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Novel cyanocombretastatins as potent tubulin polymerisation inhibitors

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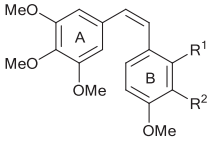
ABSTRACT

A series of novel cyanocombretastatins bearing a 3,4,5-trimethoxyphenyl moiety combined with a variety of substituted phenyl rings, were synthesised and their antitumour activity was evaluated. The *Z*-cyanocombretastatins were synthesised in a one-step protocol in high purity and yield. Fluoro, bromo, iodo, and derivatives with boronic acid and an ethyne function at meta position of the B ring were synthesised. In vitro MTT bioassays against human chronic myelogenous leukaemia (K562) and transfected breast adenocarcinoma (MDA NQO1) cell lines, revealed promising IC_{50} inhibitory values in nanomolar range (<50 nM). Introduction of a nitrile function on the olefinic bond not only increased the cytotoxicity of the less active *Z*-isomers but rendered the analogues as moderate to potent inhibitors of tubulin polymerisation comparable to that of CA-4 (IC_{50} = 2.2 μM).

There is a continuing effort in cancer research to discover and develop new drugs that are capable of rapidly destroying tumour vasculature leading to tumour necrosis and antitumour efficacy. Such vasculature disrupting agents are promising anticancer drugs.[1,2] The combretastatins are a class of *cis*-stilbinoid natural products isolated from the genus *Combretum* that comprises numerous trees and shrubs. Whilst being structurally simple these materials are exceptionally potent inhibitors of cell division.[3] One of the most prominent lead compound is Combretastatin A-4 **1**. Stilbene **1** was first isolated from the African Willow Tree, *Combretum caffrum*, by Pettit et al.[4] Stilbene **1** has been found to be a potent inhibitor of tubulin polymerisation and a cytotoxic agent against a wide variety of human cancer cell lines, including multi-drug resistant cell lines.[5] As a result of its capacity to dock in the colchicine binding site of tubulin, it displays a potent antimitotic property that has led into its development as an anti-vascular agent.[6]

Reversible high affinity binding to the colchicine site of tubulin appears to be the major mechanism of action of stilbene **1**. Poor aqueous solubility, bioavailability, short biological half life, isomerisation of the olefinic bond and a number of detrimental adverse side effects are amongst the disadvantages of CA-4 as an efficient therapeutic agent.[7] Acute coronary and other thrombophlebitic syndromes, alterations in blood pressure, heart rate, and ventricular conduction, as well as tumour pain have raised a question regarding the safety aspect of vascular disrupting agents in anticancer therapy.[8] To improve the therapeutic index of **1** as a potential antimitotic drug, several hundred analogues have been synthesised that retain the biological activity of the parent molecule but increase solubility and improve other pharmacokinetic properties. The water-soluble prodrugs, combretastatin A-4 phosphate (CA-4P **2**), combretastatin A-1 diphosphate (CA-1P **4**), AC-7739 **5**, and AVE8062 **6** are such promising candidates for the treatment of cancer (Fig. 1).[9] Recent clinical trials demonstrated that the phosphate prodrug **2** prolonged the survival of patients with advanced anaplastic thyroid cancer without any adverse side effects, but failed to reduce the growth of tumour in murine CT-26 adenocarcinoma colon cancer model.[10] However, neither of candidates has entered into clinical use and there remains a need to design and develop new vascular disrupting agents to treat solid tumours.[11]



1 (CA-4)	R^1 = H, R^2 = OH
2 (CA-4P)	R^1 = H, R^2 = O-$PO(ONa)_2$
3 (CA-1)	R^1 = R^2 = OH
4 (CA-1P)	R^1 = R^2 = O-$PO(ONa)_2$ Oxi4503
5 AC-7739	R^1 = H, R^2 = $NH_3^+Cl^-$
6 AVE-8062	R^1 = H, R^2 = NH-Ser.HCl salt (Ombrabulin)

Figure 1. Structures of naturally-occurring CA-4 **1** & CA-1 **3**, their corresponding phosphate prodrugs 2 and 4, along with other candidates (5 and 6) currently under clinical investigation.

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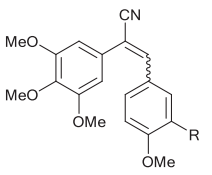


Compound	R	Configuration
11a	F	*E*
14a	F	*Z*
15a	Br	*Z*
18	I	*Z*
20	C≡C-H	*Z*
24a	$B(OH)_2$	*Z*

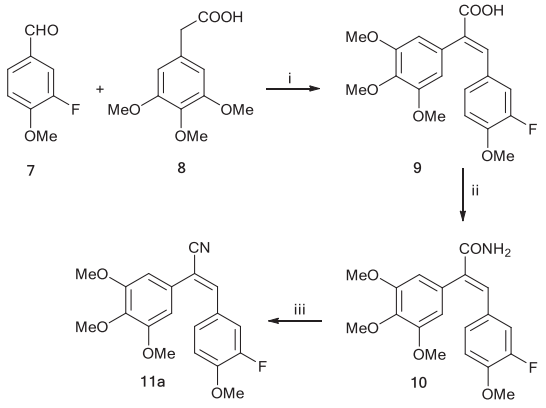
Figure 2. *E* and *Z*-cyanocombretastatins.

The SAR studies[12–14] of **1** revealed that 3′,4′,5′-trimethoxy A ring and *cis*-configuration of the olefinic bond is essential for biological activity. The key structural factor is the presence of an ethylene bridge connecting the two rings which holds them at an appropriate distance and maintains the correct dihedral angle to maximise the interactions with protein tubulin. McGown and Fox[15] showed that in order to obtain a compound capable of binding to colchicine site on tubulin the two planar rings should be tilted at 50–60° to each other.

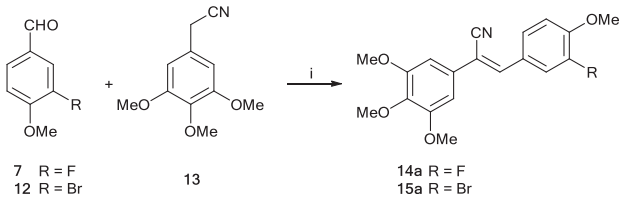
The work herein stated is based on the paper published by Ohsumi et al.[12] which was the first group to report the synthesis of nitrile bearing CA-4 analogues. As a part of our ongoing research towards characterising novel antitumour agents, we have synthesised a series of nitrile bearing *E* and *Z*-combretastatins as shown in Figure 2. Introduction of a heteroatom via a moderately polar nitrile function is believed to have a net effect on polarity of the analogues and increase their water solubility in return.

Owing to the Lewis acidity of boronic acids and the hydrogen bond donating capability of their hydroxyl groups, it was thought that its insertion into CA-4 analogues would bring about a change in biological activity. It is predictable that boron may interact with tubulin not only through hydrogen bonding but also by forming covalent bonds, and this interaction would have an impact on the kinetics of ligand–protein binding.[11] Therefore, it was decided to synthesise an analogue containing a boronic acid at *meta* position of the B ring **24a**. Combretastatins possessing an ethyne unit have shown potential as tubulin-disrupting agents and stilbene **20** was prepared in order to test its biochemical efficacy.[16]

Cinammic acid **9** was synthesised by the published procedure from 3,4,5-trimethoxyphenylacetic acid **8**, and 3-fluoro-4-methoxybenzaldehyde **7** (Scheme 1).[17] The cinnamic acid **9** was converted to the amide **10** using EDCI/HOBt and ammonium hydroxide which was dehydrated to the nitrile **11a** in good yield. The aldehydes used in this study are either purchased from commercial sources or synthesised as shown in Schemes 3–5. The *Z*-isomers of the cyanocombretastatin were synthesised by base-catalysed Perkin condensation of 3,4,5-trimethoxyphenylacetonitrile



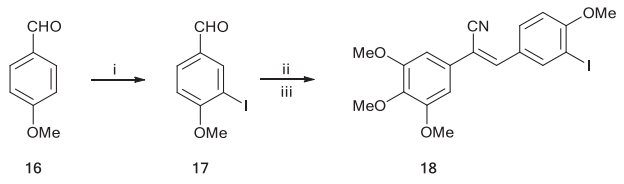
Scheme 1. Synthesis of cyanocombretastatin **11a**. Reagents and conditions: (i) $(CH_3CO)_2O$, TEA, HCl 100 °C, 70%; (ii) EDCI, HOBt, *N*,*N*-diisopropylethylamine, NH_4OH/THF 35 °C, 55%; (iii) $SOCl_2$/Pyridine RT, 67%.



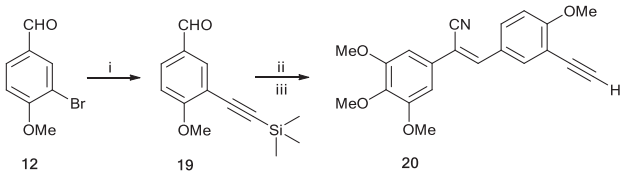
Scheme 2. Synthesis of analogues 14a and 15a. Reagents and conditions: (i) NaOMe/MeOH 45–65 °C.

13 and substituted aldehydes (Scheme 2). Similarly stilbenes **18**, **20**, and **24a** were prepared in good yield (Schemes 3–5). The synthesis of the alkyne **20** was achieved by condensation of benzaldehyde **19** and phenylacetonitrile **13** as well as concomitant desilylation (Scheme 4). Based on the previous findings[5,18–21] it was decided to synthesise an analogue bearing a boronic acid functionality at the *meta* position of the ring B of the parent structure **24a**.

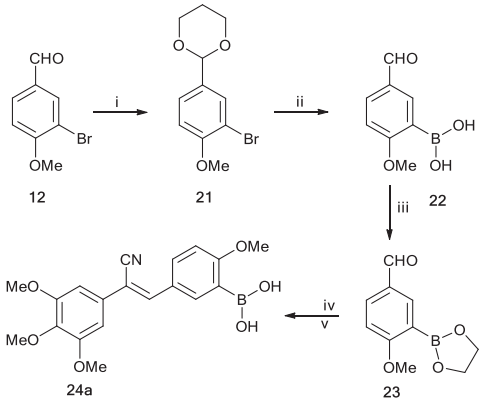
Acetal-protection of aldehyde **12** was achieved using triethyl orthoformate, tetrabutylammonium tribromide, and 1,3-propanediol.[22] The aldehyde-functionalised boronic acid **22** was synthesised using *n*BuLi/trimethyl borate in high yield (86%) (Scheme 5).



Scheme 3. Synthesis of analogue 18. Reagents and conditions: (i) I_2, CF_3COOAg RT, 53%; (ii) 3,4,5-trimethoxyphenylacetonitrile; (iii) NaOMe/MeOH 45 °C, 93%.

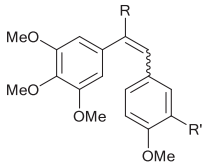


Scheme 4. Synthesis of analogue 20. Reagents and conditions: (i) ethynyltrimethylsilane, TPP, TEA, $Pd(OAc)_2$ 85-90 °C, 79%; (ii) 3,4,5-trimethoxyphenylacetonitrile; (iii) NaOMe/MeOH 55 °C, 50%.



Scheme 5. Synthesis of analogue 24a. Reagents and conditions: (i) triethyl orthoformate, TBATB/propanediol RT, 98%; (ii) $(CH_3O)_3B$, *n*-BuLi/THF −78 °C, HCl, 86%; (iii) ethylene glycol, $MgSO_4$ RT, 75%; (iv) 3,4,5-trimethoxyphenylacetonitrile; (iv) NaOMe/MeOH 50 °C, 66%.

Table 1
^{13}C GD NMR experiment



Compound	R	R′	*J* values (Hz)*	Configuration
7	COOH	F	6.6	*E*
8	$CONH_2$	F	6.9	*E*
9a	CN	F	9.4	*E*
12a	CN	F	14.4	*Z*
13a	CN	Br	14.3	*Z*
16	CN	I	13.7	*Z*
18	CN	C≡C–H	14.9	*Z*
22a	CN	$B(OH)_2$	18.2	*Z*

Table 2
Antiproliferative activity, and tubulin polymerisation inhibition

Compound	IC_{50} ± SD nM[a]		IC_{50} ± SD μM*
	K562	MDA NQO1	Tubulin assembly assay
1	2.3 (±1.6)	3.1 (±1.8)	2.2 (±1.2)
11a	6.7 (±2.8)	4.1 (±1.5)	4.2 (±0.5)
14a	18.7 (±10.5)	20.3 (±11.8)	18.5 (±0.7)
15a	49.5 (±4.8)	70.6 (±9.6)	35.6 (±1.6)
18	42.6 (±7.2)	85.3 (±10.2)	43.8 (±2.3)
20	30.2 (±6.3)	46.5 (±7.7)	25.3 (±2.8)
24a	20.4 (±12.6)	35.7 (±13.2)	17.4 (±1.1)

[a] Values are means of at least three experiments; standard deviation is given in parentheses.

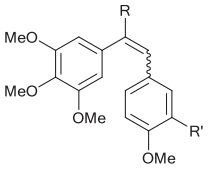
Following a similar method[23] the boronic acid was then converted into the dioxaborolane **23**. Subsequent reaction with phenylacetonitrile **13** yielded cyanocombretastatin **24a** (Scheme 5). The stereochemistry of the analogues was elucidated using ^{13}C Gated Decoupling NMR. Presence of a doublet at about δ 118 ppm showed the coupling between the olefinic proton and the nitrile carbon. In the *E*-configuration we observed a lower *J* value (typically 6–9 Hz) compared to the *Z*-configuration (>12 Hz) (Table 1).

All the analogues were tested for their ability to inhibit the proliferation of MDA NQO1[24] and K562[25] cancer cell lines. Many of the analogues exhibited excellent antiproliferative activity (Table 2). The cell lines were mycoplasma-free and proliferation tests were carried out using the MTT bioassay described previously.[26] The IC_{50} value was calculated by reference to a standard curve constructed for control cells. The isolation of the tubulin and the assembly assay were carried out with minor modifications to the methods described previously.[14,15,27] The results enabled the calculation of the drug dose required to inhibit the assembly of tubulin by 50% (IC_{50} value), determined by graphical means as percentage of the control assembly (Table 2).

The antiproliferative activity of compound **11a** rivals that of CA-4 itself. Selected analogues that exhibited significant antiproliferative activities were also tested for their ability to inhibit the polymerisation of tubulin. The *Z*-isomers of the combretastatin are more cytotoxic than their *E*-counterparts by about a factor of 1000. In these instances the *Z*-isomers are where the aryl rings are *cis*. The addition of a cyano group on the double bond changes the *E/Z* nomenclature so that for *E*-compounds the aryl rings are *cis* and for *Z*-compounds the aryl rings are *trans*.

The double bond bearing a nitrile would be an effective replacement for the *Z*-olefin of CA-4 analogues by comparing the activities of compounds **14a** with that of **14b**, and **15a** with that of **15b** (Table 3). It can be clearly seen that although the presence of the nitrile function did not make a significant change in the activity of

Table 3
Introduction of nitrile function and its effect on antiproliferative activity



Compound	R	R′	Configuration	IC_{50} (K562) nM[a]	IC_{50} (MCF-7)[b] μM
1	H	OH	*Z*	2.3 (±1.6)	—
11a	CN	F	*E*	6.7 (±2.8)	—
11b	H	F	*Z*	7[27]	—
14a	CN	F	*Z*	18.7 (±10.5)	—
14b	H	F	*E*	>2500	—
15a	CN	Br	*Z*	49.5(±4.8)	—
15b	H	Br	*E*	7830[28]	—
24a	CN	$B(OH)_2$	*Z*	20.4 (±12.6)	—
24b	H	$B(OH)_2$	*E*	—	470 (±140)[20]

[a] Values are means of at least three experiments; standard deviation is given in parentheses.
[b] Human breast carcinoma Cells.

the *Z*-isomers (**11a** and **11b** where aryl rings are *cis*), insertion of a nitrile group to the *Z*-isomers (aryl rings *trans*) significantly increased the antiproliferative activity as compared to the corresponding counterpart (**14a**, **15a**, **24a** with **14b**, **15b** and **24b**). Although the data (**24a** and **24b**) is derived from different cell lines and precaution is required when comparing, the large difference in IC_{50} values proves that nitrile addition to the double is tolerated and enhances the biological activity.

It is unusual that these *Z*-isomers (aryl rings *trans*) show such potent cytotoxicities compared to their *cis*-counterparts.[29,30] This may arise because the cyano group is large compared to a proton and may twist these *Z*-isomers so that they can adopt a conformation where the aryl rings are twisted from a flat *trans* arrangement to a more twisted *cis* arrangement. This configurational change occurs when an ethyl or methyl group is attached to an olefinic bond on a combretastatin.[30] However, the agent with the most potent ability to inhibit the assembly of tubulin is fluoro stilbene **11a**. As expected, this molecule has the aryl rings in a *cis* arrangement.

In the course of this study we have synthesised a series of combretastatin analogues bearing a nitrile function on the olefinic bridge, and have found many of these agents to be potent *in vitro* inhibitors of the proliferation of two cancer cell lines, viz. K562 and MDA NQO1. Compounds **11a**, **14a**, and **24a** exhibited moderate to potent tubulin-binding activity. In contrast with the general belief that the thermodynamically more stable *trans*-isomers are biologically inactive,[6] the *Z*-analogues reported herein have exhibited significantly high antiproliferative activity against MDA NQO1 and K562 cell lines. The results herein published suggest that replacing the olefinic hydrogen with a nitrile function is well-tolerated and does not diminish the ability of appropriately substituted analogues to prevent microtubule assembly. We identify **11a** as a lead candidate for further evaluation.

Acknowledgment

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Supplementary data

Supplementary data (copies of ^{1}H, ^{13}C NMR, IR and mass spectra) associated with this article can be found, in the online version,

at http://dx.doi.org/10.1016/j.bmcl.2012.08.089. These data include MOL files and InChiKeys of the most important compounds described in this article.

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