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FIRST SYNTHESIS OF RACEMIC CONCENTRICOLIDE, AN ANTI-HIV-1 AGENT ISOLATED FROM THE FUNGUS *DALDINIA CONCENTRICA*

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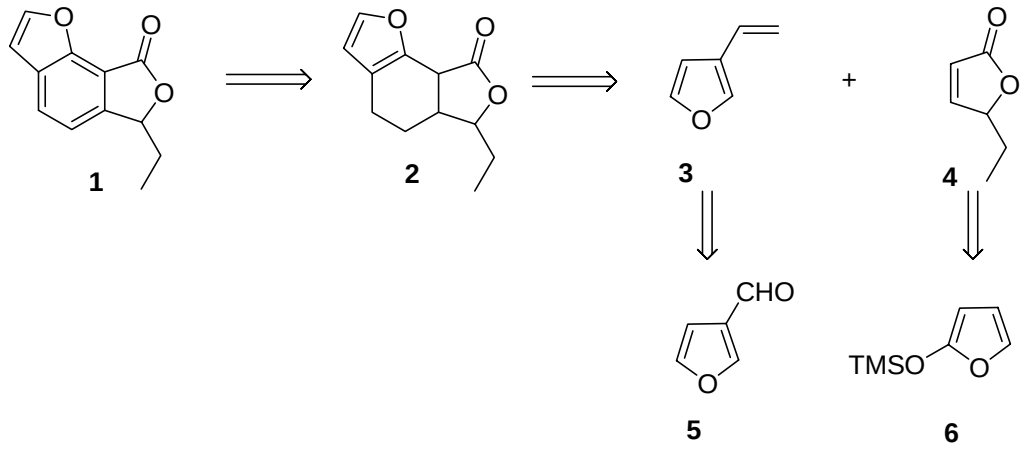
Abstract – Concentricolide, a novel compound with anti-HIV-1 activity and isolated from ascomycete *Daldinia concentrica*, has been synthesised for the first time as a racemate from furan-3-carbaldehyde and dihydrofuran-2(3*H*)-one via a Diels-Alder reaction. The identity of the synthetic sample was verified by comparison of its spectral data with those of an authentic sample.

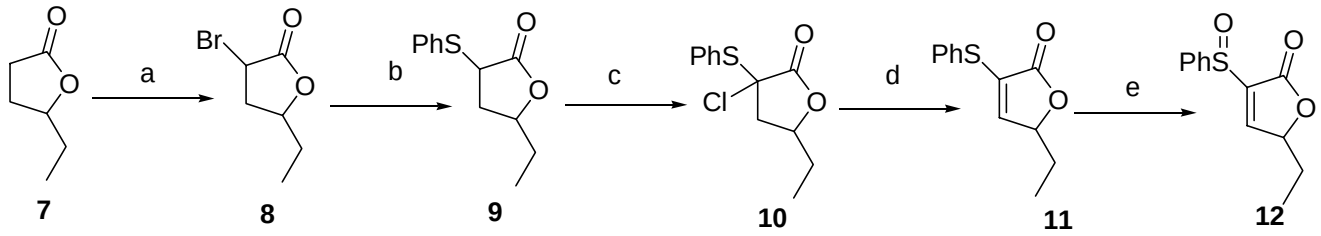
Concentricolide (**1**) is a new natural product isolated by us from ascomycete *Daldinia concentrica* in 2005, and found to exhibit significant anti-HIV-1 activity.[1] It inhibited HIV-1-induced cytopathic effects with an EC_{50} value of 0.31 μg/mL. The therapeutic index (TI) of this product was 247. Concentricolide is also effective in the blockage (EC_{50} 0.83 μg/mL) of syncytium formation between HIV-1 infected cells and normal cells. In the following investigation, several new compounds with this type of structure were isolated from the culture broth of *D. concentrica*.[2,3] Because these compounds might show promise as antiviral agents[3] and anti-AIDS agents, synthetic studies are needed. Herein, we describe the first synthesis of (±)-concentricolide (**1**).

Our synthetic strategy is presented in Scheme 1. (±)-Concentricolide (**1**) could be obtained via a Diels-Alder reaction with known precursors 3-vinylfuran (**3**) and 4-ethylbut-2-en-4-olide (**4**), which are derived from commercially available 3-carbaldehyde (**5**) and 2-(trimethylsiloxy)furan (**6**), respectively. 3-Vinylfuran (**3**) was obtained as a mixture via a Grignard reaction that occurred under refluxing with Mg turnings and $TMSCH_2Cl$ in dry Et_2O, under nitrogen. This was followed by vigorous stirring with aqueous 1.0 M HCl until all traces of **5** disappeared, as observed by TLC (AcOEt: hexane 1:5). This

mixture was kept at -20 °C, protected from light, and used directly in the Diels-Alder reaction. 2-Trimethylsiloxyfuran (**2**) was alkalated with iodoethane in the presence of a molar excess of silver trifluoroacetate to give the 4-ethylbut-2-en-4-olide (**4**) in 60% yield. Unfortunately, the reaction between synthons **3** and **4** did not give the anticipated Diels-Alder product. Instead, a complex mixture of compounds resulted despite repeated trials, and with added Lewis acid catalyst $BF_3.Et_2O$. This outcome indicated that the two synthons were mismatched, and the reactivity of synthon **4** with synthon **3** must be improved to give the Diels-Alder product. Addition of an α-sulfinyl group to the electron-withdrawing group of the dienophile can be used to increase reactivity in normal Diels-Alder reactions.[4] The desired adduct was formed by the addition of the dienophile to the furan 2,3-double bond 3-vinyl group diene system.[5] An added advantage of this activating group was observed: it readily underwent elimination after the cycloaddition, and introduced the double bond. We thus altered synthons **4** to **12** in this way, as shown in the synthetic pathway depicted in Scheme 2.

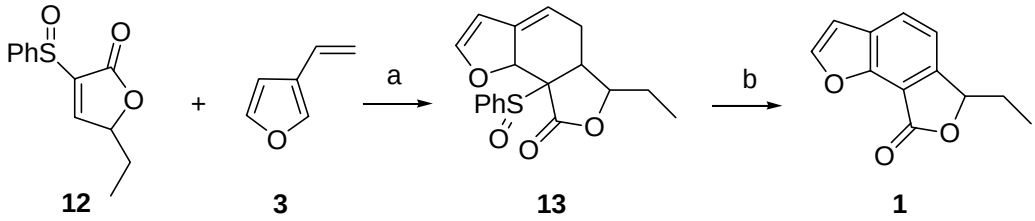


Scheme 1. Retrosynthetic Analysis of Concentricolide (**1**).



Scheme 2. Synthesis of **12**. *Reagents and conditions:* a) PBr_3/Br_2, 70-90 ºC (57%). b) PhSH, Et_3N, Et_2O (98%). c) NCS, CCl_4, reflux (82%). d) Li_2CO_3, LiBr, THF, reflux (35%). e) *m*-CPBA, DCM, 0 °C (82%).

Preparation of 5-ethyl-3-(phenylsulfinyl)furan-2(5*H*)-one (**12**) began with inexpensive dihydrofuran-2(3*H*)-one (**7**) using a modification of the method of outlined by Sweeney and Smith.[7,8] Bromination of dihydrofuran-2(3H)-one gave 3-bromo-5-ethyl-dihydrofuran-2(3*H*)-one (**8**), which underwent nucleophilic attack by thiophenol to offer 5-ethyl-3-(phenylthio)-dihydrofuran-2(3*H*)-one (**9**) in excellent yield. α-Chlorination of **9** with *N*-chlorosuccinimide (NCS) provided 3-chloro-5-ethyl-3-phenylthio-dihydrofuran-2(3*H*)-one (**10**) in 82% yield, and this compound underwent conjugative elimination of HCl upon reaction with a mixture of lithium bromide and lithium carbonate in refluxing THF to give 5-ethyl-3-(phenylthio)furan-2(5*H*)-one (**11**) in 35% yield. The corresponding sulphoxide was prepared via *m*-CPBA oxidation of **11** in 82% yield.



Scheme 3. Synthesis of (±)-concentricolide (**1**). *Reagents and conditions:* a) hydroquinone (0.1 equiv), rt, toluene, 72 h. b) $CaCO_3$, toluene, reflux, 19 h.

The Diels-Alder reaction was carried out in the presence of 10.67 mg of hydroquinone to prevent the formation of a complex mixture.[9] Treatment of a solution of **3** in dry toluene at reflux with three equivalents of **12** in for 72 h afforded **13**, which was immediately stirred with calcium carbonate in anhydrous toluene without isolation for 19 h at 90 ºC, providing the precursor by elimination of PhSOH. The precursor mixtures were swiftly converted into the concentricolide by oxidation, which occurred during purification by column chromatography, affording the target product **1**. In our experiment, only about 7.6 mg of concentricolide was obtained from the 91.0 mg (0.97 mmol) of **3** and 677.6 mg (2.87 mmol) of **12**. It was noted that the yield was low and the further improvements are the subject of ongoing research.

EXPERMENTAL

General Experimental Procedures

Optical rotation was measured using a Horiba SEPA-300 polarimeter. IR spectra were obtained on a Bruker Tensor 27 with KBr pellets. NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 spectrometers. EI-MS was recorded with a VG Autospec-3000 spectrometer and HR-ESI-MS was recorded with an API QSTAR Pulsar 1 spectrometer. Silica gel (200-300 mesh, Qingdao Marine

Chemical Inc., China) was used for column chromatography. Fractions were monitored by TLC and spots were visualised by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. All commercial agents were produced by Sigma-Aldrich.

*Synthesis of 3-Vinylfuran (**3**)*: Chlorotrimethylsilane (1.02 g, 8.28 mmol) was added dropwise to magnesium turnings (0.24 g, 9.9 mmol) in dry Et_2O (5 mL) under nitrogen, and the mixture was refluxed (45 °C) for 9 h, then cooled to 0 °C and treated with a solution of furan-3-carbaldehyde (0.69 g, 7.2 mmol) in dry Et_2O (7 mL). After stirring for 4 h at 0 °C and then 14 h at 23 °C, the reaction mixture was quenched with sat. aq. NH_4Cl (5 mL) at 0 °C. The layers were separated, the aqueous layer was extracted with Et_2O (3 × 5 mL), and the combined organic layers were washed with sat. aq. $NaHCO_3$ (2 × 5 mL), brine (10 mL), and dried over Na_2SO_4. Following solvent evaporation *in vacuo*, the residue was dissolved in Et_2O (5 mL) and stirred vigorously with 1.0 M HCl (3.4 mL) for 80 min. until it was not visible by TLC (AcOEt: hexane =1:5). The layers were separated, the aqueous layer was extracted with Et_2O (2 × 5 mL), and the combined organic layers were washed with sat. aq. $NaHCO_3$ (2 × 5 mL) and brine (5 mL), and dried over Na_2SO_4. The solution was carefully concentrated *in vacuo* to *ca.* 5 mL with no heating to give the product (676.9 mg, ca. 50%) as a mixture with TMSOH and Et_2O. This mixture was kept at -20 °C, protected from light and was used immediately. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.10 (br s, 1H), 7.04 (br s, 1H), 6.23 (br s, 2H), 5.13 (dd, 1H, *J* = 16 Hz, 7.5 Hz), 4.78 (dd, 1H, *J* =7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 142.78 (CH), 139.90 (CH), 126.07 (CH), 124.34 (C), 112.23 (CH_2), 106.50 (CH).

Synthesis of 3-bromo-5-ethyldihydrofuran-2(3H)-one (***8***): PBr_3 (1.44 g, 5.31 mmol) was combined with dihydrofuran-2(3*H*)-one (6.28 g, 55.09 mmol) in an ice bath, stirred for 20 min, treated with Br_2 (8.80 g, 55.09mmol), and stirred for an additional 30 min at rt. The mixture was heated at 90 ºC, and Br_2 (8.80 g, 55.00 mmol) was added. After stirring for 4 h, the mixture was poured into ice water (250 mL), and washed with sat. aq. $NaHCO_3$ until the red-brown colour disappeared. The layers were separated, the aqueous layer was extracted with Et_2O (3 × 20 mL), and the combined organic layers were washed with sat. aq. $NaHCO_3$ (2 × 5 mL), brine (5 mL), and dried over Na_2SO_4. The solution was concentrated *in vacuo*, and the crude product was purified by silica gel column chromatography using petroleum ether-AcOEt (20:1) to afford the product as an oil (6.06 g, 57%). IR ν_{max} (KBr) cm^{-1}: 2971, 2939, 1782, 1185, 965, 946, 669. FAB-MS *m/z* (%): 193 (3), 109 (20), 95 (35), 81 (51), 69 (69), 55 (100). HR-ESI-MS *m/z*: $C_6H_9O_2NaBr$, $[M+Na]^+$ (found: 214.9694, calcd.: 214.9683). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.69 (m, 1H), 4.46 (dd, 1H, *J* = 6.7 Hz, 1.5 Hz), 2.55 (m, 1H), 2.41 (m, 1H), 1.78 (m, 1H), 1.04 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.48 (C), 80.86 (CH), 39.01 (CH), 38.84 (CH_2), 27.15 (CH_2),

9.16 (CH_3).

*Synthesis of 5-ethyl-3-(phenylthio)dihydrofuran-2(3H)-one (**9**):* PhSH (3.93 g, 35.73 mmol) and Et_3N (3.61 g, 35.73 mmol) were added to a solution of 3-bromo-5-ethyldihydrofuran-2(3*H*)-one (6.86 g, 35.73 mmol) in dry THF (50 mL) at 0 ºC over 30 min. The mixture was heated to reflux and stirred for 10 h, then partitioned between water (100 mL) and Et_2O (90 mL). The layers were separated, and the aqueous phase was extracted with Et_2O (90 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 to give **9** as yellow oil (7.79g, 98%). IR ν_{max} (KBr) cm^{-1}: 2970, 2938, 1772, 1480, 1462, 1440, 1189, 948, 746, 692. FAB MS *m/z* (%): 223 (100), 177 (24), 149 (5), 113 (7), 57 (9). HR-ESI-MS *m/z*: $C_{12}H_{15}O_2S$, $[M+H]^+$, (found: 223.0790, calcd.: 223.0792). ^1H NMR (500 MHz, $CDCl_3$): δ 7.50 (m, 2H), 7.29 (m, 3H), 4.30 (m, 1H), 3.98-3.86 (m, 1H), 2.27 (m, 1H), 1.84-1.50 (m, 3H), 0.91 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.61 (C), 133.00 (CH), 131.72 (C), 128.94 (CH), 80.26 (CH), 44.90 (CH), 35.06 (CH_2), 27.96 (CH_2), 9.14 (CH_3).

*Synthesis of 3-chloro-5-ethyl-3-phenylthiodihydrofuran-2(3H)-one (**10**):* To a solution of 5-ethyl-3-(phenylthio)dihydrofuran-2(3*H*)-one (10.21 g, 45.99 mmol) in CCl_4 (90 mL) under nitrogen was added *N*-chlorosuccinimide (6.16 g, 46.14 mmol). After refluxing for 8 h, water (50 mL) was poured into the reaction mixture, the layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with sat. aq. $NaHCO_3$ (2 × 50 mL), brine (50 mL), and water (50 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give the crude product. The product was further purified by silica gel column chromatography using petroleum ether-AcOEt (20:1) to afford **10** as a golden oil (9.75 g, 82%). IR ν_{max} (KBr) cm^{-1}: 2972, 2938, 1775, 1440, 1350, 1185, 1024, 956, 751, 691. FAB-MS *m/z* (%): 257 (25), 221 (100), 95 (35), 175 (22), 69 (17), 57 (25). HR-ESI-MS *m/z*: $C_{12}H_{14}O_2SCl$, $[M+H]^+$ (found: 257.0405, calcd.: 214.0403) ^1H NMR (400 MHz, $CDCl_3$): δ 7.71-7.34 (m, 5H), 4.58 (m, 1H), 2.90-2.26 (m, 2H), 1.88-1.60 (m, 2H), 1.05-0.85 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.73 (C), 136.64 (CH), 143.84 (CH), 133.82 (CH), 132.85 (C), 129.10 (CH), 83.56 (CH), 46.34 (CH_2), 27.68 (CH_2), 9.20 (CH_3).

*Synthesis of 5-ethyl-3-(phenylthio)furan-2(5H)-one (**11**):* Lithium bromide (354.8 mg, 4.08 mmol) and lithium carbonate (1.50 g, 20.27 mmol) were dried for 3 h at 90 ºC under reduced pressure, and added to a solution of 3-chloro-5-ethyl-3-phenylthiodihydrofuran-2(3*H*)-one (5.23 g, 20.35 mmol) in anhydrous THF (40 mL). The mixture was heated to the point of reflux for 30 min until it became orange-brown in colour. The inorganic salts were filtered off by passage through Celite, and the organic solution was

washed with sat. aq. $NaHCO_3$ (50 mL), brine (50 mL), water (50 mL), and dried over Na_2SO_4. The solution was concentrated *in vacuo* and the residue was purified via silica gel column chromatography using petroleum ether-AcOEt (15:1) to afford **11** as a yellow oil (1.59 g, 35%). IR ν_{max} (KBr) cm^{-1}: 2972, 2938, 1775, 1440, 1350, 1185, 1024, 956, 751, 691. FAB-MS *m/z* (%): 221 (100), 203 (28), 175 (22), 175 (22), 111 (15), 97 (22), 83 (30), 69 (37), 55 (70). EI-MS *m/z* (%): 220 (76), 191 (29), 164 (62), 135 (100), 111 (18), 91 (33), 77(10). HR-ESI-MS *m/z*: $C_{12}H_{13}O_2S$, $[M+H]^+$ (found: 221.0644, calcd.: 221.0636). ^1H NMR (400 MHz, $CDCl_3$): δ 7.54 (m, 2H), 7.42 (m, 3H), 6.55 (d, 1H, *J* = 1.8 Hz), 1.83-1.61 (m, 2H), 0.95 (t, 3H, *J* = 7.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.55 (C), 144.26 (CH), 133.26 (CH), 131.91 (C), 129.47 (CH), 128.94 (CH), 83.30 (CH), 26.22 (CH_2), 8.54 (CH_3).

Synthesis of 5-ethyl-3-(phenylsulfinyl)furan-2(5H)-one ***(12)***: A solution of 3-chloro-5-ethyl-3-phenylthiodihydrofuran-2(3*H*)-one (0.698 g, 3.17 mmol) in CH_2Cl_2 (25 mL) was added to a solution of 86% *m*-chloroperoxybenzoic acid (*m*-CPBA) (643.6 mg, 3.17 mmol) in CH_2Cl_2 (9 mL), and the reaction mixture was stirred for 1 h at -10 ºC. Additional CH_2Cl_2 (25 mL) and 10% aqueous $NaHSO_3$ (15 mL) were added, and the layers were separated. The organic phase was washed with 10% aqueous $NaHCO_3$, 10% brine, and dried over Na_2SO_4. The solution was concentrated *in vacuo*, and the residue was purified via silica gel column chromatography using petroleum ether-AcOEt (15:1) to afford **12** as a yellow oil (614.6 mg, 82% yield). HR-ESI-MS *m/z*: $C_{12}H_{12}O_3NaS$, $[M+Na]^+$ (found: 259.0394, calcd.:, 259.0404). EI-MS *m/z* (%): 236 (15), 188 (15), 125 (96), 111 (60),103 (33), 97 (42), 77 (100), 65 (38), 57 (55); ^1H NMR (400 MHz, $CDCl_3$): δ 7.83 (m, 1H), 7.63 (m, 2H), 7.33 (m, 3H, *J* = 1.8 Hz), 4.99-4.80 (t, 1H, *J* = 6.1 Hz),1.75-1.45 (m, 2H), 0.83-0.66 (t, 3H, *J* = 7.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.87 (C), 154.46 (CH), 140.75 (C), 131.60 (CH), 128.91(CH), 83.20 (CH), 25.37 (CH_2), 8.11 (CH_3).

Synthesis of Concentricolide ***(1)***: 5-Ethyl-3-(phenylsulfinyl)furan-2(5*H*)-one (677.6 mg, 2.87 mmol) and hydroquinone (10.67 mg, 0.097 mmol) were added to a solution of 3-vinylfuran (91.0 mg, 0.97 mmol) in dry toluene (1.5 mL) and stirred for 72 h at rt. $CaCO_3$ (9.7 mg, 0.97 mmol) was added, and the reaction mixture was heated to 90 °C with stirring for 15 h. The solvent was evaporated *in vacuo*, and the residue was purified via silica gel column chromatography using petroleum ether-AcOEt (10:1) before the product was crystallised (7.6 mg, 3.8%). HR-ESI-MS *m/z*: 225.0522, $[M+Na]^+$, 225.0523 calcd. for $C_{12}H_{10}O_3Na$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.92 (dd, 1H, *J* = 8.0, 3.6 Hz), 7.80 (d, 1H, *J* = 2.1 Hz), 6.91 (d, 1H, *J* = 2.1 Hz), 5.58 (dd, 1H, *J* = 7.0 Hz, 4.1 Hz), 2.19 (m, 1H), 1.87 (m, 1H), 1.01 (t, 3H, *J* = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.14 (C), 146.58 (CH), 127.88 (C), 116.04 (CH), 106.64 (CH),

82.97 (CH), 27.89 (CH_2), 8.81 (CH_3). These data were in agreement with those reported for an authentic sample.[1]

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