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Design, synthesis, biochemical, and biological evaluation of nitrogen-containing trifluoro structural modifications of combretastatin A-4

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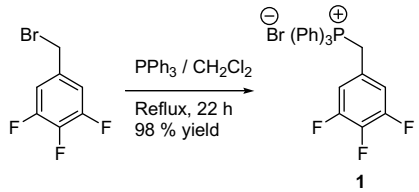
ABSTRACT

A new trifluorinated amino-combretastatin analogue, (*Z*)-2-(4′-methoxy-3′-aminophenyl)-1-(3,4,5-trifluorophenyl)ethene, prepared by chemical synthesis, was found to be a potent inhibitor of tubulin assembly (IC_{50} = 2.9 μM), and cytotoxic against selected human cancer cell lines. This new lead compound is among the most active from a group of related structural modifications.

The combretastatin family of compounds was isolated from the African bush willow tree [*Combretum caffrum* Kuntze (Combretaceae)] by Pettit and colleagues.[1,2] The stilbenoids combretastatin A-4 (CA4)[3] and combretastatin A-1 (CA1)[4] are potent inhibitors of tubulin assembly into microtubules resulting in both antimitotic activity and tumor vascular disruption. The corresponding CA4 and CA1 phosphate prodrugs, CA4P[5,6] and CA1P,[7] are examples of clinically relevant vascular disrupting agents (VDAs). VDAs selectively hinder blood flow by occluding tumor vasculature, resulting in hypoxia and ultimately necrosis of tumors. Both CA4P (Zybrestat™) and CA1P (OXi4503) are currently in human clinical trials.[8] These compounds function through a proposed mechanism involving vascular damage that has been described.[9,10]

Structure–activity relationship (SAR) studies of the *Z*-stilbenoid combretastatin molecular scaffold led to the discovery of biologically active CA4 analogues, where an amine is substituted at the 2′-position[10] and the 3′-position[11–15] (Fig. 1). A review of the literature revealed that fluoro-substitutions can be well tolerated on the combretastatin scaffold,[16–24] and that a 3,4,5-trifluorinated derivative of CA4, first reported by Hadfield and co-workers, inhibits tubulin polymerization.[16] Accordingly, a design paradigm centered on trifluorinated nitrogen-substituted combretastatin derivatives was utilized for the preparation of eight new analogues. Of significance is the (*Z*)-2-(4′-methoxy-3′-aminophenyl)-1-(3,4,5-trifluorophenyl)ethene analogue **8** which is an excellent inhibitor of tubulin polymerization, and is moderately inhibitory against both the NCI-H460 lung cancer and the DU-145 prostate cancer cell lines. The excellent biochemical and biological activity of compound **8** is significant and establishes it as a new lead compound among analogues bearing nitrogen substitution on the trifluorinated combretastatin platform. The synthesis of these nitrogen-containing fluorinated combretastatin analogues as well as preliminary biochemical and biological results are presented herein.

The synthesis of newanalogues **2**–**9** utilized a Wittig reaction as the key synthetic transformation. Commercially available 3,4,5-trifluorobenzyl bromide was reacted with triphenylphosphine to form the corresponding phosphonium bromide salt **1** (Scheme 1), which was treated with NaH followed by



1

Scheme 1. Synthesis of phosphonium bromide salt **1**.

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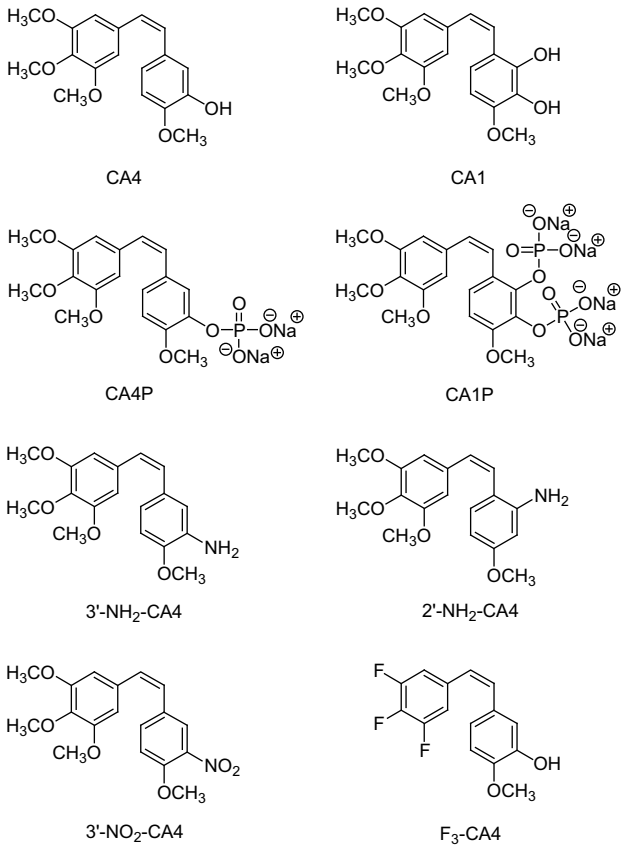


Figure 1. Combretastatin A4, A1, and related analogues.

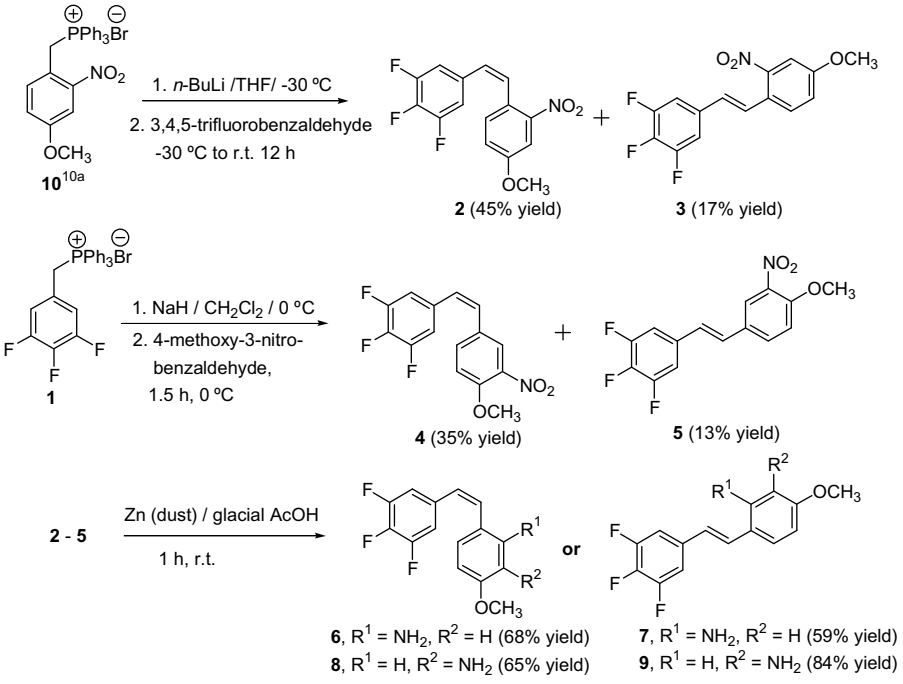
4-methoxy-3-nitrobenzaldehyde to afford (*Z*/*E*)-2-(4′-methoxy-3′-nitrophenyl)-1-(3,4,5-trifluorophenyl)ethene (**4** and **5**) isolated in a 2.7:1 ratio, respectively (Scheme 2).

Wittig salt **10**[10a] was treated with *n*-BuLi to generate the ylide, which was then reacted with 4-methoxy-2-nitrobenzaldehyde to afford a mixture of *Z*- and *E*-combretastatin isomers (**2** and **3**, respectively) isolated in a 2.7:1 ratio (Scheme 2). The *Z*-alkenes were separated from their corresponding *E* isomers using flash column chromatography. Reduction of nitrostilbene analogues **2**–**5** to their corresponding amines **6**–**9** was achieved by treatment with zinc and glacial acetic acid at room temperature. Interestingly, reduction of a mixture of nitrostilbenes **4** and **5** using iron and acetic acid with toluene as solvent afforded the corresponding stilbenes **8** and **9** in much lower yields (11% and 20%, respectively).

For purposes of comparison, 2′-amino-CA4 and F_3-CA4, both of which are excellent inhibitors of tubulin assembly, were evaluated for inhibition against two cancer cell lines, and found to be active. The series of eight new trifluorinated nitrogen-containing combretastatin derivatives (**2**–**9**) was evaluated for their cytotoxicity against NCI-H460 (lung) and DU-145 (prostate) human cancer cell lines using a standard SRB assay.[25] In addition, selected analogues were screened for their ability to inhibit tubulin polymerization.[26]

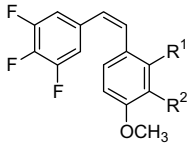
The amino derivative **8** was an excellent inhibitor of tubulin polymerization having an IC_{50} value of 2.9 μM, and demonstrated cancer cell growth inhibition (GI_{50} = 0.11 μg/mL, and GI_{50} = 0.072 μg/mL) against the NCI-H460 and the DU-145 cancer cell lines, respectively. The trifluorinated *Z*-stilbenes with nitro substitution in either the 2′- (analogue **2**), or 3′- (analogue **4**) position, and an amino substituent in the 2′-position (analogue **6**) showed no inhibition against the NCI-H460 and the DU-145 human cancer cell lines (Table 1) at a concentration of 5 μg/mL. The corresponding nitrogen-containing *E*-fluoro combretastatins (**3**, **5**, **7**, and **9**) were not cytotoxic against these cancer cell lines (Table 2). In general, combretastatin analogues of the *E*-configuration reported in the literature are inactive in these assays.[9,14,16,27]

Although, the trifluorinated-3′-amino stilbene **8** inhibited tubulin polymerization at a concentration comparable to that of 3′-amino-CA4 (Table 1), its cytotoxicity was somewhat diminished. It is interesting to note that the cytotoxicity of F_3-CA4 is also less than the corresponding parent compound CA4.



Scheme 2. Synthesis of *E* and *Z* nitrogen-containing stilbene analogues (**2**–**9**).

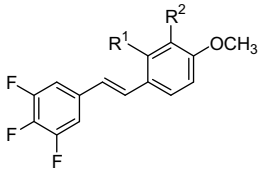
Table 1
Inhibition of microtubule formation and cytotoxicity for *Z* nitrogen-containing trifluorostilbenes **2**, **4**, **6**, and **8**



Compound	R^1	R^2	Tubulin Inhibition IC_{50} (μM)	NCI-H460 GI_{50} (μg/mL)	DU-145 GI_{50} (μg/mL)
2	NO_2	H	nd	>5	>5
4	H	NO_2	>40	>5	>5
6	NH_2	H	nd	>5	4.3
8	H	NH_2	2.9	0.11	0.072
CA4	—	—	1.2[a]	0.0006[a]	0.0008[a]
3′-NH_2-CA4	—	—	2.6[b]	0.00068[b]	0.00096[b]
2′-NH_2-CA4	—	—	1.4[c]	0.0012	0.0016
F_3-CA4	—	—	4.5[d]	0.20	0.040

nd, not determined.
[a] Ref. 27.
[b] Ref. 14.
[c] Ref. 10a.
[d] Refs. 16 and 28.

Table 2
Inhibition of microtubule formation and cytotoxicity for *E* nitrogen-containing trifluorostilbenes **3**, **5**, **7**, and **9**



Compound	R^1	R^2	Tubulin Inhibition IC_{50} (μM)	NCI-H460 GI_{50} (μg/mL)	DU-145 GI_{50} (μg/mL)
3	NO_2	H	nd	>5	>5
5	H	NO_2	>40	>5	>5
7	NH_2	H	nd	>5	>5
9	H	NH_2	>40	>5	>5

nd, not determined.

In conclusion, we have synthesized eight trifluorinated nitrogen-containing combretastatin analogues and carried out initial biochemical and biological evaluations. The activity of compound **8** is impressive and warrants its further development as a potential VDA.

Tubulin polymerization assay:[18,14] Tubulin was purified from calf brain following a method reported by Hamel and Lin.[18] Polymerization was followed turbidimetrically at 350 nm. IC_{50} values of the various analogues were determined from the data using nonlinear regression analysis with Prism software (GraphPad) 3.02 version.

SRB assay:[25] Inhibition of human cancer cell line growth was assessed using the National Cancer Institute's standard sulforhodamine B assay, as previously described.[25] Briefly, cells in an appropriate culture media solution supplemented with 5% fetal bovine serum were inoculated into 96-well plates and incubated for 24 h. Serial dilutions of the compounds were then added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI_{50} or the drug concentration causing a 50% reduction in net protein increase) was calculated from optical density data.

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Supplementary data

Detailed experimental syntheses, ^1H NMR, ^{13}C NMR, ^{19}F NMR, HRMS, and HPLC data have been made available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.070.

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