



Synthesis and antitumor-evaluation of cyclopropyl-containing combretastatin analogs

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ABSTRACT

Several derivatives of combretastatin have been prepared bearing a cyclopropyl unit instead of the natural occurring cis-double bond. Final products and synthetic intermediates were evaluated for their cytotoxic properties in two human cancer cell lines.

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In 1982, Pettit and co-workers isolated highly oxygenated stilbene derivatives from the bark of the African willow tree *Combretum caffrum*.¹ The newly isolated compounds were named combretastatins and were identified as natural products with remarkable biological properties. Combretastatin A4 (**1**, Fig. 1), is among the most potent cytotoxic compounds known to date.^{2,3} Combretastatins induce apoptotic cell death by selectively binding to tubulin at the colchicine binding site resulting in disruption of the formation of microtubules and cell cycle arrest at the transition of meta- to ana-phase.^{4,5}

Since their discovery in 1982, numerous derivatives of combretastatin have been prepared and subjected to biological activity testing.^{6–10} As indicated in Figure 2, structural modifications are generally possible either at the aromatic moieties (structural motif A and C) or the two atom bridge connecting the aromatic rings (structural motif B). Structure activity relationship studies revealed that the methoxy substituents at ring A are required for biological activity. Furthermore, free hydroxyl functionalities or other equivalent hydrogen bonding donors at ring C and the cis-stilbene moiety are essential for high levels of cytotoxicity.^{11,12}

Derivatives of combretastatin used in clinical studies are shown in Figure 1.^{13–17} Although these compounds express high levels of in vitro activity, the in vivo activity is limited because of the high tendency of the system to undergo cis/trans isomerization. Several publications describe the synthesis and biological evaluation of derivatives with heterocyclic moieties instead of the cis-stilbene unit.^{18–21} However, the overall polarity of the compounds is strongly modified by such an electronic modification. One way to bypass this problem is to incorporate a structural motif which

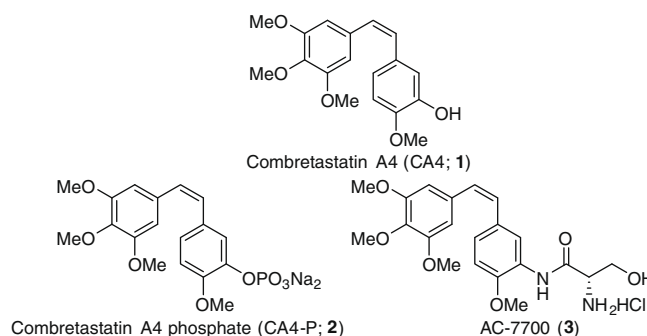


Figure 1. Combretastatin A4 (**1**) and derivatives used in clinical studies.

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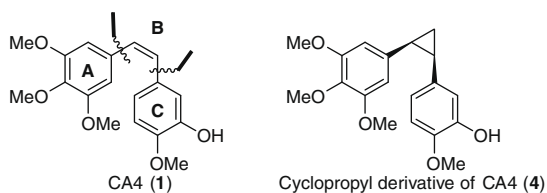
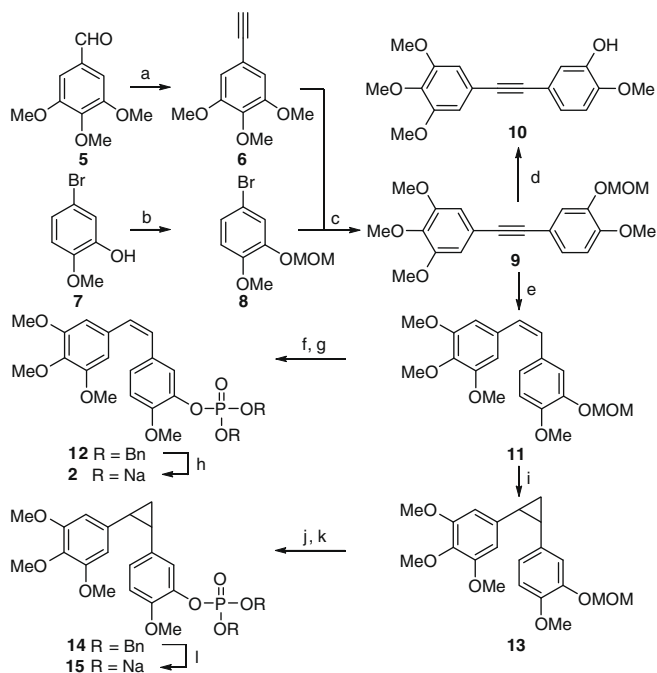


Figure 2. Comparison of CA4 (**1**) and the cyclopropyl derivative of the natural product (**4**).

maintains the overall polarity of the natural product and ensures binding of the natural product derivative to tubulin but at the same time locks the *cis* isomer of the two atom bridge. A possibility to achieve this goal is to incorporate a fully saturated carbocycle instead of the stilbene moiety. In addition to the advantage of the fixed *cis*-relationship of the aromatic rings, variation of the ring size allows fine-tuning of the angle and exact position of the aromatic portions.

Herein, we describe the synthesis and biological evaluation of novel derivatives of combretastatin with cyclopropyl units instead of the *cis*-double bond. All compounds containing the cyclopropyl moiety were synthesized as racemates as we were mainly interested in investigating the effect of the angle and spatial arrangement of the aromatic rings.

The synthesis of the natural product and the cyclopropyl derivative is shown in Scheme 1. The reaction sequence started by conversion of the carbonyl moiety of trimethoxy benzaldehyde (**5**) into the corresponding alkyne functionality with TMS-diazomethane.²² Alkyne **6** was coupled under Suzuki conditions with aromatic bromide **8**,²³ available by MOM-protection of phenol **7**, to afford bis-functionalized alkyne **9** in good yield. For biological activity studies, small amounts of this material were deprotected to afford phenol **10** in 90% yield. Selective reduction of the triple bond in **9** to the *cis*-stilbene motif was achieved by hydroboration with $\text{BH}_3\cdot\text{THF}$ complex



Scheme 1. Synthesis of CA4 and cyclopropyl-containing derivatives. Reagents and conditions: (a) TMS-diazomethane, *n*-BuLi, -78°C , 90%; (b) MOMCl, DIPEA, 95%; (c) triisopropylborate, *n*-BuLi, $\text{Pd}(\text{PPh}_3)_4$, 90°C , 56%; (d) MeOH/HCl, 90%; (e) H_2 , Pd/CaCO₃, quinoline, 81%; (f) MeOH/HCl, 95%; (g) $(\text{BnO})_2\text{P}(\text{O})\text{H}$, DIPEA, DMAP, 67%; (h) (i) TMSBr, NaI, 48%; (ii) NaOMe, 70%; (i) CH_2N_2 , $\text{Pd}(\text{OAc})_2$, 55%; (j) MeOH/HCl, 68%; (k) $(\text{BnO})_2\text{P}(\text{O})\text{H}$, DIPEA, DMAP, 67%; (l) (i) TMSBr, NaI; (ii) NaOMe, 32% (two steps).

and cyclohexene in 80% yield.²⁴ Deprotection of the MOM ether afforded the natural product (**1**) which was further converted to phosphate **2** by treatment with dibenzyl phosphite and subsequent cleavage of the benzyl groups with TMSBr.²⁵ MOM-protected stilbene derivative **11** was used in the cyclopropanation reaction with CH_2N_2 and $\text{Pd}(\text{OAc})_2$. Subsequent deprotection afforded the cyclopropyl-containing analog of combretastatin (**4**). Following the reaction sequence described above, introduction of the phosphate group for higher water solubility concluded the synthesis with the isolation of **15** (Scheme 1).

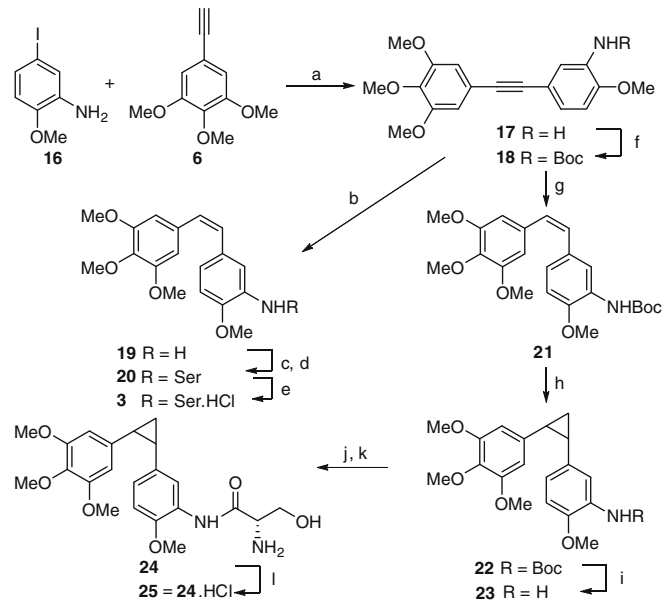
The preparation of amine **23** and amide **25** is carried out in close analogy. (Scheme 2). Sonogashira coupling²⁶ of iodide **16** and alkyne **6** followed by hydrogenation under Lindlar conditions using Pd on CaCO₃ afforded amine **19** in 83% yield. For reference purposes, AC-7700 was prepared by benzotriazole promoted coupling of amine **19** with Fmoc-protected serine acetate, basic cleavage of the protecting groups (**20**) and precipitation of the hydrochloride.¹⁰

Cyclopropanation of free amine **19** could not be carried out successfully. The problem was solved by introduction of a Boc group on the nitrogen (**21**). Treatment of carbamate **21** with CH_2N_2 and a catalytic amount of $\text{Pd}(\text{OAc})_2$ afforded cyclopropyl derivative **22** in 58% yield. Cleavage of the Boc group (**23**) followed by installation of the serine side chain as described above allowed the isolation of the desired cyclopropyl derivative of AC-7700 (**25**).

All compounds reported herein were evaluated for *in vitro* cytotoxicity in HeLa (cervical adenocarcinoma) and MCF-7 (breast adenocarcinoma)—using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.²⁷ Combretastatin A4 and other derivatives already used in clinical trials were included as control substances. Results of this biological activity study are summarized in Table 1.

Alkyne derivatives obtained as intermediates in the synthesis of the cyclopropane containing substrates were also employed in biological activity studies. As expected, these substrates were found to be completely inactive and these findings confirm previous studies.²⁸

Combretastatin A4 and derivatives containing the *cis*-stilbene moiety were found to be most active and these compounds express



Scheme 2. Preparation of N-containing combretastatin analogs. Reagents and conditions: (a) $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, NEt_3 , Cul, 70%; (b) H_2 , Pd/CaCO₃, quinoline, 83%; (c) Fmoc-L-Serine(Ac), DIC, HOBT, 70%; (d) NaOH, 61%; (e) HCl, 91%; (f) Boc_2O , K_2CO_3 , 90%; (g) H_2 , Pd/CaCO₃, quinoline, 60%; (h) CH_2N_2 , $\text{Pd}(\text{OAc})_2$, 58%; (i) MeOH/HCl, 85%; (j) Fmoc-L-Serine(Ac), DIC, HOBT, 76%; (k) NaOH, 92%, (l) HCl, 91%.

Table 1

Calculated IC₅₀ values for synthetic intermediates and final cyclopropyl derivatives of combretastatin

Compound	Structural motif	Calculated IC ₅₀ value (μM)	
		Hela	MCF-7
9	Triple bond	26.81	25.63
10	Triple bond	1.54	1.64
17	Triple bond	>30	>30
18	Triple bond	>30	>30
1	Cis-double bond	0.00051	0.0024
2	Cis-double bond	0.00043	0.0020
3	Cis-double bond	0.0037	0.025
11	Cs-double bond	0.197	0.269
12	Cis-double bond	0.246	1.17
19	Cis-double bond	0.00053	0.0035
20	Cis-double bond	0.00072	0.0031
21	Cis-double bond	1.17	3.85
4	Cyclopropyl ring	0.028	0.102
13	Cyclopropyl ring	23.96	14.01
14	Cyclopropyl ring	10.17	10.77
15	Cyclopropyl ring	0.37	0.297
22	Cyclopropyl ring	10.07	11.84
23	Cyclopropyl ring	0.041	0.212
24	Cyclopropyl ring	0.032	0.063
25	Cyclopropyl ring	0.432	0.670

IC₅₀ values in the nanomolar range. Highest level of activity was observed in compounds which are currently employed in clinical studies. Reference compounds **1** and **2** are equipotent as dephosphorylation takes place in the medium and **1** as active agent enters the cells. If the free hydroxyl (or amino) functionality on ring C is blocked by substituents lacking hydrogen bonding donors (**11** and **21**), the activity level drops. This result is in agreement with findings by other groups.²⁹

Substrates with the cyclopropyl unit as structural motif are significantly less active than the corresponding stilbene derivatives but still possess moderate to good cytotoxic properties. As pointed out above, cyclopropyl derivatives were prepared and tested as racemic mixtures and therefore the activity of the enantiopure material is not known. However, since all racemic cyclopropyl compounds are substantially less potent than the corresponding stilbenes, even enantiopure substrates would express lower potency than the natural product.

It is interesting that phosphate **15** is less active than **4** suggesting that dephosphorylation proceeds at lower rate than in **2**.

Again, the hydroxyl- or amino-functionality at ring C plays a major role and the activity is much higher when hydrogen bonding donors are available. Intermediates lacking acidic protons are nearly inactive.

The serine moiety is cleaved rapidly in the stilbene- and cyclopropyl-series as **20** and **24** showed similar activities compared to **19** and **23**.

We were surprised that the hydrochloric salts **3** and **25** were significantly less active than the free amine. This observation is poorly understood. Counter ions often play a major role in the potency of biologically active compounds and protein binding is also determined by ion concentration in the medium. A similar counter ion depending activity was also observed by Pettit in the case of phosphorylated combretastatins.³⁰

Preliminary docking studies of compounds **4** and **23** into the colchicine binding site of tubulin (pdb code 1sa0)³¹ further strengthen the importance of H-bonds. The phenol **4** shows an H-bond to Thr179 whereas the aniline **23** form an H-bond Ser178. However, considering side chain rotamers compound **4** might additionally form an H-bond with Ser178 (Figs. 3a and 3b). Docking studies also propose two different binding hypotheses for combretastatin analogs. Phenol **4** aligns well with DAMA-colchicine, whereas aniline **23** adopts a conformation with good

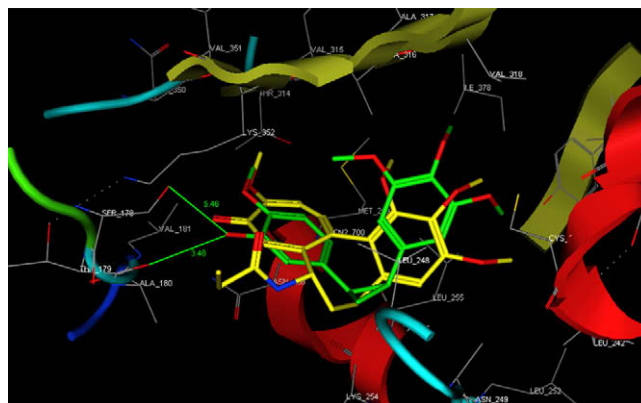


Figure 3a. Docking of cyclopropyl derivatives **4** into the colchicine binding site of tubulin. Yellow: DAMA-colchicines, green: Compd **4**.³²

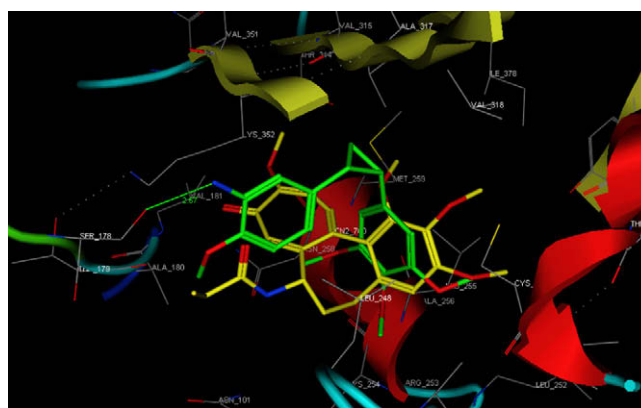


Figure 3b. Docking of cyclopropyl derivatives **23** into the colchicine binding site of tubulin. Yellow: DAMA-colchicines, green: Compd **23**.³²

alignment of the trimethoxy-phenyl rings but a different orientation of ring B pointing towards the β-sheets with Lys352.

Despite the lower in vitro activity of cyclopropanes in comparison to the natural product, cyclopropanes lack the ability to undergo cis/trans isomerization and therefore represent highly interesting lead compounds for in vivo studies. The spatial demand of the cyclopropyl moiety is rather small and the incorporation of a cyclopropane ring does not change the overall polarity of the substrate. The main difference between natural stilbene derivatives and the corresponding cyclopropanes emerges from different angles between the aromatic rings. By preparing substrates with varied ring size (cyclobutyl or cyclopentyl moieties), the angle of the aromatic rings can be adjusted. Fine tuning of the spatial arrangement of the aromatic rings by synthesis of such compounds and subsequent evaluation of the biological activity is currently under investigation in our laboratories and will be reported in due course.

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References and notes

- Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavaniyaya, P. *Can. J. Chem.* **1982**, *60*, 1374.
- Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984.
- Cirla, A.; Mann, J. J. *Nat. Prod. Rep.* **2003**, *20*, 558.

4. Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H. M.; Lin, C. M.; Hamel, E. J. *Med. Chem.* **1992**, *35*, 2293.
5. Chaudhary, A.; Pandeya, S. N.; Kumar, P.; Sharma, P.; Gupta, S.; Soni, N.; Verma, K. K.; Bhardwaj, G. *Mini-Rev. Med. Chem.* **2007**, *7*, 1186.
6. Brown, T.; Holt, H.; Lee, M. In *Topics in Heterocyclic Chemistry*; Gupta, R. R., Ed.; Springer: Berlin, Heidelberg, 2006; pp 1–51.
7. Hatanaka, T.; Fujita, K.; Ohsumi, K.; Nakagawa, R.; Fukuda, Y.; Nihei, Y.; Suga, Y.; Akiyama, Y.; Tsuji, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3371.
8. Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. J. *Med. Chem.* **2006**, *49*, 3033.
9. Borrel, C.; Thoret, S.; Cachet, X.; Guenard, D.; Tillequin, F.; Koch, M.; Michel, S. *Bioorg. Med. Chem.* **2005**, *13*, 3853.
10. Monk, K. A.; Siles, R.; Hadimani, M. B.; Mugabe, B. E.; Ackley, J. F.; Studerus, S. W.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Rhodes, M. R.; Pettit, G. R.; Pinney, K. G. *Bioorg. Med. Chem.* **2006**, *14*, 3231.
11. Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200.
12. Nandy, P.; Banerjee, S.; Gao, H.; Hui, M. B. V.; Lien, E. J. *Pharm. Res.* **1991**, *8*, 776.
13. Hori, K.; Saito, S.; Kubota, K. *Br. J. Cancer* **2002**, *86*, 1604.
14. Salmon, H. W.; Mladinich, C.; Siemann, D. W. *Eur. J. Cancer* **2006**, *42*, 3073.
15. Robinson, S. P.; McIntyre, D. J. O.; Checkley, D.; Tessier, J. J.; Howe, F. A.; Griffiths, J. R.; Ashton, S. E.; Ryan, A. J.; Blakey, D. C.; Waterton, J. C. *Br. J. Cancer* **2003**, *88*, 1592.
16. West, C. M. L.; Price, P. *Anti-Cancer Drugs* **2004**, 179.
17. Dowlati, A.; Robertson, K.; Cooney, M.; Petros, W. P.; Stratford, M.; Jesberger, J.; Rafie, N.; Overmoyer, B.; Makkar, V.; Stambler, B.; Taylor, A.; Waas, J.; Lewin, J. S.; McCrae, K. R.; Remick, S. C. *Cancer Res.* **2002**, *62*, 3408.
18. Wang, L.; Woods, K. W.; Li, Q.; Barr, K. J.; McCroskey, R. W.; Hannick, S. M.; Gherke, L.; Credo, R. B.; Hui, Y. H.; Marsh, K.; Warner, R.; Lee, J. Y.; Zielinski-Mozng, N.; Frost, D.; Rosenberg, S. H.; Sham, H. L. *J. Med. Chem.* **2002**, *45*, 1697.
19. Kim, Y.; Nam, N. H.; You, Y. J.; Ahn, B. Z. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 719.
20. Simoni, D.; Grisolia, G.; Giannini, G.; Roberti, M.; Rondanin, R.; Piccagli, L.; Baruchello, R.; Rossi, M.; Romagnoli, R.; Invidiata, F. P.; Grimaudo, S.; Jung, M. K.; Hamel, E.; Gebbia, N.; Crosta, L.; Abbadessa, V.; Di Cristina, A.; Dusonchet, L.; Meli, M.; Tolomeo, M. *J. Med. Chem.* **2005**, *48*, 723.
21. Jonnalagadda, S. S.; terHaar, E.; Hamel, E.; Lin, C. M.; Magarian, R. A.; Day, B. W. *Bioorg. Med. Chem.* **1997**, *5*, 715.
22. van de Sande, M.; Gais, H. J. *Chem.-Eur. J.* **2007**, *13*, 1784.
23. Castanet, A. S.; Colobert, F.; Schlama, T. *Org. Lett.* **2000**, *2*, 3559.
24. Bui, V. P.; Hudlicky, T.; Hansen, T. V.; Stenstrom, Y. *Tetrahedron Lett.* **2002**, *43*, 2839.
25. Pettit, G. R.; Minardi, M. D.; Rosenberg, H. J.; Hamel, E.; Bibby, M. C.; Martin, S. W.; Jung, M. K.; Pettit, R. K.; Cuthbertson, T. J.; Chapuis, J. C. *J. Nat. Prod.* **2005**, *68*, 1450.
26. Li, H. B.; Yang, H.; Petersen, J. L.; Wang, K. K. *J. Org. Chem.* **2004**, *69*, 4500.
27. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
28. Hadfield, J. A.; McGown, A. T. *Synth. Commun.* **1998**, *28*, 1421.
29. Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200.
30. Pettit, G. R.; Minardi, M. D.; Rosenberg, H. J.; Hamel, E.; Bibby, M. C.; Martin, S. W.; Jung, M. K.; Pettit, R. K.; Cuthbertson, T. J.; Chapuis, J. C. *J. Nat. Prod.* **2005**, *68*, 1450.
31. Ravelli, R. B. G.; Gigant, B.; Curmi, P. A.; Jourdin, I.; Lachkar, S.; Sobel, A.; Knossow, M. *Nature* **2004**, *428*, 198.
32. Docking was performed with the software package MOE version 2008.10 (Chemical Computing Group). All amino acids in a distance of 4.5 Å to colchicine were used for defining the receptor. Ligands were docked using the Triangle Matcher as placement routine with the rotate bonds function activated. Rescoring 1 was performed with London dG, GridMin was used for refinement and Affinity dG was selected for rescoring 2. The top 10 ranked poses for each ligand were analyzed.