4538 J. Med. Chem. 2009, 52, 4538–4542
DOI: 10.1021/jm900321u

Journal of Medicinal Chemistry
Brief Article

*Iso*combretastatins A versus Combretastatins A: The Forgotten *iso*CA-4 Isomer as a Highly Promising Cytotoxic and Antitubulin Agent

Samir Messaoudi,[†] Bret Tréguier,[†] Abdallah Hamze,[†] Olivier Provot,[†] Jean-François Peyrat,[†] Jordi Rodrigo De Losada,[†] Jian-Miao Liu,[‡] Jérôme Bignon,[‡] Joanna Wdzieczak-Bakala,[‡] Sylviane Thoret,[‡] Joelle Dubois,[‡] Jean-Daniel Brion,[†] and Mouâd Alami*[,†]

[†]*Univ Paris-Sud, CNRS, BioCIS-UMR 8076, Laboratoire de Chimie Thérapeutique, Faculté de Pharmacie, 5 rue J.-B. Clément, Châtenay-Malabry F-92296, France, and* [‡]*Institut de Chimie des Substances Naturelles (ICSN), UPR 2301 CNRS, Avenue de la Terrasse, F-91198 Gif-sur-Yvette, France*

Received March 25, 2009

Herein is reported a convergent synthesis of *iso*combretastatins A, a novel class of potent antitubulin agents. These compounds having a 1,1-diarylethylene scaffold constitute the simplest isomers of natural *Z*-combretastatins A that are easy to synthesize without need to control the Z-olefin geometry. The discovery of *iso*CA-4 with biological activities comparable to that of CA-4 represents a major progress in this field.

Introduction

The discovery of natural substances capable of interfering with the assembly or disassembly of microtubules has drawn much attention because microtubules are recognized as an attractive pharmacological target for anticancer drugs.[1,2] Natural substances that are able to modulate the microtubule assembly can be broadly divided into two groups. In the first one, paclitaxel, currently used in the treatment of ovarian and breast cancer, is well-known to promote the polymerization of microtubules, resulting in highly stable, nonfunctional assembled microtubules.[3] In contrast to taxanes, the vinca alkaloids (e.g.; vincristine, vinblastine, etc.), dolastatins as well as colchicine inhibit microtubule assembly.[4] However, all these complex molecules have limitations resulting from high toxicity, difficulty of synthesis, and some of them become rapidly prone to resistance phenomena.[5] Among the large class of natural substances known as inhibitors of tubulin assembly, combretastatins A-1 to A-6, isolated from the African willow tree *Combretum caffrum*, are currently the simplest structures and have attracted the attention of many medicinal chemists for the rational design of antitubulin agents (Figure 1).[6] The cell growth inhibition of combretastatins of the series A-1 to A-6 was evaluated in vitro in a panel of 60 human tumor cell lines.[6c] The most potent member of these natural stilbenes family is CA-4 ($GI_{50} = 3.2$ nM[a]), while CA-1 ($GI_{50} = 16.2$ nM) and CA-2 ($GI_{50} = 31.6$ nM), respectively, were about one-fifth and one-tenth as potent as CA-4. In another study,[6b] CA-3 was found to retain significant growth cell inhibition against the murine P-388 lymphocytic leukemia ($ED_{50} = 26$ nM), while CA-5 displayed a significant loss of potency in the cytotoxicity assays ($ED_{50} = 900$ nM) and CA-6 was essentially inactive. Furthermore, combretastatins A-1 to A-4 were identified as potent inhibitors of tubulin polymerization at the micromolar concentration (2−5 μM) due to their ability to rapidly bind to tubulin at the colchicine site. The recent interest in the anticancer potential of the combretastatin A-series has gained momentum following the observation that CA-4 and CA-1 induce rapid and reversible vascular shutdown in established tumors in vivo, consistent with an antivascular mechanism of action.[7] The water-soluble prodrugs CA-4P and CA-1P have reached the most advanced stage of preclinical development. Currently, CA4-P[8] either as a single agent or in combination therapy is undergoing several advanced clinical trials worldwide for the treatment of age-related macular degeneration (AMD) or anaplastic thyroid cancer.[9]

The relative simplicity of the 1,2-diarylethylene scaffold of combretastatin A-series, along with their biological properties, resulted in extensive structure−activity relationship (SAR) studies.[10] In fact, over the past decade, the literature search, with regard to combretastatins, has exploded, and just for the last five years, more than 250 publications and 170 patents concern combretastatins, many of which show the great interest in the discovery of new small molecules targeting tubulin assembly.

In spite of these SAR studies and the impressive number of analogues synthesized (>28000), only the CA-4 and CA-1 remain the most promising molecules as their prodrugs, CA-4P and CA-1P, have a significant impact on the clinical management of cancer.[9]

Despite their remarkable therapeutic interest, these *Z*-natural stilbene compounds are prone to double-bond isomerization during storage and administration, leading to the *E*-isomer, which dramatically displayed a reduced inhibition of cancer cell growth and tubulin assembly.[11] It should be noted that the third isomer of the combretastatin A-series, named *iso*combretastatins A (*iso*CA), has never been studied despite the impressive number of synthetic analogues.

*To whom correspondence should be addressed. Phone: 33(0)1.46.83.58.87. Fax: 33(0)1.46.83.58.28. E-mail: mouad.alami@u-psud.fr.

[a]Abbreviations: CA, combretastatin A; isoCA, isocombretastatin A; IC_{50}, the half maximal inhibitory concentration; GI_{50}, the half maximal growth inhibitory concentration; ED_{50}, the half maximal effective dose concentration).

 Published on Web 06/16/2009

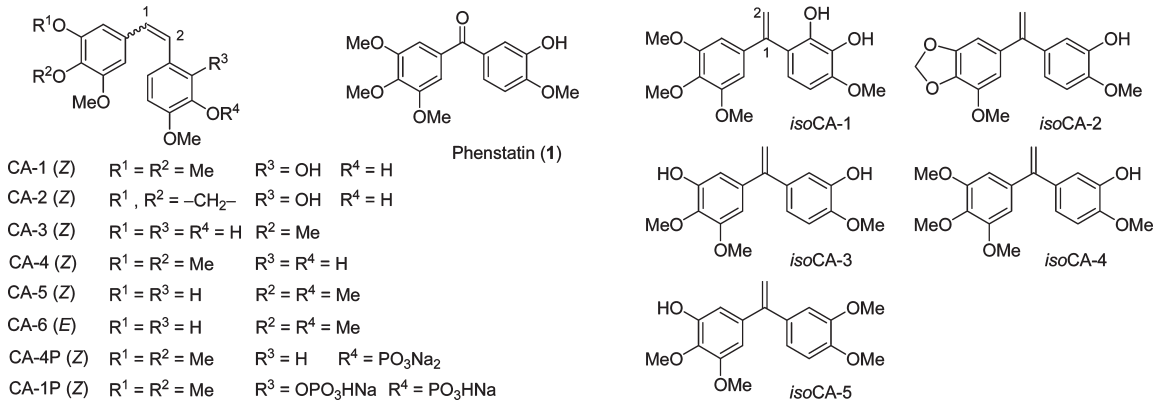
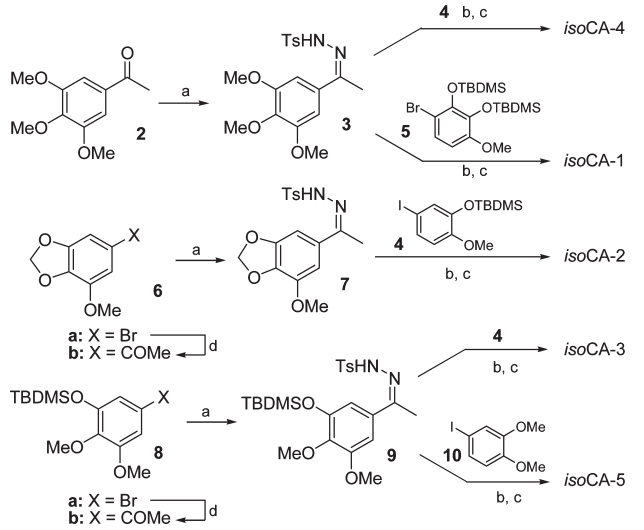


Figure 1. Combretastatins A, synthetic tubulin assembly inhibitor phenstatin (**1**), and structures of the synthesized *iso*combretastatins A-1 to A-5 (*iso*CA).

Scheme 1[a]



[a] Reagents and conditions: (a) $TsNHNH_2$, EtOH, reflux. (b) ArX, $Pd_2(dba)_3$, Xphos, *t*BuOLi, dioxane, 70 °C. (c) K_2CO_3, MeOH. (d) (i) *n*-BuLi, THF, −78 °C then MeCHO; (ii) PCC, CH_2Cl_2, 20 °C.

Moreover, *iso*CA derivatives share a structural similarity with phenstatin, a very strong anticancer substance serendipitously discovered[12] (Figure 1) and, therefore, could potentially be an attractive class of compounds for cancer treatment.

In our efforts to discover novel tubulin assembly inhibitors,[13] we hypothesized that *iso*combretastatins A with 1,1-diarylethylene scaffold could be as active as those of natural combretastatins A. Here, we report on synthesis of *iso*combretastatins A-1 to A-5 (Figure 1) and the evaluation of their biological properties. This study would bring additional information on the importance of the carbonyl oxygen of phenstatin to form crucial interactions at the colchicine site when compared to *iso*CA. The potencies of newly synthesized *iso*CA were examined for their capacity to inhibit cancer cell growth and to act as potential antimitotic agents.

Chemistry. Because the CA-4 is the most active compound of the combretastatin A-series, we first focused our attention on the synthesis of *iso*CA-4. Thus, the reaction of *N*-tosylhydrazone[14] (**3**), readily available from **2**, with aryl iodide (**4**) in the presence of $Pd_2(dba)_3$ and Xphos provided the resulting coupling product, which was subsequently desilylated under alkaline media to obtain the target *iso*CA-4 (Scheme 1). In the same way, starting from hydrazones **3**, **7**, and **9**, the coupling with appropriate aryl halides **4**, **5**, and **10** furnished after alkaline deprotection the desired *iso*CA-2 to *iso*CA-5 in good yield (Scheme 1). It should be noted that *iso*CA-5 is either the isomer of position of the CA-5 or its trans isomer CA-6.

Table 1. Cytotoxicity of *iso*CA on the Human Cancer Cell Lines and Antitubulin Activities

	IC_{50} [nM][a]				
	HCT116	K562	H1299	MDA-MB231	ITP IC_{50} [μM][b]
phenstatin	33	41	21	28	2.0
colchicine	32	29	30	26	2.1
CA-4	2.3	3.6	5.0	3.0	1.0
*iso*CA-1	850	750	2000	820	2.1
*iso*CA-2	38	38	32	48	3.4
*iso*CA-3	34	52	25	8	5.0
*iso*CA-4	2.5	5.0	5.0	4.0	2.2
*iso*CA-5	2800	3100	5000	3000	NA[c]

[a] IC_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). [b] ITP = Inhibition of tubulin polymerization; IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly (average of three experiments). [c] Nonactive.

Results and Discussion

The *iso*combretastatins A-1 to A-5 were evaluated for their cytotoxic activities against four types of human cell lines, human colon carcinoma HCT116 cells, chronic myelogenous leukemia K562 cells, nonsmall lung human carcinoma H1299 cells, and human breast cancer MDA-MB231 cells. The results of this study summarized in Table 1 demonstrated that the newly synthesized *iso*CA-4 exhibited approximatively a 10-fold greater cytotoxic activity (nanomolar level) than that of phenstatin and colchicine on the four tested cell lines. Interestingly, *iso*CA-4 activity against the above cell lines is comparable to the natural CA-4[15] isomer (2−5 nM). These results suggest that switching an aromatic ring from the C2 to the C1 position retained potent cytotoxicity and that, in this series, the 1,1-ethylene bridge would be regarded as a bioisostere[16] of the *Z*-1,2-ethylene one. Next, other *iso*combretastatins A were examined. Again, *iso*CA-2 and *iso*CA-3 showed strong cytotoxicities (IC_{50} values of 32−48 nM and 8−52 nM, respectively), which are comparable to their CA-2 and CA-3 parents.[6] Inverting a MeO substituent by a OH group on the aromatics led to a dramatic reduction in cytotoxicity when *iso*CA-4 and *iso*CA-5 were compared. The *iso*CA-5

cytotoxicity is comparable to that of the CA-5 parent product but is 1000-fold less important than *iso*CA-4.

As combretastatin A-series have been well documented to interact with tubulin, *iso*combretastatins A-1 to A-5 were evaluated for their antitubulin assembly activities (Table 1). According to our assumption about the similarity between the natural isomer CA-4 and *iso*CA-4, the latter showed an inhibition of tubulin assembly in the same order of magnitude (2.2 μM) than that exhibited by CA-4 (1.0 μM) or colchicine (2.1 μM). More interestingly, the activity of *iso*CA-4 is comparable with that of phenstatin (2.0 μM) despite the absence of the carbonyl function that was suggested to play a positive role for strong antitubulin activity.[17] This trend was also observed by the level of the inhibition of tubulin assembly displayed by *iso*CA-2 (3.4 μM), *iso*CA-3 (5.0 μM), and the less cytotoxic *iso*CA-1 (2.1 μM).

The effects of the most active agent *iso*CA-4 on cell cycle distribution were analyzed in K562 cells cultured for 24 h in the presence of increasing amounts of *iso*CA-4. The results presented in Figure 2 demonstrate a dose-dependent increase in the proportion of cells in G2/M phase and the simultaneous decrease in the number of cells in S and G1 phases, suggesting that *iso*CA-4 induces selectively arrested cell division in the G2/M phase of the cell cycle.

The X-ray crystal structure of *iso*CA-4 depicted in Figure 3A shows that the conformation of the 1,1-diarylethylene scaffold is not planar and the planes of the two aromatic rings are inclined toward each other (dihedral angle = 68°). A similar nonplanarity was observed in the case of the natural substance CA-4.[18] It seems that in combretastatin A-series, *switching the trimethoxyaryl nucleus from the C(1) to the C(2) position of the ethylene bridge preserves the spatial relationship of the two aromatic rings* (*cis-relationship*) *in the 1,1-diarylethylene scaffold* and, therefore, possibly could explain similar activities exhibited by these substances.

Adopting the conformation found in its X-ray crystal structure, *iso*CA-4 was computer docked inside the colchicine binding site. For this purpose, the X-ray structure of tubulin-colchicine complex (Code PDB: 1sa0)[19] was used. Figure 3B shows the docking-derived superimposition of *iso*CA-4, CA-4, phenstatin, and colchicine (blue, green, red, and orange, respectively). As expected, *iso*CA-4, CA-4, and phenstatin show a nice fitting between them and the colchicine X-ray structure into the binding site (Figure 3B). As previously reported for a set of colchicine site inhibitors,[17] the trimethoxyphenyl moieties occupy very similar Cartesian space. Hydroxyl and methoxy groups belonging to the rest of the system are

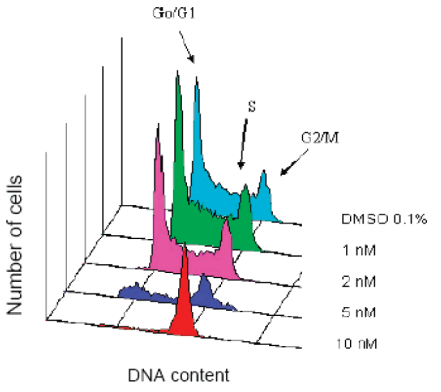


Figure 2. Effect of *iso*CA-4 on cell-cycle distribution in chronic myelogenous leukemia K562 cells determined by flow cytometry analysis. DNA content was assessed via propidium iodide staining.

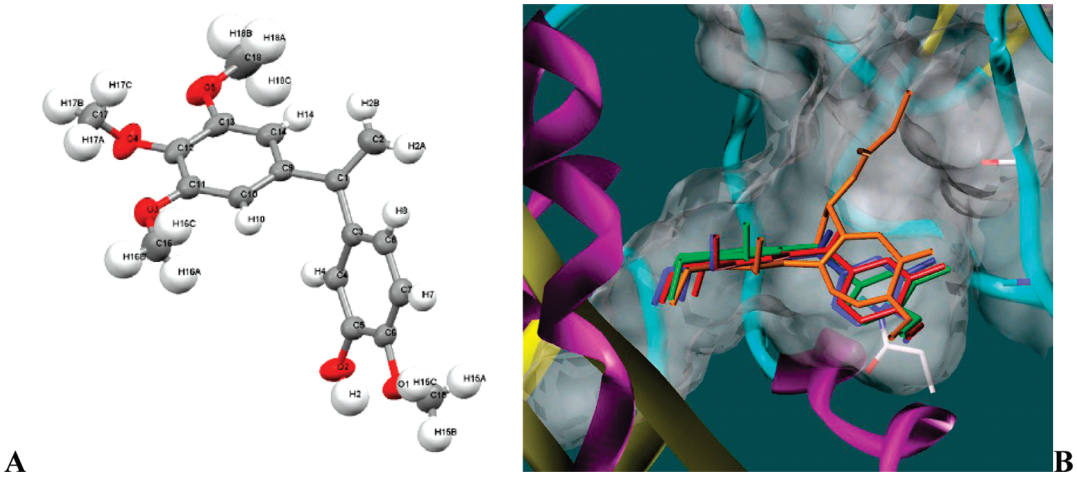


Figure 3. (A) X-ray crystal structure of *iso*CA-4. (B) Putative binding mode of *iso*CA-4 (blue), CA-4 (green), phenstatin (red), and colchicine (orange) in the colchicine binding site.

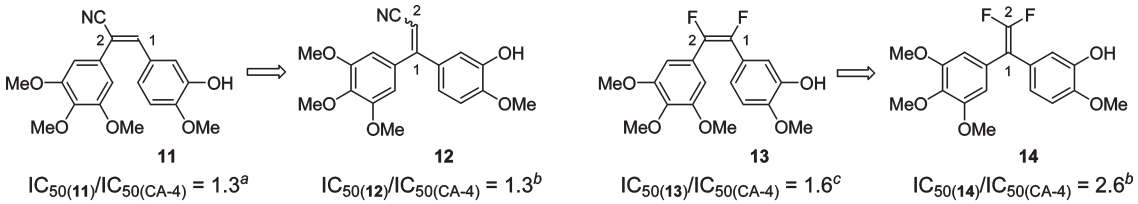


Figure 4. *Iso*CA-4 analogues **12** and **14** with a tri- and tetra-substituted double bonds.

also well fitted. Hydroxyl groups belong to CA-4, *iso*CA-4, and phenstatin show a hydrogen bond with backbone of Val181 as proposed by Nguyen et al.[17]

Because the 1,1-ethylene bridge appears to be a suitable bioisosteric replacement for the *Z*-1,2-ethylene one in this series, we attempted to extend this finding to other potent antitubulin agents. We anticipated that substances **12** and **14** with a one-carbon sp^2 bridge would be as active as their known synthetic parent compounds **11**[20] and **13**[21] having a tri- or tetra-substituted double-bond, respectively (Figure 4). To this end, the synthetic strategy that allowed the preparation of **12** and **14** utilized a Wittig olefination of phenstatin silyl ether intermediate[12] with the appropriate ylides followed by an *O*-desilylation step. The substances **12** and **14** were then evaluated for their cytotoxic effects against HCT116 cell lines and for tubulin polymerization inhibitory activity using CA-4 as reference compound (Figure 4). Diarylacrylonitrile **12** related to CC-5079[22] was tested as a mixture of *E* and *Z* isomers (1/1) and was found to exhibit a high activity with an IC_{50} of 3.0 nM (IC_{50} = 2.3 nM for CA-4) and difluorinated substance **14** showed almost the same activity as the parent compound **13** (H460 nonsmall cell lung carcinoma). Interestingly, **12** and **14** displayed strong antitubulin activities with IC_{50} of 4.3 and 2.5 μM, respectively. These results clearly demonstrate that the modification made on the ethylene bridge maintains cytotoxic and antitubulin polymerization activities and, consequently, validate our bioisosteric replacements wherein a 1,1-ethylene moiety mimics a *Z*-1,2-ethylene one.

Conclusion

In the present study, we have described the synthesis and identification of active 1,1-diarylethylenes, named *iso*combretastatins A, with strong anticancer activities. These compounds, in contrast to their parent 1,2-diarylethylene derivatives, are easy to synthesize without the need to control the olefin geometry and constitute the simplest isomers of natural combretastatins A. The most active agent, *iso*CA-4, shares a striking structural similarity with phenstatin and displayed a 10-fold better cytotoxic activity (2 nM). Moreover, *iso*CA-4 appears to elicit its cytotoxicity in a fashion similar to CA-4, via inhibition of tubulin polymerization, which then leads to cell cycle arrest in G2/M.[1]

As the replacement of the 1,2-ethylene by the 1,1-ethylene bridge resulted in retention of biological activities, our results may encourage the use of this scaffold in future structure−activity relationship studies. All of this data make these compounds worthy of further in vitro and in vivo investigation. The design of other antitumor agents based on the above findings is currently underway; the results of synthetic and biological studies will be reported in due course.

Experimental Section

Procedure for the synthesis of *iso*CA-1 to *iso*CA-5. To a dioxane (6 mL) solution of *N*-tosylhydrazone (0.42 mmol), *t*BuOLi (84 mg, 1.05 mmol), Pd_2dba_3 (44 mg, 0.042 mmol), and X-Phos (40 mg, 0.084 mmol) was added the aryl halide (0.42 mmol) in dioxane (1 mL). The mixture was stirred at 75 °C for 5 h. CH_2Cl_2 (10 mL) was added to the cooled mixture and then filtered over a pad of celite. After concentration, the residue was dissolved in MeOH (3 mL), K_2CO_3 (116.0 mg, 0.84 mmol) was added, and stirring was continued for 2 h. Water (10 mL) was added and the aqueous phase was extracted with Et_2O (3 × 10 mL). The organic phase was washed with brine (15 mL), dried over $MgSO_4$, and concentrated in vacuo to give a residue which was purified by silica gel chromatography to yield *iso*CA. Purity was determined by elemental analysis and HPLC; purity of key target compounds *iso*CA-1 to *iso*CA-5 was >98%.

Acknowledgment. We thank the CNRS for support of this research and the ICSN for a doctoral fellowship to B.T.

Supporting Information Available: Typical experimental procedure, analytical data, crystallographic information files (CIF), modeling programs, ^1H, ^{13}C NMR, and MS data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Alami, M.; Brion, J.-D.; Provot, O.; Peyrat, J.-F.; Messaoudi, S.; Hamze, A.; Giraud, A.; Bignon, J.; Bakala, J.; Liu, J.-M. *Iso*CA-4 and analogues thereof as potent cytotoxic agents inhibiting tubulin polymerization. Patent WO 122620 A1, 2008.
(2) Hadfield, J. A.; Ducki, S.; Hirst, N.; McGown, A. T. Tubulin and microtubules as targets for anticancer drugs. *Prog. Cell Cycle Res.* **2003**, *5*, 309–325.
(3) Schiff, P. B.; Fant, J.; Horwitz, S. B. Promotion of microtubule assembly in vitro by taxol. *Nature* **1979**, *277*, 665–667.
(4) Bhattacharayya, B.; Panda, D.; Gupta, S.; Banerjee, M. Antimitotic activity of colchicine and the structural basis for its interaction with tubulin. *Med. Res. Rev.* **2008**, *28*, 155–183.
(5) Jordan, M. A. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Curr. Med. Chem. Anticancer Agents* **2002**, *1*, 1–17.
(6) (a) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. Isolation, structure, and synthesis of combretastatins A-1 and B-1, potent new inhibitors of microtubule assembly, derived from *Combretum caffrum*. *J. Nat. Prod.* **1987**, *50*, 119–131. (b) Pettit, G. R.; Singh, S. B. Isolation, structure, and synthesis of combretastatin A-2, A-3, and B-2. *Can. J. Chem.* **1987**, *65*, 2390–2396. (c) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. Antineoplastic agents. 291. Isolation and synthesis of combretastatins A-4, A-5, and A-6. *J. Med. Chem.* **1995**, *38*, 1666–1672.
(7) Tozer, G. M.; Kanthou, C.; Parkins, C. S.; Hill, S. A. The biology of the combretastatins as tumour vascular targeting agents. *Int. J. Exp. Pathol.* **2002**, *83*, 21–38.
(8) Siemann, D. W.; Chaplin, D. J.; Walicke, P. A. A review and update of the current status of the vasculature-disabling agent combretastatin-A4 phosphate (CA4P). *Expert Opin. Investig. Drugs* **2009**, *18*, 189–197.
(9) (a) Patterson, D. M.; Rustin, G. J. S. Combretastatin A-4 phosphate. *Drugs Future* **2007**, *32*, 1025–1032. (b) Lippert, J. W. Vascular disrupting agents. *Bioorg. Med. Chem.* **2007**, *15*, 605–615.
(10) Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Dusacca, S.; Genazzani, A. A. Medicinal chemistry of combretastatin A4: present and future directions. *J. Med. Chem.* **2006**, *49*, 3033–3044.
(11) Pettit, G. R.; Toki, B.; Herald, D. L.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Chapuis, G. C. Antineoplastic Agents. Asymmetric Hydroxylation of *trans*-Combretastatin A-4. *J. Med. Chem.* **1999**, *42*, 1459–1465.
(12) Pettit, G. R.; Toki, B.; Herald, D. L.; Verdier-Pinard, P.; Boyd, M. R.; Hamel, E.; Pettit, R. K. Antineoplastic agents. 379. Synthesis of phenstatin phosphate. *J. Med. Chem.* **1998**, *41*, 1688–1695.
(13) (a) Provot, O.; Giraud, A.; Peyrat, J.-F.; Alami, M.; Brion, J.-D. Synthetic approach to enyne and enediyne analogues of anticancer agents. *Tetrahedron Lett.* **2005**, *46*, 8547–8550. (b) Mousset, C.; Giraud, A.; Provot, O.; Hamze, A.; Bignon, J.; Liu, J. M.; Thoret, S.; Dubois, J.; Brion, J.-D.; Alami, M. Synthesis and antitumor activity of benzils related to combretastatin A-4. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3266–3271.
(14) Barluenga, J.; Moriel, P.; Valdés, C.; Aznar, F. *N*-Tosylhydrazones as reagents for cross-coupling reactions: a route to polysubstituted olefins. *Angew. Chem., Int. Ed.* **2007**, *46*, 5587–5590.
(15) Giraud, A.; Provot, O.; Hamze, A.; Brion, J. D.; Alami, M. One-pot hydrosilylation−protodesilylation of functionalized diarylalkynes: a highly selective access to *Z*-stilbenes. Application to the synthesis of combretastatin A-4. *Tetrahedron Lett.* **2008**, *49*, 1107–1110.
(16) Patani, G. A.; LaVoie, E. J. Bioisosterism: a rational approach in drug design. *Chem. Rev.* **1996**, *96*, 3147–3176.

(17) Nguyen, T. L.; McGrath, C.; Hermone, A. R.; Burnett, J. C.; Zaharevitz, D. W.; Day, B. W.; Wipf, P.; Hamel, E.; Gussio, R. A common pharmacophore for a diverse set of colchicine site inhibitors using a structure-based approach. *J. Med. Chem.* **2005**, *48*, 6107–6116.
(18) Lara-Ochoa, F.; Espinosa-Pérez, G. A new synthesis of combretastatins A-4 and AVE-8062. *Tetrahedron Lett.* **2007**, *48*, 7007–7010.
(19) Ravelli, R. B.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature* **2004**, 198–202.
(20) Ohsumi, K.; Nakagawa, R.; Fukuda, Y.; Hatanaka, T.; Morinaga, Y.; Nihei, Y.; Ohishi, K.; Suga, Y.; Akiyama, Y.; Tsuji, T. Novel combretastatin analogues effective against murine solid tumors: design and structure−activity relationships. *J. Med. Chem.* **1998**, *41*, 3022–3032.
(21) Alloatti, D.; Giannini, G.; Cabri, W.; Lustrati, I.; Marzi, M.; Ciacci, A.; Gallo, G.; Tinti, M. O.; Marcellini, M.; Riccioni, T.; Guglielmi, M. B.; Carminati, P.; Pisano, C. Synthesis and biological activity of fluorinated combretastatin analogues. *J. Med. Chem.* **2008**, *51*, 2708–2721.
(22) Zhang, L.-H.; Wu, L.; Raymon, H. K.; Chen, R. S.; Corral, L.; Shirlei, M. A.; Narla, R. K.; Gamez, J.; Muller, G. W.; Stirling, D. I.; Bartlett, J. B.; Schafer, P. H.; Payvandi, F. The synthetic compound CC-5079 is a potent inhibitor of tubulin polymerization and tumor necrosis factor-α production with antitumor activity. *Cancer Res.* **2006**, *66*, 951–959.