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Reprint of: Antiproliferative activity of the *Antrodia camphorata* secondary metabolite 4,7-dimethoxy-5-methylbenzo[*d*][1,3]dioxole and analogues☆

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Dedicated to the memory of Professor Emilio Ghisalberti.

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ABSTRACT

Both the traditional Chinese medicinal fungus, *Antrodia camphorata*, and its secondary metabolite, 4,7-dimethoxy-5-methylbenzo[*d*][1,3]dioxole, have been reported to possess promising anticancer activity. In this work the natural product and analogues bearing more polar substituents were synthesised and assessed for antiproliferative activity in the NCI-60 screen. Although each compound inhibited the growth of some cell lines at 10 μM, none had sufficient activity to warrant further investigation.

1. Introduction

4,7-Dimethoxy-5-methylbenzo[*d*][1,3]dioxole (**1**) (Fig. 1) is a constituent of the fruiting body of the fungus *Antrodia camphorata*[1] (also known as *Antrodia cinnamomea*, *Taiwanofungus camphoratus, niu-chang-chih, and jang-jy*), which grows only on the stout camphor tree, *Cinnamomum kanehirae*, a native of Taiwan. *A. camphorata* is a rare and highly valued traditional Chinese medicine used for the treatment of food and drug intoxication, diarrhoea, abdominal pain, hypertension, itchy skin, liver diseases, and cancer [2–13]. As such, much interest has been shown in its secondary metabolites [14–41].

The benzodioxole **1** has been shown to possess anti-inflammatory activity, suppressing the fMLP[1]-induced generation of superoxide in human neutrophils, with a potency matching ibuprofen [18], and modestly inhibiting nitric oxide synthases [42], and other mediators of inflammation [43].

In 2008, a patent claiming that **1** inhibits the growth of tumour cells of breast cancer, liver cancer, and prostate cancer was filed [44,45]. Subsequently a number of studies have further investigated the anticancer potential of the natural product [42,46–48]. In this work we synthesised **1** and a small series of analogues and screened their antiproliferative activity against 60 human tumour cell lines.

2. Results and discussion

We recently reported a synthesis of **1** and its biaryl dimer **5**, which takes advantage of a double Baeyer–Villiger oxidation of chrysazin dimethyl ether (**2**) to rapidly assemble the 1,2,3,4-tetraoxygenated core in dioxocin **3** (Scheme 1) [40].

Given the reported promising anticancer activity of the natural product **1**[42,46–48], we became interested in whether the precursor, apiolaldehyde (**4**), shared its antiproliferative effects. In addition, the formyl group provided a convenient handle for the generation of a small series of analogues. In particular, we were interested in whether antiproliferative activity would be maintained in more polar/water soluble analogues that were expected to be more drug-like. The alcohol **9**, apiolaldehyde (**4**) and apiolic acid (**6**) (Scheme 2), are also likely metabolites of **1**, and may be relevant to pharmacological activity of the natural product *in vivo*.

The synthesis of the analogues of **1** is set out in Scheme 2. Pinnick oxidation [49] of apiolaldehyde (**4**), gave apiolic acid (**6**). The same conversion has previously been achieved with urea-hydrogen peroxide [50]. The acid **6** was converted to the primary amide **7**, by treatment of the corresponding acid chloride with aqueous ammonia. Synthesis of the nitrile **8** was achieved by direct oxidative amination [51] of apiolaldehyde (**4**); this transformation has also been achieved using ammonia and iodine [52]. Finally, the reduction of **4** with sodium borohydride gave primary alcohol **9**[53].

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[1] fMLP = *N*-formylmethionyl-leucyl-phenylalanine.

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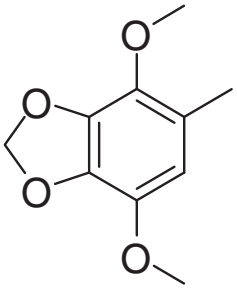


1

Fig. 1. Structure of the bioactive natural product 4,7-dimethoxy-5-methylbenzo[*d*][1,3]dioxole.

The natural product **1** and analogues **4**, **6–9** were assessed for antiproliferative activity in the US National Cancer Institute's Human Tumour Cell Lines Screen (NCI-60) [54] at 10 μM. Selected results are presented in Table 1 (see Supporting information for the complete dataset).

Unfortunately, neither the natural product nor any of the analogues showed sufficient activity against any of the 60 cell lines to warrant IC_{50} determinations, and any interpretation of structure–activity relationships based on testing at a single concentration is therefore tentative. Surprisingly, neither the natural product **1**, which has been reported to "profoundly decreased the growth of COLO-205 human colon cancer cell tumor xenografts in an athymic nude mouse model" [48], nor any of the analogues inhibited the growth of this cell line at 10 μM. The natural product **1** was most active against SR and UO-31 cell lines, which derive from leukemia and a renal tumour, respectively. Indeed all analogues modestly inhibited the growth of these cell lines, with the exception of the nitrile **8**, which actually slightly promoted the growth of UO-31 cells. In contrast, the nitrile **8** most potently inhibited the growth of HL-60(TB) (leukemia) and HOP-92 (non-small cell lung cancer) cell lines.

3. Conclusion

The benzodioxole natural product **1**, which has been reported to have anticancer potential, and a small series of more polar analogues, were synthesised and assessed for antiproliferative activity in the NCI-60 screen. None of the compounds showed any significant activity at 10 μM. Hence, although **1** should not necessarily be abandoned as inspiration for the discovery of anticancer agents, substantial improvements in potency of analogues will be required to identify a genuine lead. The possibility that **1** contributes synergistically to an anticancer effect of *Antrodia camphorata*, or that a metabolite of the natural product is responsible for its *in vivo* activity anticancer activity, cannot be ruled out at this stage.

4. Experimental

4.1. General details

4,7-Dimethoxybenzo[*d*][1,3]dioxole-5-carbaldehyde (*apiolaldehyde*) (**4**) and 4,7-dimethoxy-5-methylbenzo[*d*][1,3]dioxole (**1**) were synthesised as described previously [40]. Solvents were distilled before use. 'Petrol' refers to the hydrocarbon fraction distilling from 64 to 67 °C; DCM = dichloromethane. Anhydrous THF (tetrahydrofuran) was obtained from a Pure Solv 5-Mid Solvent Purification System (Innovative Technology Inc.). All other reagents and materials were purchased from commercial suppliers and used as received.

Reaction progress was monitored by analytical thin layer chromatography (TLC) using Merck aluminium-backed TLC_{F254} plates. Spots were visualised with a UV lamp (254 nm). Organic extracts were dried over anhydrous $MgSO_4$. Solvents were evaporated under reduced pressure at approximately 45 °C, and then traces of solvent were removed under a flow of nitrogen.

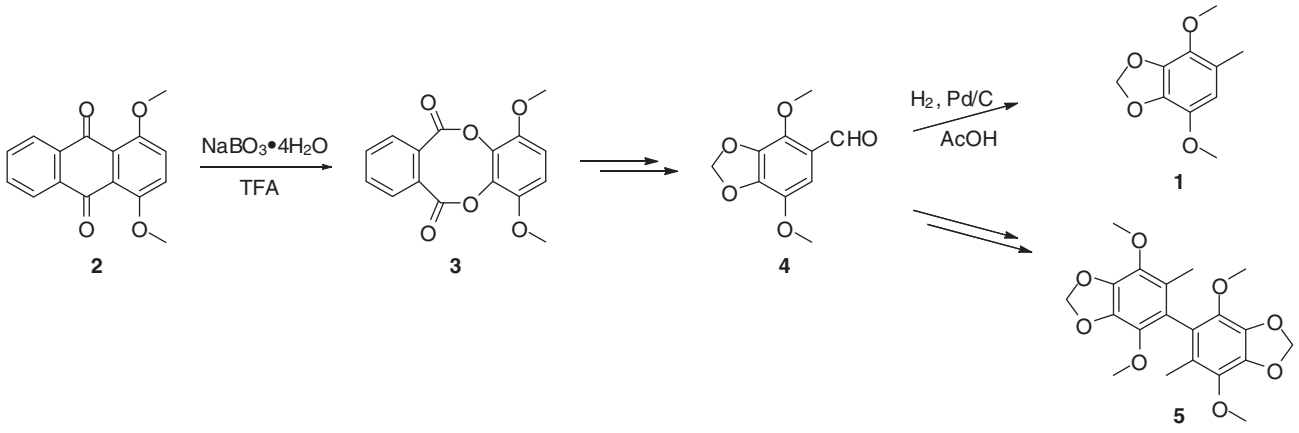
^1H and ^{13}C NMR spectra were obtained using a Varian Gemini-400 (400 MHz, ^1H, 100 MHz, ^{13}C), spectrometer. Deuterochloroform ($CDCl_3$) was used as the solvent, with residual $CHCl_3$ (^1H, δ = 7.26 ppm) or $CDCl_3$ (^{13}C, δ = 77.16 ppm) being used for calibration.

The infrared spectrum was recorded on a neat sample using a Perkin-Elmer Spectrum One FT-IR spectrometer with Attenuated Total Reflectance (ATR). Melting points were determined on a Reichert hot stage melting point apparatus.

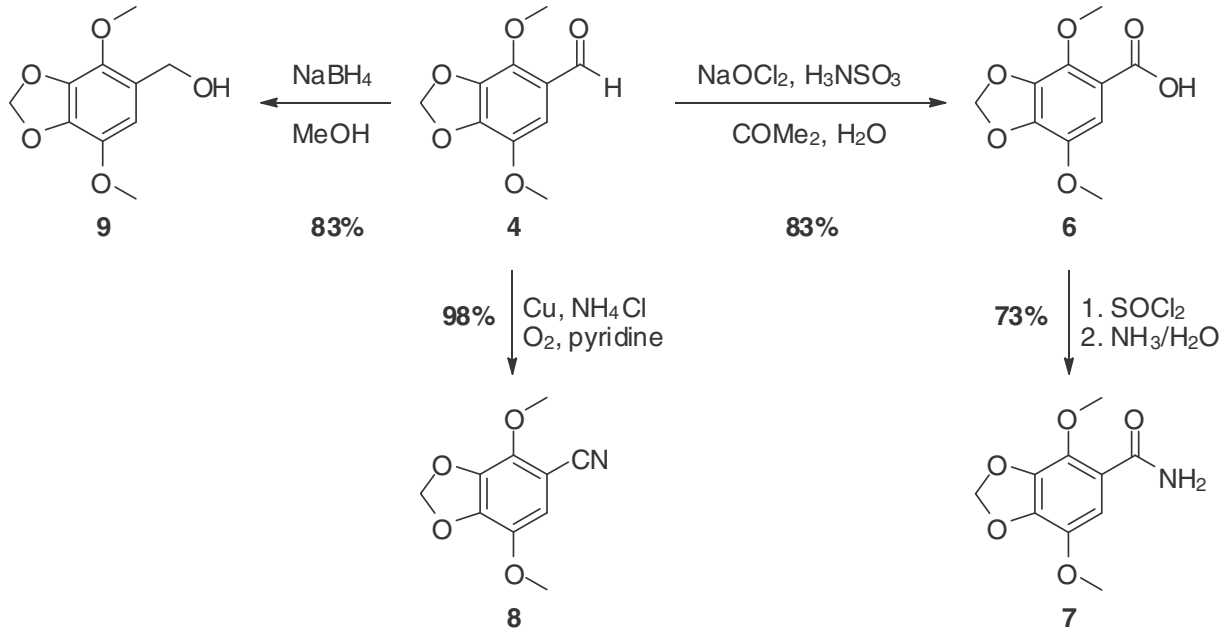
4.2. Synthesis

4.2.1. 4,7-Dimethoxybenzo[d][1,3]dioxole-5-carboxylic acid (apiolic acid) (6)

Sulfamic acid (0.39 g, 1.0 mmol) was added to a stirred solution of aldehyde **4** (0.10 g, 1.0 mmol) in acetone/water (3:1) (5 mL), followed by sodium chlorite (0.14 g, 1.5 mmol). After 2 h, the reaction mixture was diluted with DCM (100 mL) and the organic phase was washed with brine (3 × 100 mL). The aqueous phase was extracted with DCM (50 mL) and the combined organic phase was dried and evaporated to give the carboxylic acid **6** as yellow solid (0.19 g, 83%), which crystallized from DCM as off-white needles, mp = 176–178 °C [lit [55]. = 176 °C]. R_f = 0.2 (1:1 petrol/EtOAc); ^1H NMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H, ArH), 6.10 (s, 2H, OCH_2O), 4.14 (s, 3H, OCH_3), 3.88 (s, 3H, OCH3); ^{13}C NMR (100 MHz, $CDCl_3$): 165.3 (CHO), 141.7

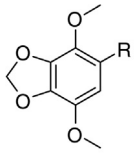


Scheme 1. Previous synthesis of natural products **1** and **5**[40].



Scheme 2. Synthesis of analogues of the natural product **1**.

Table 1
Percentage growth inhibition[a] of selected cell lines caused by **1** and analogues at 10 μM.



#	R	Cell line							
		HL-60(TB)	MOLT-4	SR	HOP-92	NCI-H522	COLO 205	UO-31	MDA-MB-231/ATCC
1	CH_3	2	5	20	n.t.[b]	–3	1	16	–2
4	CHO	–9	–6	20	n.t.	11	–6	21	–11
6	CO_2H	–14	–3	14	n.t.	20	–4	20	–5
7	$CONH_2$	–1	5	11	1	0	–2	6	14
8	CN	20	0	9	27	10	–11	–11	–17
9	CH_2OH	–12	24	21	n.t.	15	–3	21	–16

[a] Negative values indicate increases growth relative to the control.
[b] n.t. = not tested.

(ArO), 139.9 (ArO), 138.1 (ArO), 137.8 (ArO), 113.9 (Ar), 111.4 (ArH), 103.2 (OCH_2O), 61.5 (OCH_3), 56.8 (OCH_3).

4.2.2. 4,7-Dimethoxybenzo[d][1,3]dioxole-5-carboxamide (apiolamide) (7)

A mixture of thionyl chloride (0.5 mL, 5 mmol) and carboxylic acid **6** (0.22 g, 0.97 mmol) was stirred under reflux with moisture guard ($CaCl_2$) for 3 h. The excess thionyl chloride was evaporated and the residue was dissolved in anhydrous THF (1 mL) and cooled to 0 °C, then treated dropwise with conc. ammonia (0.4 mL). After stirring overnight, the reaction mixture was diluted with DCM (100 mL) and the solution was washed with water (2 × 100 mL), $NaHCO_3$ (2 × 100 mL) and brine (100 mL), dried and evaporated to give the amide **7** an off-white solid (0.16 g, 73%), which crystallized from EtOAc/DCM as yellow prisms, mp = 184–185 °C [lit [56]. (*i*-PrOH) = 202–203 °C]. R_f = 0.1 (1:1 petrol/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (s, 1H, NH), 7.45 (s, 1H, ArH), 6.07 (s, 2H, OCH_2O), 5.68 (s, 1H, NH), 4.03 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.6 ($CONH_2$), 140.1 (ArO), 139.6 (ArO), 138.8 (ArO), 137.6 (ArO), 117.8 (Ar), 110.2 (ArH), 102.7 (OCH_2O), 60.8 (OCH3), 56.7 (OCH3). NMR data have not previously been reported for this compound.

4.2.3. 4,7-Dimethoxybenzo[d][1,3]dioxole-5-carbonitrile (8)

A stirred mixture of aldehyde **4** (0.21 g, 1.0 mmol), copper powder (95 mg, 1.5 mmol), NH_4Cl (0.11 g, 2.0 mmol) and pyridine (5 mL) was heated at 100 °C under a balloon of O_2 for 24 h. The reaction mixture was allowed to cool, then diluted with 1 M HCl (100 mL) and extracted with Et_2O (5 × 50 mL). The extract was washed with brine (100 mL), dried and evaporated to give the nitrile **8** as yellow solid (0.20 g, 98%), which crystallized from petrol/DCM as yellow needles, mp = 134–137 °C [lit [52]. = 134–135 °C]. R_f = 0.6 (1:1 petrol/EtOAc); IR (ATR) cm^{-1}: 2221 (CN); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.70

(s, 1H, ArH), 6.10 (s, 2H, OCH_2O), 4.04 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.7 (ArO), 141.2 (ArO), 139.3 (ArO), 137.8 (ArO), 116.5 (Ar), 112.3 (ArH), 102.8 (OCH_2O), 97.4 (CN), 60.6 (OCH3), 57.1 (OCH3). The ^1H NMR spectrum matched the reported data [52].

4.2.4. (4,7-Dimethoxybenzo[d][1,3]dioxol-5-yl)methanol (9) [53]

Sodium borohydride (45 mg, 1.2 mmol) was added portion-wise to a stirred solution of aldehyde **4** (210 mg, 1.00 mmol) in MeOH (2.5 mL) at 0 °C. After 5 min the reaction was quenched with water (10 mL) and extracted with EtOAc (3 × 5 mL). The organic extract was washed with water (5 mL), brine (5 mL), dried and evaporated to give alcohol **9** as a colourless solid (176 mg, 83%), which crystallized from petrol/DCM as colourless needles, mp = 86–87 °C [lit [57]. = 85 °C]; R_f = 0.35 (1:1 petrol/EtOAc); ^1H NMR (400 MHz, $CDCl_3$): δ 6.48 (s, 1H, ArH) 5.95 (s, 2H, OCH_2O), 4.56 (s, 2H, CH_2OH) 3.94 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 2.28 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.8 (ArO), 138.0 (ArO), 136.5 (ArO), 136.2 (ArO), 126.1 (Ar), 107.7 (ArH), 101.7 (OCH_2O), 61.6 (CH_2OH), 60.1 (OCH_3), 56.0 (OCH_3).

4.3. NCI-60 screen

Details of the NCI-60 one-dose screening methodology can be found at https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm. NSC numbers and results against the entire 60 cell lines are provided in the Supplementary information.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2017.08.019.

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