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Synthesis of the first unnatural schisantherins and their effects in multidrug-resistant cancer cells

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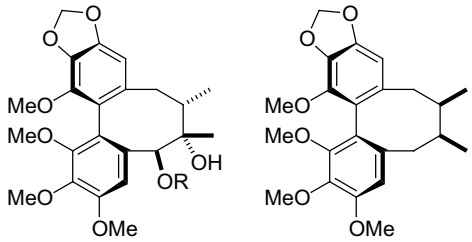
Abstract

Schisandrol A, a dibenzocyclooctadiene lignan, was obtained by a simplified procedure from *Schisandra chinensis* fruits. Its reaction with carboxylic acids to give new esters (schisantherins) required special conditions such as microwave irradiation. An X-ray single crystal structure analysis of schisandrol A revealed a sterical shielding of the secondary OH group as the likely reason. The cinnamoate inhibited the P-gp drug transporters of multidrug-resistant human Kb-V1 cervix carcinoma cells better than the natural benzoate and comparable to the clinical sensitizer verapamil.

Keywords: *Schisandra chinensis*; Esterification; Phosphorus ylides; Microwave; Multidrug resistance

Fruits of *Schisandra chinensis* (Turcz.) Baill (*Schisandraceae*) belong to the top 10 most widely used plants in traditional Chinese and Japanese herbal medicine due to their hepatoprotective, antiasthmatic, antidiabetic, sedative and tonic properties.[1] Dibenzo[*a*,*c*]cyclooctadiene lignans[2] are the constituents believed responsible for the majority of these biological effects. Dozens of such lignans have been chromatographically isolated from *Schisandra* extracts and identified mainly by mass spectrometry and 2D NMR spectroscopy.[3–6] Lately, the focus of medicinal interest has shifted from the hepatological aspects to their beneficial interactions with cancer related processes such as oxidative stress[7,8] and multidrug resistance (MDR).[9] Schisandrol A (**1**), its naturally occurring mono esters (schisantherins) **2a**–**c**,[10–12] and schisandrin B (**3**)[13–15] (Fig. 1) have been intensively studied for their ability to block the ABC-transporter mediated efflux of drugs. Overexpression of such transporters in cancer cells is the most frequent cause for MDR and eventual failure of chemotherapy. In vitro studies[9] revealed that schisandrin B (**3**) and schisantherin A (**2a**) can overcome MDR and fully restore the intracellular drug accumulation in cells overexpressing ABCB1 (P-glycoprotein; P-gp) type transporters while diol **1** itself was far less efficacious. Yet, more systematic studies of other, especially unnatural schisandrol A esters, were hampered by the inavailability of the parent diol **1** in larger than just minute quantities by the established extraction/HPLC procedures.[16,17]



	R	name
1	H	schisandrol A
2a	PhCO	schisantherin A
2b	angeloyl	schisantherin B
2c	tigloyl	

Fig. 1. Structures of schisandrol A (**1**), its natural esters **2a**–**c** and schisandrin B (**3**).

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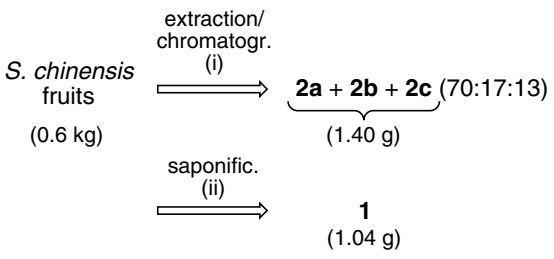
In a simplified extraction procedure we now obtained 1.40 g of a 70:17:13 mixture of schisandrol A esters **2a**:**2b**:**2c** from 0.6 kg of dried ground berries of *S. chinensis*. The fruit powder was first extracted at room temperature with hexane. This extract was discarded, the remainder was filtered off, dried and re-extracted with ethyl acetate. A single column chromatography run of the concentrated extract on Silica Gel 60 with cyclohexane/ethyl acetate (2:1) as the eluent afforded the mixture of esters **2a**–**c**. This procedure is less tedious than the early one by Kobayashi et al.,[16] which implied six extraction steps, a steam distillation and three different column chromatography runs. It is also faster than the more recent small-scale protocols terminated by HPLC purification steps. Saponification of the inseparable mixture of esters **2a**–**c** with 15% aqueous NaOH/MeOH (1:1) took a full eight hours at 80 °C, and so much longer than anticipated, but eventually yielded ca. 1 g of pure schisandrol A (**1**) (Scheme 1).

We then tried out several standard esterification methods to convert **1** into new esters **2** of various alkanoic and (het)aromatic carboxylic acids. These included the protocols by Hassner–Steglich, Mitsunobu, Yamaguchi and Mukaiyama. Much to our surprise they all failed even under forced conditions or after protracted reaction periods and starting materials were recovered in most cases. So, both the formation and the cleavage of esters of schisandrol A appear to be hindered. An X-ray single crystal structure analysis of **1** revealed, as a possible explanation, a secondary OH-group (O4 in Fig. 2) shielded by the adjacent methyl group and the benzodioxolane, and so difficult to reach for carboxylic acids or condensation agents.[18]

After some experimentation we found three practicable ways to esterify schisandrol A (**1**) (Scheme 2). Benzoates and cinnamoates were obtained by reaction of **1** with the respective benzoyl or cinnamoyl chlorides and an excess of triethylamine under microwave irradiation (300 W, xylene, 170 °C, 3 h). In this way, esters **2a** and **2d**[19] were obtained in ca. 50% yield. Minor by-products which we think result from transannular elimination of water (MS: 414) were easily removed by column chromatography. Acetate **2e**[20] was the only ester accessible under normal thermal conditions from acetyl chloride and triethylamine, also in about 50% yield. Finally, our well-established three-component reaction[21] between alcohol **1**, saturated, unsaturated or (het)aromatic aldehydes and the cumulated phosphorus ylide Ph_3PCCO,[22] when carried out under microwave conditions, also afforded the corresponding α,β-unsaturated esters, for example, cinnamoate **2d** from benzaldehyde or ester **2f**[23] from furfural. This domino reaction proceeds by addition of the OH group across the C=C bond of the ylide to give a new ester ylide which in turn undergoes Wittig alkenation with the aldehyde present in the mixture. Ph_3PCCO itself does not readily react with aldehydes. Its slim C=C=O moiety is obviously capable of reaching the secondary alcohol group of **1** despite the large terminal Ph_3P residue.

The ability of schisantherins **2a**,**d**,**e** to inhibit the cancer-relevant P-gp transporters was assessed in fluorometric Calcein-AM assays. Functional transporters extrude the nonfluorescent calcein-AM before intracellular esterases



Scheme 1. Preparation of schisandrol A (**1**) from *Schisandra chinensis*. Reagents and conditions: (i) ground berries (0.6 kg) extracted with *n*-hexane (10 L), rt, 8 h; extract discarded, residue extracted with EtOAc (8 L), rt, 6 h; extract concentrated and purified by column chromatography (silica gel; cyclohexane/EtOAc, 2:1), R_f 0.26; (ii) 15% aq NaOH (30 mL), MeOH (30 mL), 80 °C, 8 h.

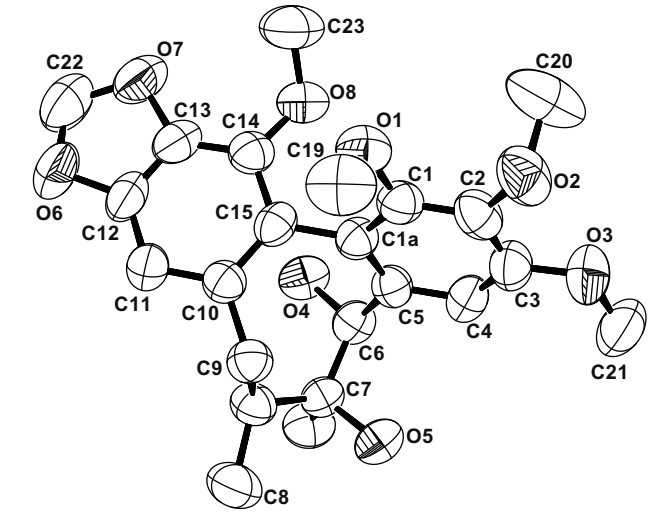
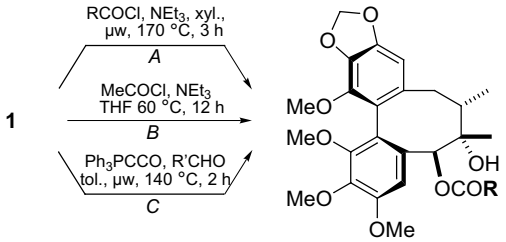


Fig. 2. Molecular structure of schisandrol A (**1**) as obtained from an X-ray single crystal structure analysis (ORTEP representation, 50% probability ellipsoids); hydrogen atoms are omitted (CCDC 675525).[18]



	R	method	yield[%]
2a	Ph	A	50
2d	PhCH=CH	A	50
2d	PhCH=CH	C	47
2e	Me	B	50
2f		C	64

Scheme 2. Syntheses of esters **2** of schisandrol A (**1**) by three different routes A–C.

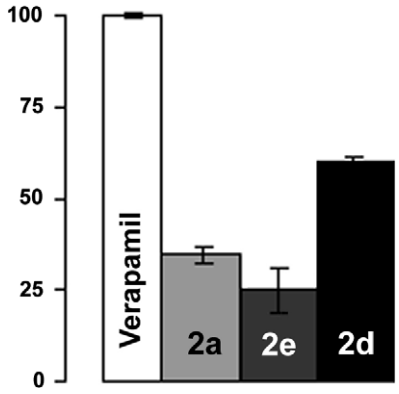


Fig. 3. Calcein-AM assays: % inhibition of the ABCB1 (P-gp) drug transporter in Kb-V1 cells by esters of schisandrol A at 50 μM relative to verapamil after 90 min exposure. Compound **2a**: 34.5 ± 2%, **2d**: 59.7 ± 2%, **2e**: 25.0 ± 6%.

can cleave the ester bonds, and so fluorescent calcein is not accumulated. Therefore, modulators of the P-gp function can easily be recognized and their effect be quantified by measuring the intracellular fluorescence.[24] In tests with multidrug-resistant human Kb-V1 cervix carcinoma cells, a subclone[25] of Kb-3-1 cells (DSM ACC 158) overexpressing exclusively P-gp type transporters, the natural benzoate displayed an inhibitory effect after 90 min of ca. 35% of that of the clinically used chemosensitizer verapamil which is in line with previous reports.[9] While the new acetate was less active (ca. 25%), the new cinnamoate reached even 60% of the verapamil activity (Fig. 3).[26] This is encouraging especially as the schisantherins are supposed to be only very weakly (cyto)toxic if at all.[27] P-gp transporters recognize most of the anticancer drugs nowadays indispensable in the clinic such as anthracyclines (doxorubicin, epirubicin), etoposide, mitomycin C, the taxanes (paclitaxel), and the vinca alkaloids (vincristine, vinblastine). A systematic study of a larger array of new unnatural esters of schisandrol A is now underway to optimize the pharmacological profile of this compound class, including activity against other types of cancer-relevant ABC-transporters.

Acknowledgements

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18. *Crystal data for* **1**: $C_{23}H_{28}O_8$, $M = 432.45$, hexagonal, space group $P6(1)$, $a = b = 11.4978(16)$, $c = 29.290(6)$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$, $V = 3353.4(10)$ Å^3, $Z = 6$, $\lambda = 0.71073$ Å, $\mu = 0.097$ mm^{-1}, $T = 293$ K; 24976 reflections, 4144 unique; final refinement to convergence on F^2 gave $R = 0.0353$ (F, 1479 obsd data only) and $R_w = 0.0772$ (F^2, all unique data), GOF = 0.549. CCDC 675525.
19. Schisandryl A cinnamoate (**2d**) by method A—typical procedure: A mixture of **1** (58 mg, 0.13 mmol), Et_3N (25 μL, 0.18 mmol), cinnamoyl chloride (27 mg, 0.16 mmol), and xylene (7 mL) in a sealed vial was placed in the cavity of a microwave appliance (CEM Discover™). The mixture was irradiated with 300 W power input for 3 h while being kept at a temperature of 170 °C. The volatiles were removed on a rotary evaporator, the remainder was taken up in ethyl acetate (10 mL), washed with dilute aqueous $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated. The crude product thus obtained was purified by column chromatography on Silica Gel 60 to give **2d** as faintly yellow crystals of mp 73–76 °C; R_f 0.50 (ethyl acetate/cyclohexane, 1:1, v/v); yield: 35 mg (50%). ν_{max} (KBr)/cm^{-1} 3573, 2926, 1692, 1621, 1597, 1497, 1462, 1449, 1267, 1103, 1046, 768; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14 (3H, d, J 7.2 Hz), 1.33 (3H, s), 1.50 (1H, s), 1.90–2.05 (1H, m), 2.18 (1H, dd, J 14.0, 3.0 Hz), 2.31 (1H, dd, J 14.0, 10.3 Hz), 3.55 (3H, s), 3.64 (3H, s), 3.86 (3H, s), 3.89 (3H, s), 5.23 (1H, d, J 0.5 Hz), 5.69 (1H, s), 5.70 (1 H, d, J 0.5 Hz), 5.95 (1H, d, J 16.0 Hz), 6.54 (1H, s), 6.76 (1H, s), 6.93 (1H, d, J 16.0 Hz), 7.35–7.42 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.8, 26.9, 28.0, 36.4, 42.5, 55.9, 59.0, 60.6, 60.8, 72.2, 84.1, 100.4, 102.4, 109.9, 117.5, 121.4, 122.2, 128.0, 128.3, 128.9, 130.3, 130.4, 134.3, 135.2, 140.3, 141.8, 148.5, 151.9, 152.2, 164.8; m/z (EI) 562 (M^+, 19%), 414 (100%), 371 (63%), 342 (65%), 312 (27%), 300 (43%), 285 (13%), 267 (10%), 251 (11%), 207 (14%), 147 (22%), 131 (30%), 103 (15%).
20. Schisandryl A acetate (**2e**) by method B: Compound **2e** (130 mg, 0.27 mmol, 50%) was obtained from **1** (237 mg, 0.55 mmol), Et_3N (92 μL, 0.66 mmol), acetyl chloride (47 μL, 0.66 mmol) and THF (20 mL) as a colourless viscous oil by stirring the mixture of starting materials at 60 °C for 12 h followed by a chromatographical purification on silica gel; R_f 0.46 (ethyl acetate/cyclohexane, 1:1, v/v). ν_{max} (KBr)/cm^{-1} 3590, 2915, 1715, 1620, 1495, 1380, 1270, 1040; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (3H, d, J 7.2 Hz), 1.26 (3H, s), 1.56 (1H, s), 1.57 (3H, s), 1.85–1.90 (1H, m), 2.13 (1H, dd, J 14.0, 3.0 Hz), 2.22 (1H, dd, J 14.0, 10.5 Hz), 3.53 (3H, s), 3.84 (3H, s), 3.86 (3H, s), 3.89 (3H, s), 5.54 (1H, s), 5.88 (1H, d, J 0.5 Hz), 5.91 (1H, d, J 0.5 Hz), 6.46 (1H, s), 7.24 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.7, 20.2, 27.9, 36.3, 42.1, 55.9, 59.2, 60.6, 60.8, 72.0, 84.2, 100.6, 102.7, 110.0, 119.4, 121.0, 130.5, 134.0, 135.3, 139.5, 140.5, 148.6, 151.8, 152.2, 169.4; m/z (EI) 474 (M^+, 74%), 414 (85%), 398 (7%), 371 (51%), 343 (100%), 312 (73%), 300 (53%), 281 (24%), 207 (30%).

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23. *Schisandryl A furan-2-propenoate (***2f***) by method C–typical procedure*: A mixture of **1** (30 mg, 0.06 mmol), furfural (5.8 μL, 0.06 mmol), Ph_3PCCO (42 mg, 0.14 mmol), benzoic acid (1 mg) and toluene (6 mL) in a sealed vial was irradiated in the microwave oven with 300 W power input for 2 × 1 h while being kept at a temperature of 140 °C. The solution was washed with 2 × 15 mL of 1 M aqueous $NaHCO_3$, dried and concentrated in vacuum. The crude product was purified by column chromatography on Silica Gel 60 to give **2f** as a colourless semicrystalline oil; R_f 0.30 (ethyl acetate/cyclohexane, 1:1, v/v); yield: 25 mg (64%). ν_{max} (ATR)/cm^{-1} 3529, 2976, 2938, 1837, 1691, 1635, 1597, 1474, 1423, 1409, 1267, 1109, 1048, 1016; ^1H NMR (300 MHz, $CDCl_3$): δ 1.15 (3H, d, *J* 7.1 Hz), 1.33 (3H, s), 1.45 (1H, s), 1.95–2.06 (1H, m), 2.19 (1H, dd, *J* 13.8, 3.0 Hz), 2.33 (1H, dd, *J* 13.8, 9.8 Hz), 3.58 (3H, s), 3.67 (3H, s), 3.89 (3H, s), 3.90 (3H, s), 5.56 (1H, d, *J* 1.6 Hz), 5.71 (1H, s), 5.81 (1H, d, *J* 1.6 Hz), 5.87 (1H, d, *J* 15.8 Hz), 6.48 (1H, dd, *J* 1.8, 3.4 Hz), 6.55 (1H, s), 6.60 (1H, d, *J* 3.0 Hz), 6.62 (1H, d, *J* 15.8 Hz), 6.76 (1H, s), 7.47 (1H, d, *J* 1.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.8, 28.0, 36.4, 42.4, 55.9, 59.1, 60.1, 60.9, 72.2, 84.0, 100.5, 102.5, 109.9, 112.3, 114.8, 115.2, 121.5, 122.2, 130.4, 130.6, 134.4, 135.2, 140.4, 141.8, 144.8, 148.6, 150.9, 151.9, 152.2, 164.8; *m*/*z* (EI) 552 (M^+, 15%), 414 (100%), 398 (9%), 371 (75%), 356 (11%), 343 (53%), 300 (45%), 281 (11%), 267 (5%), 207 (17%), 138 (10%), 121 (31%). Satisfactory microanalyses (C, 0.2; H, 0.1) were obtained for **2d**–**f**.
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26. The Calcein-AM efflux assay was carried out with slight modifications according to the cited literature.[24,25] Kb-V1 cells (DSM ACC 158) were incubated in 96-well plates with 50 μM of test compounds **2** (in DMF) at 37 °C for 10 min. Calcein-AM solution (1 mM in DMSO) was added to achieve a concentration of 0.25 μM. Plates were immediately placed in a TECAN fluorescence plate reader and calcein fluorescence was continuously monitored at 37 °C for 90 min at 485 nm excitation and 535 nm emission wavelength. Three wells on each plate contained the internal standard (positive control) verapamil (50 μM). From the time-dependent increase of cellular fluorescence the initial rate of fluorescence generation (IRF) was determined. Quantification of P-gp inhibition was done using the equation % inhibition = [IRF (test compound) − IRF(background)]/[IRF (verapamil) − IRF (background)] × 100, where IRF (verapamil) was the IRF in the presence of 50 μM verapamil and IRF (background) was the IRF in the absence of test compound.
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