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Antimitotic and Cell Growth Inhibitory Properties of Combretastatin A-4-like Ethers

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Abstract—A series of diarylamines, diaryl and arylbenzyl ethers based on combretastatin A-4 was prepared and evaluated for anticancer activity. 2-Methoxy-5-(3′,4′,5′-trimethoxyphenoxymethyl)phenol was the most active (IC_{50}, K562 20 nM) and caused significant G2/M cell cycle arrest. © 2000 Elsevier Science Ltd. All rights reserved.

Combretastatin A-4 (**1a**), isolated from the African bush willow, *Combretum caffrum*,[1] shows exciting potential as an anticancer agent binding strongly to tubulin at a site shared with, or close to, the colchicine binding site.[2] It inhibits cell growth at low concentrations (IC_{50}, P388 murine leukaemia cell line 2.6 nM) and shares many structural features common to other tubulin-binding agents[3] such as colchicine and podophylotoxin. The phosphate salt **2**,[4] which has better water solubility than **1a**, is soon to enter phase II clinical trials.[5] It is the ability of **1a** and **2** to damage tumour vasculature, thereby effectively starving tumours of nutrients, which makes them such exciting molecules.[6] It is now clear that inhibition and modulation of angiogenesis and vasculature function will have a significant impact on the clinical treatment of cancer.[7]

The spatial relationship between the two aromatic rings of combretastatin A-4, colchicine and similar drugs is an important structural feature that determines their ability to bind to tubulin.[8] We have already reported the synthesis and biological evaluation of chalcones similar to **1a**. Chalcone **3**, the most potent compound of the series, is highly cytotoxic and binds to tubulin at the colchicine-binding site.[9] It is clear that the X group in the combretastatin A-4-like compounds **1**–**j** is important in determining their biological activity (Table 1).[10]

Evidently lacking from this series were the ether and amine analogues of **1a** where X = O or NH and OCH_2. We now report both the synthesis and biological activity of such a series of compounds.

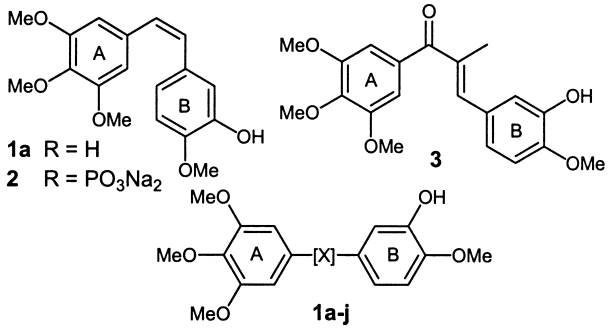

Table 1. Cell growth inhibitory properties of diaryl containing compounds **1a**–**j**

1	X	IC_{50}/μM	1	X	IC_{50}/μM
a	*Z*-CH=CH-[11]	0.0026[a]	**e**	(*S*,*S*)-C(OH)HC(OH)H-[12]	2[a]
b	*E*-CH=CH-[13]	0.16[a]	**f**	(*R*,*R*)-C(OH)HC(OH)H-[12]	19[a]
c	-C≡C-[14]	21[b]	**g**	-(*RS*)-C(OH)HCH$_2$-[15]	0.033[a]
d	-CH$_2$CH$_2$-[13]	0.11[a]	**h**	-(*RS*)-CH$_2$CH(OH)-[12]	33[a]
e	-CO-[16]	0.01[a]	**j**	—[d,17]	>50[c]

[a]P388 murine leukemia cell line.
[b]K562 human chronic myelogenous leukaemia cell line.
[c]L1210 murine leukemia cell line.
[d]The aryl rings are bonded directly to each other.

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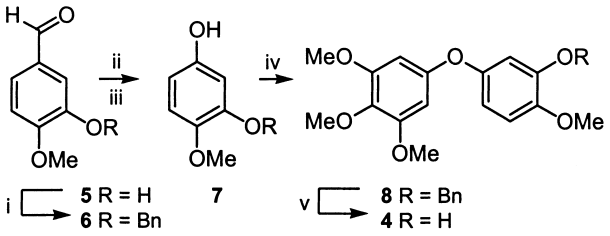
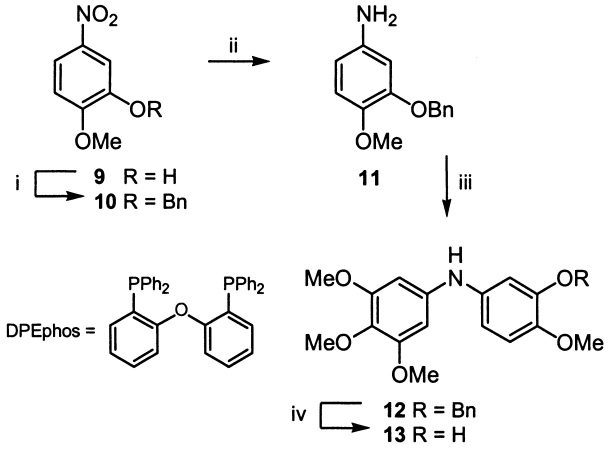
The target ether **4** was prepared via the new copper-catalyzed Ullmann-type coupling recently described by Buchwald and co-workers (Scheme 1).[18] This involved the treatment of benzyl-protected phenol **7** with 3,4,5-trimethoxybromobenzene. We found that the yield of the ether **8** was improved if xylene was used as the reaction solvent. The phenol **7** was prepared by protection of isovanillin (**5** → **6**), followed by sequential Baeyer–Villiger oxidation (MCPBA) and ester methanolysis. Deprotection of the benzyl ether **8**, via palladium-catalyzed hydrogenolysis, proceeded in excellent yield to reveal the required diaryl ether **4**.



Scheme 1. Reagents and conditions: (i) $PhCH_2Cl$, K_2CO_3, DMF, rt 18 h. 82%; (ii) MCPBA, CH_2Cl_2, rt, 2 h, 84%; (iii) NaOMe, MeOH, rt, 30 min, 79%; (iv) 3,4,5-$(MeO)_3C_6H_2Br$, Cu, Ce_2CO_3, xylene, 140 °C, 4 days, 52%; (v) Pd/C (10%), H_2, CH_2Cl_2, 94%.

The combretastatin A-4 diaryl aniline analogue **13** was prepared in a similar manner via Pd(II)-catalyzed coupling of 3,4,5-trimethoxybromobenzene and the benzyl-protected aniline **11** using another of the Buchwald protocols (Scheme 2).[19]



Scheme 2. Reagents and conditions: (i) K_2CO_3, $PhCH_2Br$, DMF, rt, 18 h, 98%; (ii) $SnCl_2{\cdot}2H_2O$, EtOH:EtOAc 1:1, 70 °C, 3 h, 88%; (iii) 3,4,5-$(MeO)_3C_6H_2Br$, NaO-*tert*-Bu, $Pd(OAc)_2$ (8 mol%), DPEphos (12 mol%), toluene, 80 °C, 18 h, 56%; (iv) Pd/C (10%, cat.), EtOAc, H_2 (1 atm), rt, 5 days, 80%.

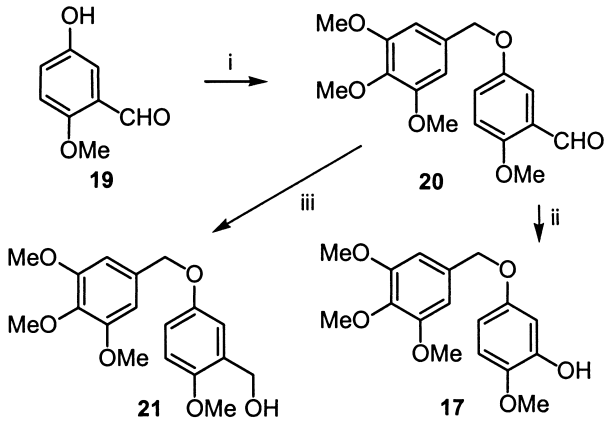
The aniline **11** was prepared from the commercially available nitrophenol **9** by sequential benzylation and reduction with tin(II) chloride. The coupling of the trimethoxybromobenzene and aniline **11** proceeded smoothly in the presence of the bisphosphine DPEphos and palladium(II) acetate. Deprotection of the benzyl group within the aniline **12** gave the required combretastatin analogue **13**.

In a similar fashion we prepared two other diaryl ethers **14** and **15** and the diarylamine **16**, from the commercially available phenols or aniline and aryl halides via the Buchwald protocols. The yields of these coupling reactions are reported in Table 2.

Table 2. Synthesis of diaryl ethers and anilines Ar^1XAr^2

	Ar^1	Ar^2	X	Yield (%)
14	3,4,5-$(MeO)_3C_6H_2$	4-$MeOC_6H_4$	O	84
15	3,4-$Me_2C_6H_4$	4-$MeOC_6H_4$	O	58
16	3,4,5-$(MeO)_3C_6H_2$	4-$MeOC_6H_4$	NH	60

We also prepared the two benzyl ethers **17** and **18**, which have the two aryl groups separated by two atoms, thereby mimicking more closely the arrangement present in combretastatin A-4 (Schemes 3 and 4). Firstly benzyl ether **20** was prepared from the phenol **19**[20] and 3,4,5-trimethoxybenzyl bromide, itself made from commercially available 3,4,5-trimethoxybenzyl alcohol.

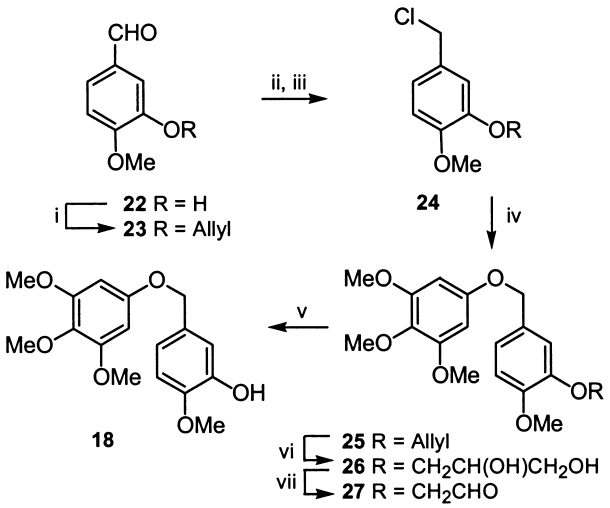


Scheme 3. Reagents and conditions: (i) 3,4,5-$(MeO)_3C_6H_2CH_2Br$, K_2CO_3, DMF, rt 18 h, 41%; (ii) H_2O_2, H_2SO_4 (cat.), MeOH, rt, 2 days, 59%; (iii) $NaBH_4$, EtOH, rt, 30 min, 94%.

We chose to use a formyl group as a latent hydroxyl group since a strategy incorporating a benzyl protecting group, as in the synthesis of **4**, would clearly present selectivity problems. Acid-catalyzed oxidation of the aldehyde **20** with hydrogen peroxide, a protocol developed by Matsumoto and co-workers,[21] directly gave the phenol **17**. This reaction was superior to the MCPBA promoted Baeyer–Villiger reaction, as separation from by-products was much more convenient. Reduction of the aldehyde **20** provided another ether analogue, the alcohol **21**.

The regioisomeric benzyl ether **18** was prepared, via the Williamson ether synthesis (Scheme 4), from commercially available 3,4,5-trimethoxyphenol and the substituted benzyl chloride **24** (prepared from allyl-protected isovanillin **23** by sequential treatment with sodium borohydride and thionyl chloride). Deprotection of the allyl group in **25** with Wilkinson's catalyst

and DABCO™, using conditions developed by Corey and Suggs,[22] gave the required benzyl ether **18**. Dihydroxylation of the allyl ether **25** gave the diol **26**, which is effectively a glyceryl analogue of *Z*-**1**, which we hoped would possess improved water solubility. Oxidative cleavage of this diol using sodium periodate gave the aldehyde-containing analogue **27**.



Scheme 4. Reagents and conditions: (i) $CH_2{=}CHCH_2Br$, K_2CO_3, DMF, rt 18 h, 95%; (ii) $NaBH_4$, EtOH, rt, 30 min, 99%; (iii) $SOCl_2$, rt, 120 min, 91%; (iv) 3,4,5-$(MeO)_3C_6H_2OH$, NaH, DMF, rt, 18 h, 99%; (v) $(PPh_3)_3RhCl$ (5 mol%), DABCO™ (1 eq), EtOH, reflux, 18 h, 59%; (vi) potassium osmate (0.2 mol%), NMNO (1.1 eq), aq acetone, rt, 18 h, 86%; (vii) $NaIO_4$, Et_2O:H_2O 2:1, rt, 18 h, 84%.

The cell growth inhibitory properties of the combretastatin A-4 analogues (summarized in Table 3) were determined in the K562 human chronic myelogenous leukaemia cell line using the MTT assay as detailed by Edmondson et al.[23] We have reported the full details of this procedure elsewhere.[24] The IC_{50} value represents the concentration which results in a 50% decrease in cell growth after 5 days incubation.

The ether **4** is remarkably active; the poorer activity of both **14** and **8** shows that the B-ring 3′ hydroxyl group is beneficial. Indeed it is gratifying to see that the combretastatin A-4 substitution pattern produces the most active diaryl ether. This is also the case for the diaryl amines; **13** is more active than **12** and **16**. However the amines are significantly less active than their ether counterparts (cf. **4** and **13**; **14** and **16**). The arylbenzyl ethers **17** and **18** are both significantly active. The potent activity of **18** is clearly noteworthy. Again most of the analogues lacking the B-ring 3′ hydroxyl group (**25**–**27**) are much less active. The cytotoxicity of the aldehyde **20** is relatively high and comparable to that of the phenol **17**. Reduction of the aldehyde (**20**→**21**) results in a dramatic loss of activity.

The effects of the most active compounds upon the cell cycle were measured by flow cytometry. The results in Table 4 show that all the compounds assessed caused significant arrest of the cell cycle at the G_2/M point,

Table 3. Cell growth inhibition[a] against the K562 cell line

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
18	0.02	**16**	3
4	0.05	**12**	15
17	0.12	**15**	24
20	0.17	**25**	30
14	0.38	**8**	51
27	2.5	**21**	60
13	2.6	**26**	90

[a]As measured by the MTT assay after 5 days incubation of the drug with the cells cultured at 37 °C.

relative to the untreated control, consistent with the behaviour of tubulin-binding agents. Strangely, the most potently antimitotic compound was the aniline **13**, even though it is moderately cytotoxic. Further experiments to determine the origin of its antimitotic effect reveal that it is probably not binding to tubulin. It neither prevents the assembly of microtubules nor binds to the colchicine binding site (Table 5).

Table 4. Effects upon the cell cycle[a]

Compound	G_0–G_1	*S*-phase	G_2/M
Control	25.3	47.2	27.5
4	4.3	22.3	73.4
13	3.4	9.4	87.2
17	3.8	17.9	78.3
18	3.5	14.0	82.5
20	9.1	33.1	57.8

[a]As measured by flow cytometry (according to the methods described in ref 25).

Table 5. Effects upon tubulin binding

	Tubulin assembly[a] IC_{50} (μM)	Colchicine displacement[b] IC_{50} (μM)
4	16.3	36
13	>100	298
17	22.5	226
18	6.6	36
20	0.6	4

[a]Assembly of tubulin was carried out as previously described (ref 26). The IC_{50} values shown represent the concentrations which cause a 50% decrease in tubulin assembly as measured by an increase in turbidity at 350 nm.
[b]Competition for the colchicine binding site was carried out as previously described (ref 26). The IC_{50} values shown represent the concentrations which cause a 50% decrease in the binding of ^{3}H-colchicine from purified porcine tubulin.

All of the other compounds inhibit the polymerization of tubulin. The most active compound in the tubulin assays, the aldehyde **20**, binds exceptionally strongly to the colchicine binding site. Its ability to prevent the polymerization of tubulin is impressive. Indeed, the activity is clearly comparable with that of combretastatin A-4 (**1a**) itself (IC_{50} 0.5, 2 μM).[27,28] However since **20** is much less cytotoxic than CA-4 it may have improved in vivo properties. These studies are currently underway. It is nevertheless tempting at this stage to speculate that the binding of **20** to tubulin involves

covalent reaction of the aldehyde group, by the formation of a Schiff's base. The poorer cellular activity compared to that of **4** and **18** may be due to unspecific reaction with non-tubulin amine residues and/or biodegradation of the aldehyde functionality.

In conclusion we have shown that the ether analogues of CA-4 share its impressive activity. The in vivo assessment of these agents is ongoing. The results of these studies will be reported in due course.

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