantly inhibits the growth of HL-60 cells and that the activity was stronger than that of vincristine.[11] Further studies showed that erianin inhibited angiogenesis *in vivo* and *in vitro* and induced endothelial cytoskeleton disorganisation.[12] Recently, Kang *et al*.'s studies[13] demonstrated that erianin had no mutagenicity *in vitro* and *in vivo*. These findings suggest that erianin is an anti-vascular agent exhibiting therapeutic potential *in vivo* and *in vitro* with low toxicity.

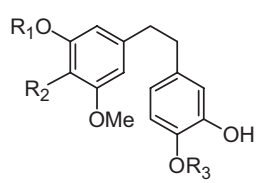
The remarkable medical importance of combretastatin A-4 and its analogues has prompted studies of their synthesis. Most of the syntheses of combretastatin A-4 involved a Wittig reaction which was developed by Pettit *et al*.[14] They reported a five-step route from a phosphonium bromide and 3,4,5-trimethoxybenzaldehyde, using *n*-butyllithium as the base. Combretastatin A-4 was obtained as a mixture of *cis* and *trans*-isomer in a ratio of 1/1.5. A stereoselective method developed by the Fürstner group,[15] was based on the Lindlar-type semi-hydrogenation of an alkyne precursor assembled by 9-MeO-9-BBN mediated Suzuki-type reaction. This gave a mixture of combretastatin A-4 and alkane in a ratio of 86/14. Both methods were limited by the availability of starting materials and the preparation of intermediates. The Perkin condensation developed by Gaukroger *et al*.[16] seems more convenient. Starting from 3,4,5-trimethoxyphenylacetic acid and isovanillin, and using a Perkin condensation and decarboxylation, combretastatin A-4 was obtained in an overall yield of 16% and with a stereoselective ratio of 99.8/0.2 after crystallisation. However, the starting material 3,4,5-trimethoxyphenylacetic acid is not commercially available and the yield is not high. Most recently, Robinson and Taylor[17] reported a Ramberg–Backlund route starting from 3,4,5-trimethylbenzyl thiol and protected 3-hydroxy-4-methoxybenzyl bromide to give combretastatin A-4 and its *trans*-isomer in a ratio of 53/47 in their optimal result, and the overall yield is 35.28%. The above-mentioned methods have shortcomings and are not ideal for large-scale applications. We report a convenient synthetic route for combretastatin A-4 **1** and erianin **2** via Perkin reaction methodology with a high level of *cis*-selectivity (*cis/trans* = 95/5 after one crystallisation) and good yields (Scheme 1). It is the most efficient synthetic route for combretastatin A-4 **1** and erianin **2** starting from commercial materials.

Results and discussions

3-Bromo-4-methoxyphenylacetic acid **4** was obtained by bromination of 4-methoxyphenylacetic acid **3**. The Perkin



R_1	R_2	R_3	
Me	OH	H	Combretastatin A-1
H	H	H	Combretastatin A-3
Me	H	H	Combretastatin A-4
H	H	Me	Combretastatin A-5



R_1	R_2	R_3	
H	OMe	Me	Combretastatin B-2
Me	OMe	H	Combretastatin B-3
Me	H	H	Combretastatin B-4
Me	OMe	Me	Erianin

Fig. 1 Selected compounds of combretastatins and erianin.

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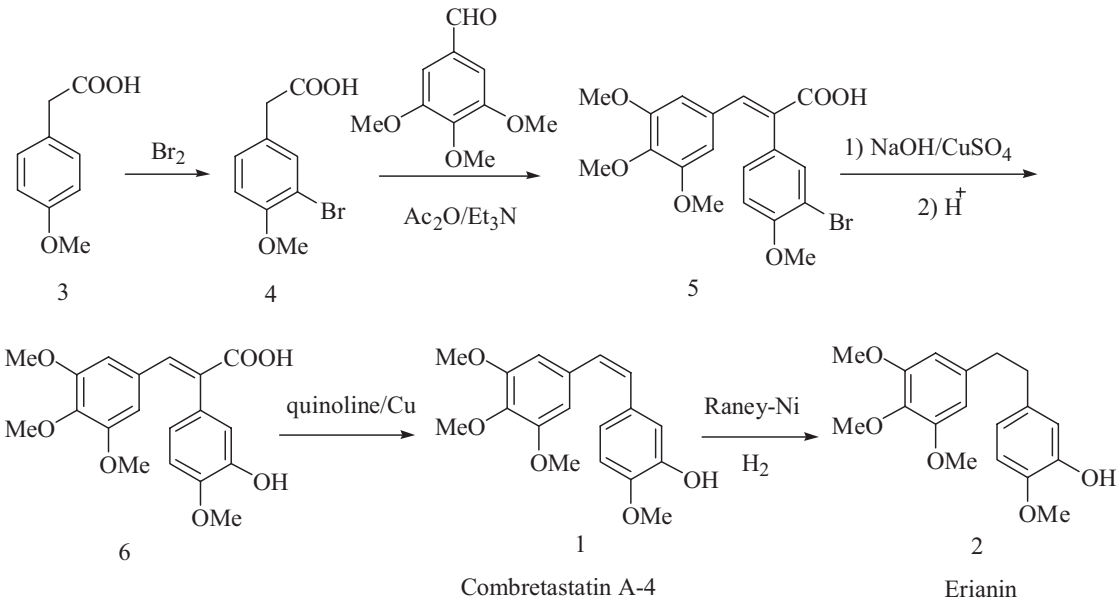

Scheme 1 Synthesis of combretastatin A-4 and erianin.

condensation between **4** and 3,4,5-trimethoxybenzaldehyde in the presence of acetic anhydride and triethylamine at 110°C gave the 1,2-diarylacrylic acids **5** in 75% yield, in which the *cis*-stilbene isomer (the *E* isomer by Cahn-Ingold rules) was the main product (*cis*/*trans* = 95/5 after one crystallisation). The *cis*-stilbene configuration of **5** was established by the ^{1}H NMR spectrum, in which the field-effect of carboxyl group in the *cis*-stilbene resulted in a remarkable down-field shift of the acrylic alkene proton, and similarly, a noticeable field-effect of carboxyl group can also be found in 2'-H in the B-ring of *trans*-stilbene. This can be confirmed by chemical shift of the acrylic alkene proton and the 2'-H in the B-ring of the isomers, for **5**, δ_{Ha} = 7.82 ppm, δ_{Hb} = 7.50 ppm, respectively, and for the *trans*-isomer of **5**, δ_{Ha} = 6.90 ppm, δ_{Hb} = 7.68 ppm, respectively (Fig.2). The hydroxyl transformation from **5** to **6** in the presence of NaOH and $CuSO_4$ at 100°C gave **6** in 75% yield. Fortunately, although the reaction was carried out in a strong base for a few days, geometrical isomerisation did not occur and the configuration of main product remained *cis*. The decarboxylation was carried out in the presence of quinoline and copper powder at 220°C under nitrogen, to give the combretastatin A-4 in 71% yield. The geometrical configuration of combretastatin A-4 was also determined by ^{1}H NMR, for which the $J_{(CH=CH)}$ = 12.4 Hz for *cis*-stilbene. Hydrogenation of combretastatin A-4 catalysed by Raney Ni then gave erianin **2** in 82% yield.

In the Perkin condensation, the product is an acid, so we used a basification/extraction/acidification procedure as an easy way to isolate the solid product and remove any non-acidic impurities from the crude product. The product was obtained in yield of 75%. In the decarboxylation reaction, dark oily byproducts in the mixture made the purification difficult. TLC analysis showed that compound combretastatin A-4 was highly lipophilic, whereas the byproducts were poorly lipophilic. Thus, we used petroleum ether to extract compound **1** from the dark oily reaction mixtures and leaving the byproducts behind. The product was thus readily purified without using column chromatography. The crude products were then recrystallised with ethyl acetate.

In conclusion, we have developed an effective process for syntheses of combretastatin A-4 and erianin in good yields. Starting from 3,4,5-trimethoxybenzaldehyde and 4-methoxyphenylacetic acid, combretastatin A-4 and erianin were synthesised with overall yields of 37.5 and 30.8% respectively, on a multigram scale.

Experimental

The melting points were determined on Thiele apparatus and were uncorrected. IR spectra were recorded on an Analect RFX-65A IR spectrometer. ^{1}H NMR were obtained from a Bruker DRX-400 MHz spectrometer with TMS as an internal standard and $CDCl_3$ was used as the solvent. EI–MS analysis was performed using a Shimadzu GCMS-QP5050A mass spectrometer. Elemental analyses were carried out by Elementar Vario EL element analyser. All reactions were monitored by TLC on silica gel GF_{254}. The ratio of *Z*/*E* was determined by HPLC. Unless otherwise mentioned, reagents were obtained commercially and used without further purification. Petroleum ether had a boiling point range 60–90°C.

3-Bromo-4-methoxyphenylacetic acid (**4**): To a stirred solution of 4-methoxyphenylacetic acid **3** (332.00 g, 2 mol) in acetic acid (800 ml), Br_2 (106 ml, 2 mol) was slowly added over 1 h. After stirring for 3 h at room temperature, the mixture was carefully poured into ice water to afford a substantial light yellow precipitate. This was filtered to give **4** as a light yellow powder in 94% yield. After recrystallising, a white crystal was obtained. M.p. 113–114°C (lit[18] 114–115°C). MS, *m/z* (%): 244 (M^+, 50), 246 (M^+ + 2, 48), 199 (100), 201 (98), 121 (10), 105 (30), 77 (70), 51 (45).

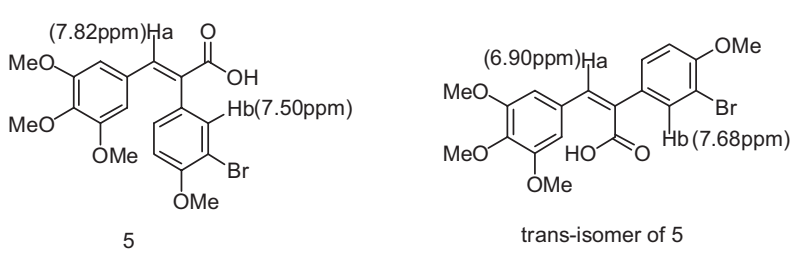

Fig. 2 Chemical shift of acrylic alkene and aromatic proton.

(E)-2-(3'-Bromo-4'-methoxyphenyl)-3-(3",4",5"-trimethoxyphenyl) acrylic acid (**5**): To a solution of compound **4** (98.40 g, 0.4 mol) and 3,4,5-trimethoxybenzaldehyde (78.40 g, 0.4 mol) in acetic anhydride (110 ml), triethylamine (110 ml) was added. The mixture was heated at 130°C and stirred for 5 h. After cooling, it was acidified with concentrated hydrochloric acid, then poured into ice-water, stirred and stored for several hours. A yellow solid was obtained. This was filtered and dissolved in 10% NaOH (400 ml), extracted and decoloured with ethyl acetate and the organic layers were separated. Hydrochloric acid was added to the aqueous phase to pH 2–3. A yellow solid was precipitated, filtered and recrystallised from ethyl acetate to afford **5** in 75% yield. M.p. 237–238°C. IR (KBr): 3318 (COOH), 3014 (CH=), 2937, 2838 (CH_3), 1708 (C=O), 1619 (C=C), 1577, 1504, 1457, 1419, 1396, 1284, 1249, 883, 742. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82 (s, 1H, CH=), 7.50 (d, 1H, J = 2.0 Hz, 2'–ArH), 7.17 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 6'–ArH), 6.93 (d, 1H, J = 8.4 Hz, 5'–ArH), 6.36 (s, 2H, 2",6"–ArH), 3.89 (s, 3H, 4'–OCH_3), 3.81 (s, 3H, 4"–OCH_3), 3.57 (s, 6H, 3",5"–OCH_3). For *trans*-isomer of **5**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (d, 1H, J = 2.2 Hz, 2'–ArH), 7.38 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.2 Hz,, 6'–ArH), 6.90 (s, 1H, CH=), 6.89 (d, 1H, J = 8.4 Hz, 5'–ArH), 6.69 (s, 2H, 2",6"–ArH), 3.90 (s, 3H, 4'–OCH_3), 3.84 (s, 3H, 4"–OCH_3), 3.59 (s, 6H, 3",5"–OCH_3). MS, *m/z* (%): 422 (M^+, 100), 424 (M^+ + 2, 98), 407 (20), 409 (18), 156 (18). Anal. Calc. For $C_{19}H_{19}BrO_6$: C 53.9; H 4.5%. Found: C 53.8; H 4.5%.

(E)-2-(3'-Hydroxyl-4'-methoxyphenyl)-3-(3", 4", 5"-trimethoxyphenyl) acrylic acid (**6**): NaOH (50.00 g) was dissolved in 500 ml water, then **5** (64.00 g 151.2 mmol) and $CuSO_4$ (20.00 g) were added to the clear solution, and the mixture was stirred at 110°C for 2.5 days. Upon cooling, filtration and acidification with 2M HCl, the solution was extracted with ethyl acetate. The organic layer was washed with brine, dried and evaporated in vacuum to afford a yellow solid which recrystallised with ethyl acetate and petroleum to afford **6** as pale yellow crystals in 75% yield. M.p. 233–234°C. IR (KBr) cm^{-1}: 3401 (OH), 2998 (CH_3), 2944 (CH_2), 2838–2518 (COOH), 1679 (C=O), 1581, 1508, 1459, 1378 (CH_3), 1334, 1184, 998, 946 (ArH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (s, 1H, CH=), 6.89 (d, 1H, J = 8.4 Hz, 5'–ArH), 6.85 (d, 1H, J = 2.0 Hz, 2'–ArH), 6.75 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 6'–ArH), 6.39 (s, 2H, 2",6"–ArH), 5.76 (w, 2H, COOH, OH), 3.89 (s, 3H, 4'–OCH_3), 3.81 (s, 3H, 4"–OCH_3), 3.57 (s, 6H, 3",5"–OCH_3). MS, *m/z* (%): 360 (M^+, 100), 345 (20, M–OCH_3), 327 (5), 285 (15). Anal. Calc. For $C_{19}H_{20}O_7$: C 63.3, H 5.6%. Found: C 63.3, H 5.6%.

Combretastatin A-4 (**1**): A mixture of compound **6** (40.00 g, 111.2 mmol) and copper powder (40.00 g, 62.5 mmol) in 200 ml quinoline was stirred at 200°C for 3 h. After cooling, ethyl acetate was added and the copper was filtered off. The filtrate was washed with 2M hydrochloric acid, and the water layer was separated and extracted with ethyl acetate. The organic layers were combined and washed with brine, dried ($MgSO_4$) and evaporated in vacuum to afford a brown viscous solid, which was recrystallised from ethyl acetate to afford combretastatin A-4 **1** as colourless crystals in 71% yield. M.p. 116–117°C (1it:[15] 115–116°C). IR (KBr): 3424 (OH), 3002 (CH=), 2938, 2836, 1610, 1579, 1508, 1459, 1419, 1328, 1182, 1025, 1004, 944, 881, 854, 796. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.91 (d, 1H, J = 2.0 Hz, 2'–ArH), 6.79 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 6'–ArH), 6.71 (d, 1H, J = 8.0 Hz, 5'–ArH), 6.51 (s, 2H, 2",6"–ArH), 6.45 (d, 1H, J = 12.4 Hz, CH=), 6.42 (d, 1H, J = 12.4 Hz, CH=), 5.49 (s, 1H, OH), 3.89 (s, 3H, 4'–OCH_3), 3.84 (s, 3H, 4"–OCH_3), 3.68 (s, 6H, 3",5"–OCH_3). MS, *m/z* (%): 316 (M^+, 100), 301 (75), 241 (8), 226 (6), 211 (5), 141 (12), 115 (8), 93 (5), 57 (8). Anal.Calc. For $C_{18}H_{20}O_5$: C 68.4, H 6.3%. Found: C 68.3, H 6.3%.

Erianin (**2**): A solution of combretastatin A-4 (20.00 g, 63.2 mmol) in ethanol was hydrogenated at 25–30atm pressure in the presence of Raney-Ni (6.00 g) at ambient temperature for 8 h. The catalyst was removed by filtration. The filtrate was evaporated in vacuum and the residue recrystallised from petroleum/chloroform to give erianin **2** as a pale reddish crystal in 82% yield. M.p. 78.5–79°C (lit[19] 79.5–80°C). IR (KBr) cm^{-1}: 3426 (OH), 2937, 2838 (CH_3), 1590, 1459, 1384, 1238, 1008; ^{1}H NMR($CDCl_3$,400 MHz) δ 6.79 (d, 1H, J = 2.0 Hz, 2'–ArH), 6.75 (d, 1H, J = 8.0 Hz, 6'–ArH), 6.62 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 5'–ArH), 6.34 (s, 2H, 2",6"–ArH), 5.55 (s, w, 1H, OH), 3.85 (s, 3H, 4"–OCH_3), 3.79 (s, 9H, 4', 3", 5"–OCH_3), 2.80 (s, 4H, CH_2CH_2). MS, *m/z* (%): 318 (M^+, 16), 181 (100), 137 (20). Anal. Calc. For $C_{18}H_{22}O_5$: C 67.9, H 6.9%. Found: C 67.9, H 6.9%.

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