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Synthesis and biological evaluation of novel heterocyclic derivatives of combretastatin A-4

Thi Thanh Binh Nguyen [a], Thierry Lomberget [a,*], Ngoc Chau Tran [a], Evelyne Colomb [b], Lore Nachtergaele [b], Sylviane Thoret [c], Joëlle Dubois [c], Joren Guillaume [a], Rawad Abdayem [b], Marek Haftek [b], Roland Barret [a]

[a] Université de Lyon, Université Lyon 1, Faculté de Pharmacie, ISPB, EA 4446 Biomolécules, Cancer et Chimiorésistances, SFR Santé Lyon-Est CNRS UMS3453 - INSERM US7, 8 avenue Rockefeller, F-69373 Lyon cedex 08, France
[b] Université Lyon 1, Faculté de Médecine et de Pharmacie, EA 4169, Fundamental, clinical and therapeutic aspects of the skin barrier, 8 avenue Rockefeller, F-69373 Lyon cedex 08, France
[c] Institut de Chimie des Substances Naturelles, UPR 2301, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France

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ABSTRACT

A novel series of combretastatin A-4 heterocyclic analogues was prepared by replacement of the B ring with indole, benzofurane or benzothiophene, attached at the C2 position. These compounds were evaluated for their abilities to inhibit tubulin assembly: derivative *cis* **3b**, having a benzothiophene, showed an activity similar to those of colchicine or deoxypodophyllotoxine. The antiproliferative and antimitotic properties of *cis* **3b** against keratinocyte cancer cell lines were also evaluated and the intracellular organization of microtubules in the cells after treatment with both stereoisomers of **3b** was also determined, using confocal microscopy.

Amongst all the chemotherapeutic classes that are used to cure cancer, microtubule-binding agents (MBAs) became over the last decades an important tool and an alternative to DNA-damaging drugs.[1]

Microtubules, which are cylindrical polymeric structures, are essential to maintain the cell shape and play a crucial role during the cell cycle. They are composed of α- and β-tubulin heterodimers and the inhibition of their dynamic assembly/disassembly into the mitotic spindle leads to apoptotic cell death.[2]

Many academic and industrial research groups have focused their efforts on the development of anti-cancer drugs based on this strategy, thus leading to numerous MBAs. Natural products were frequently the source of different series, such as combretastatin A-4 (CA-4, Fig. 1), which was isolated from the bark of the South African willow tree *Combretum caffrum* by Pettit et al.[3,4]

This stilbenoid derivative was proved to bind to the colchicine site of tubulin[5] and, regarding to very promising antimitotic activities and its very simple structure, there was an ever-growing interest for the design of CA-4 analogues. Another reason lies in the vascular targeting and disrupting properties of water-soluble CA-4 derived compounds, which would present advantage of targeting neo-vascularized tumors.[6] Using this strategy, several derivatives such as CA-4P[7] (fosbretabulin, Fig. 1) and AVE8062[8] (ombrabulin, Fig. 1) undergo clinical trials.

For these reasons, a large number of CA-4 analogues have been synthesized and evaluated for their tubulin-binding abilities,[9] therefore acting as mitotic spindle poisons and, sometimes, as vascular targeting and disrupting agents (VTA and VDA).[10]

The structure–activity relationships (SAR) have demonstrated that the *cis* configuration of the double bond and the presence of a trimethoxy aryl unit were essential for the activity.[9] In parallel, many studies aimed to appreciate the effect of the replacement of the *cis* double bond by various heterocycles (**1**, Fig. 1) such as furan/thiophene,[11] dioxolane,[12] thiazole,[13] isoxazole,[14] imidazole,[15] triazole[16] or tetrazole.[17] This approach represents a way to block a putative in vivo isomerization of the double bond to the less active *trans* alkene.[18,19]

In continuation with investigations performed by Simoni et al on heterocyclic derivatives **2** (Fig. 1),[20] we have chosen while designing our new anti-cancer drugs to keep the *cis* configuration of the double bond of the trimethoxystyrene unit and to make a variation on the nature of the substitution of the alkene. The replacement of the B ring by various heterocycles attached on their 2-position (**3**, Fig. 1), could allow to evaluate the influence of this modulation (i.e., attachment on a different position of the heterocycle) on the biological activity.

* Corresponding author. Tel./fax: +33 478 777 082.
E-mail address: thierry.lomberget@univ-lyon1.fr (T. Lomberget).

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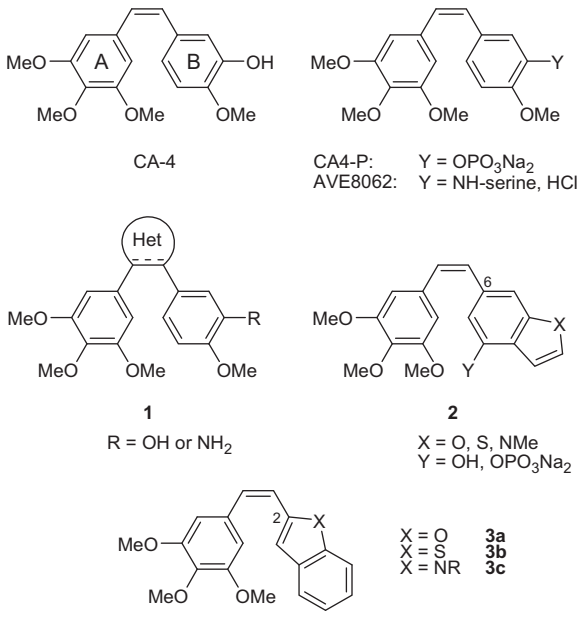


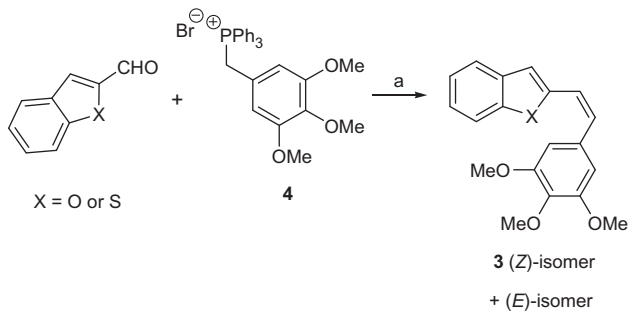
Figure 1. Representative synthetic combretastatins and CA-4 analogues synthesized in this study.

Our preliminary approach to prepare the target compounds **3** was to use a Wittig reaction between commercially available benzofuran or benzothiophene 2-carboxaldehydes and the phosponium salt **4**,[21] derived from 3,4,5-trimethoxy benzaldehyde (Scheme 1). Unfortunately, our conditions led to a mixture of *cis* and *trans* isomers that were difficult to separate, yielding after a flash chromatography the desired stereoisomers *cis*-**3a**,**b** in moderate to low isolated amounts.[22]

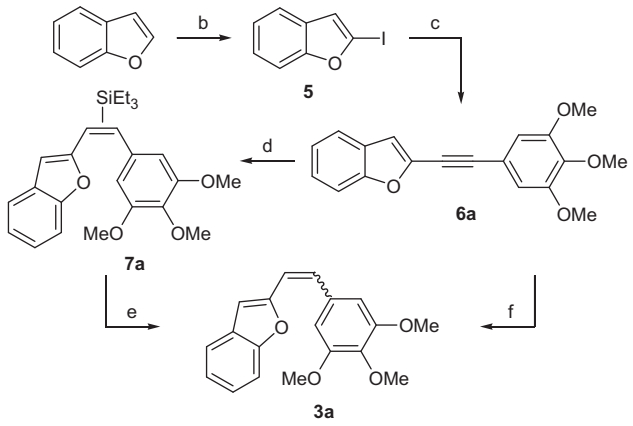
To improve the poor selectivity of the reaction, another strategy was tried: the reduction of an acetylenic derivative (Scheme 2). After deprotonation of the benzofurane, thanks to the zincate reagent $TMPLi/ZnCl_2$ developed by Mongin's group,[23] followed by iodination, 2-iodo benzofurane **5** was obtained in a quantitative yield. A Sonogashira coupling reaction[24] of this substrate with 3,4,5-trimethoxy ethynylbenzene[25] afforded compound **6a** in an excellent 94% yield (Scheme 2).

We first envisaged reducing **6a** by known procedures such as hydrogenation in the presence of Lindlar's catalyst[26] or by acidic hydrolysis of a pre-formed vinylborane derivative,[27] but these attempts led to unsatisfactory results.

We then decided to use an hydrosilylation/desilylation sequence[28] on **6a** to prepare the *cis* double bond of the target compound but, after the fluoride mediated vinylsilyl group deprotection of **7a**, a *Z*/*E* mixture was obtained.[29]



Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, 1 h then rt, 18 h; global yields 65% for **3a** (X=O) and 77% for **3b** (X=S).

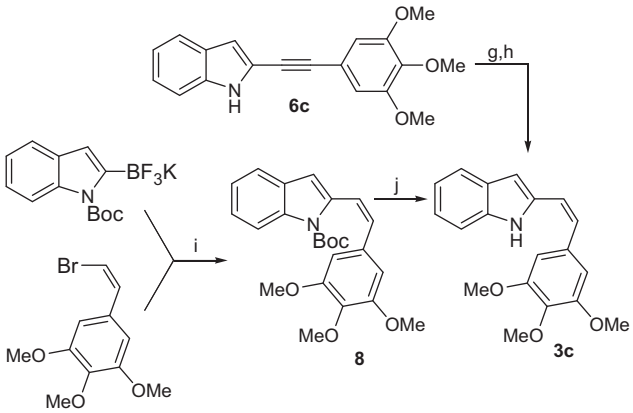


Scheme 2. Reagents and conditions: (b) (1) 2,2′,6,6′-tetramethylpiperidine/BuLi/$ZnCl_2$·TMEDA 1.5/1.5/0.5 equiv, THF, 0 °C then rt, 2 h. (2) I_2 1.5 equiv, 16 h, 99%. (c) 3,4,5-trimethoxy ethynylbenzene 1.14 equiv, $PdCl_2(PPh_3)_2$ 2 mol %, CuI 4 mol %, Et_3N 1.3 equiv, THF, rt, 5 h, 94%. (d) Et_3SiH 3 equiv, PtO_2 5 mol %, 60 °C, 4 h, 95%. (e) TBAF 1 M solution in THF 1.1 equiv, 60 °C, 2.5 h, 84%, 62/38 *Z*/*E* ratio. (f) $Pd(OAc)_2$ 2 mol %, KOH 1.5 equiv, DMF, 145 °C, 6 h, 94%, 93/7 *Z*/*E* ratio.

The solution was finally brought by a more recent reduction method: the use of potassium hydroxide and dimethylformamide, in the presence of a catalytic amount of palladium acetate.[30] Indeed, these conditions provided the desired *Z* isomer of **3a** as the major product, isolated with a 54% overall yield[31] (Scheme 2).

According to the same synthetic route, compound *Z*-**3b** was prepared from benzothiophene with a better 58% overall yield.

In order to compare oxygen and sulfur heteroatoms with the isosteric NH group on the bioactivity, the preparation of the indole derivative **3c** was then envisaged.



Scheme 3. Reagents and conditions: (g) Et_3SiH 3 equiv, PtO_2 5 mol %, 60 °C, 4 h, 78%. (h) TBAF 1 M solution in THF 1.1 equiv, 60 °C, 2.5 h, 80%, *Z* isomer only. (i) $Pd(PPh_3)_4$ 5 mol %, Na_2CO_3, DME/water 2/1, 90 °C, 18 h, 47%. (j) K_2CO_3 3 equiv, MeOH/water, rt (22 h) then 60 °C (24 h), 14%.

Table 1
Inhibition of tubulin assembly by *cis*- and *trans*-**3**

Entry	Compound	Inhibition[a]
1	Colchicine	IC_{50} = 1.7 μM
2	Deoxypodophyllotoxine	IC_{50} = 1.7 μM
3	*trans* **3a**	23% at 32 μM
4	*cis* **3a**	IC_{50} = 9.6 μM
5	*trans* **3b**	IC_{50} = 8.4 μM
6	*cis* **3b**	IC_{50} = 2.6 μM
7	*cis* **3c**	IC_{50} = 21 μM

[a] IC_{50}: Compound concentration required to decrease the rate of microtubule assembly by 50%.

The synthesis of 2-iodoindole was first carried out from indole, using literature protocols: protection of the NH group as a phenylsulfonamide,[32] LDA-mediated iodination[33] at position 2 (thanks to the *ortho*-directing properties of SO_2Ph) and removal of the protecting group using a fluoride source.[34] This iodo derivative was then engaged in a Sonogashira coupling reaction with 3,4,5-trimethoxy ethynylbenzene to get alkyne **6c** in a moderate 39% yield.

After addition of trimethylsilyl hydride under the previously described conditions, the protodesilylation surprisingly afforded the pure *Z*-isomer in an 80% yield. The *Z* configuration of compound **3c** was confirmed after ^{1}H NMR spectra comparison with a compound obtained after Suzuki coupling[35,36] of *Z*-monobromotrimethoxystyrene[37] with *N*-Boc protected indole 2-potassium trifluoroborate[38] followed by *tert*-butyl carbamate basic deprotection[39] of **8** (Scheme 3).

With these *E* and *Z* compounds **3** in hand, we then carried out the evaluation of biological properties of these new heterocyclic derivatives of CA-4.

The heteroaromatic derivatives **3** prepared above were first evaluated for their ability to inhibit tubulin assembly (Table 1). The tubulin assembly assay was realized according to a previously described protocol, using colchicine and deoxypodophyllotoxine as reference compounds, evaluated the same day under the same conditions.[40]

Firstly, it is to note that the best activity was once more observed for *cis* derivatives as described in previous literature studies. The benzothiophene analogues **3b** are much more active than the benzofurane **3a**. This first indication shows that the more voluminous sulfur heteroatom in **3b** may bring a beneficial effect, compared to the oxygen in **3a** (Table 1, entries 4 and 6). On the contrary, the introduction of a nitrogen atom seems detrimental to the activity as shown by the weaker inhibition of compound **3c** on tubulin polymerization (Table 1, entry 7). Finally, the most active compound is *cis* derivative **3b** (3 times more active than its *trans* stereoisomer),[41] which tubulin binding properties are similar to the reference compounds colchicine and deoxypodophyllotoxine (Table 1, entry 6).

We have then decided to further investigate the biological effects of the most promising **3b** compounds on two different epithelial cell lines.

Indeed, epidermal cancers are most common in Man due to the mutagenic action of long-term exposure to solar radiation and the large surface of exposed skin. Most of the skin cancers are treated with surgery. However, multiple lesions, especially when situated at locations rendering surgery difficult because of the functionality or esthetics issues, may require efficient local treatments sparing the surrounding healthy tissue. Also, the related pre-cancerous lesions, for example, actinic keratosis, are a good indication for topical therapies.

Two human keratinocyte lines were selected for this study: a spontaneously immortalized HaCaT line,[42] showing no tumorogeneicity in animal models, and SKv-a line derived from a human papillomavirus (HPV-16)-induced intraepithelial neoplasia.[43] The latter line was selected for its capacity of forming tumors after experimental subcutaneous injection in immunocompromised mice.

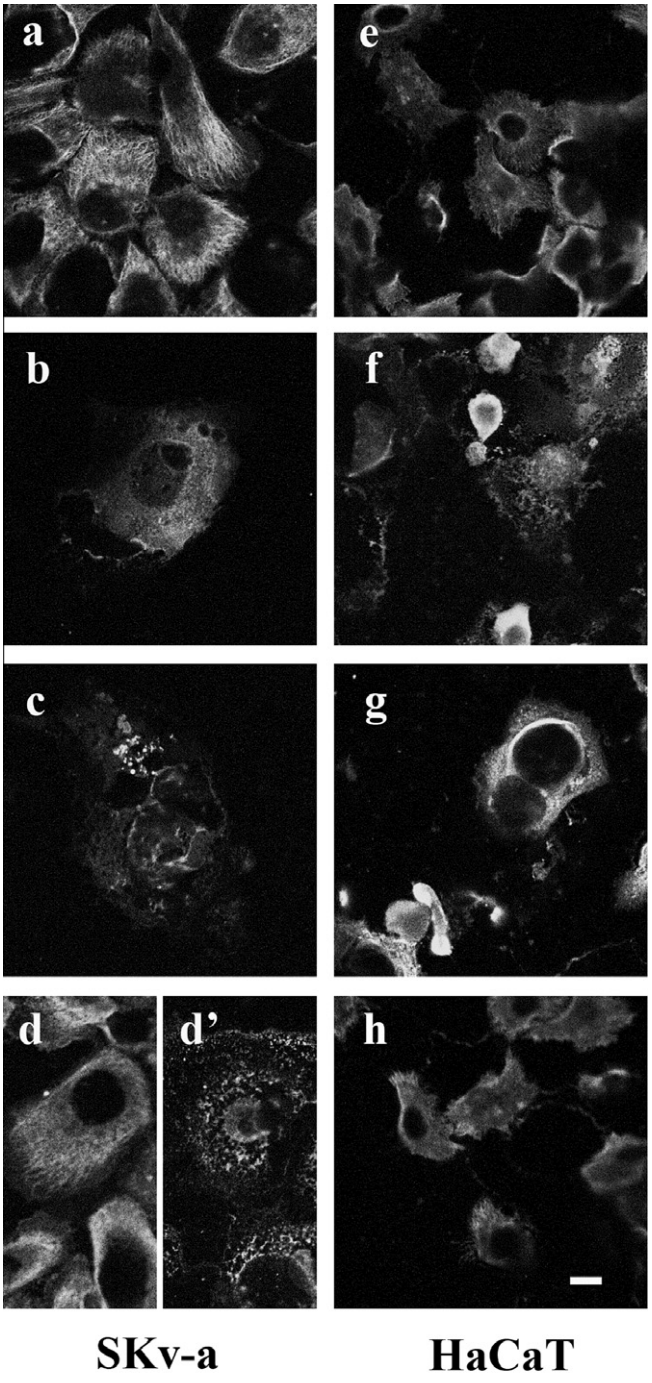


Figure 2. Effect of compounds *cis* and *trans* **3b** and colchicine (a positive control) on the intracellular organization of microtubules in SKv-a cells (left) and HaCaT cells (right). (a,e) vehicle-treated controls; (b,f) colchicine; (c,g) *cis* **3b**, (d,d′,h) *trans* **3b**. Beta-tubulin was labelled with a specific monoclonal antibody (Amersham Life Sciences; CA, USA) revealed with a goat anti-mouse FITC probe (Caltech; Carlsbad, CA, USA). Confocal optical sections were recorded at the objective magnification of ×63. Scale bar corresponds to 10 μm.

Table 2
Anti-proliferative activities of *cis* CA-4, colchicine and *cis* **3b** as measured with MTT test in HaCaT keratinocyte cell line

Entry	Compound	IC_{50}[a] (nM)
1	*cis* CA-4	1
2	Colchicine	12
3	*cis* **3b**	16

[a] A sample's concentration which produces a 50% reduction in cell activity. For details, see Supplementary data.

MTT test evaluating metabolic activity of cells, directly related to the number of viable cells remaining after 72 h exposure, showed that the *cis* isomer of **3b** derivative displayed an anti-proliferative activity on the cultured cells comparable to that of colchicine (Table 2, entries 2 and 3). Nevertheless, compound *cis*

3b was much less active than *cis* CA-4 itself (Table 2, entries 1 and 3).

We then decided to use a 10^{-6} M drug concentration for the cell microtubule organization studies.

Compounds *cis* **3b** and, to a much lesser degree, *trans* **3b** had a major impact on the microtubule network in SKv-a and HaCaT cells, as visualized in Figure 2.

Indeed, pictures in Figure 2 show representative changes induced by the drugs applied at 10^{-6} M. Colchicine and *cis* **3b** treatment resulted in radical changes in cell morphology including cell rounding, detachment, and loss of the radial distribution of structured microtubules (c–f). *Trans* **3b** isomer was less active, leaving several keratinocytes virtually intact (d) whereas the remaining cells presented characteristic microtubule depolymerisation (d′).

These results indicate that the benzothiophene analogue of CA-4 *cis* **3b** shows anti-microtubule activity similar to that of colchicine.

As cell cycle control is the major regulatory mechanism of cell growth, we analyzed the influence of the **3b** compounds on the cell lines by flow cytometry. Cells at the exponential phase of growth were treated with 5×10^{-6} M compounds for 24 h. Then, they were detached from the plates with gentle Accutase treatment (PAA, France) and exposed in suspension to propidium iodide at 0.05 mg/mL in a citrate buffer containing Nonidet P40 for 1 h at 4 °C. FACS analysis was performed on 20,000 cells using human keratinocyte cell lines HaCaT and SKv-a and showed cell cycle arrest in mitosis. This effect was most pronounced in HaCaT cells after 24 h treatment with compound *cis* **3b** (Fig. 3).

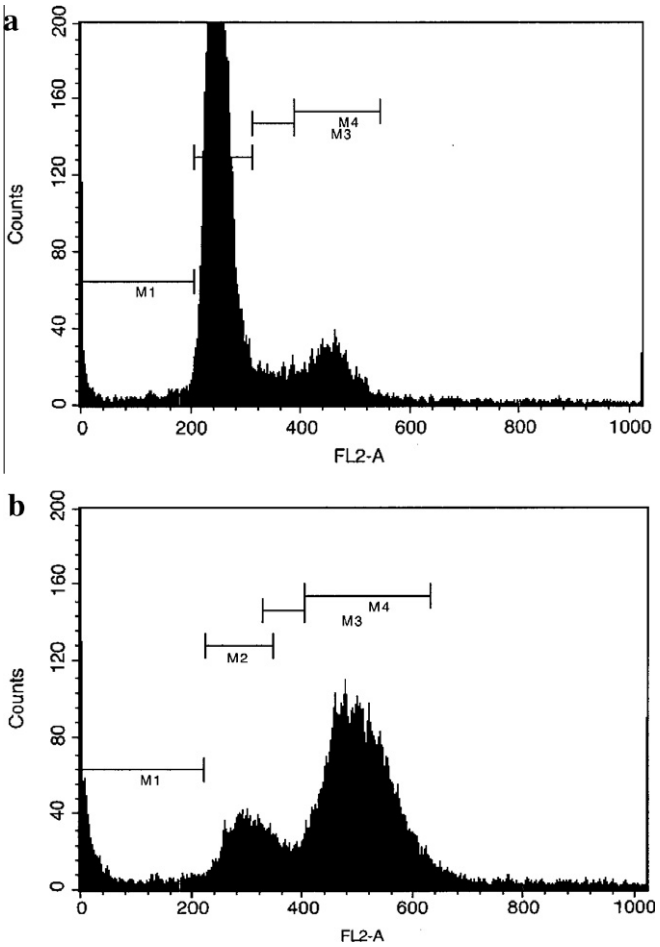


Figure 3. Induction of G2/M phase arrest and polyploid cells in HaCaT cell line treated with compound *cis* **3b**. Human keratinocyte line was treated with the vehicle solvent (0.5% DMSO) (a) or with *cis* **3b** (5×10^{-6} M) (b) for 24 h. Cells were harvested, stained with propidium iodide and 20,000 stained cells were subjected to FACS analysis to determine the distribution of cells throughout the G1, S, and G2/M phases. Experiments were performed in triplicate and yield repeatable results.

To conclude with these preliminary results, we have described herein new CA-4 derivatives, designed after replacement of the B ring with various heterocycles attached to the vicinal heteroatom position. Stereoisomers of these compounds **3a–c** were evaluated for their ability to inhibit tubulin and the benzothiophene series proved to be the most active.

Evaluations of the anti-proliferative and anti-mitotic properties on cutaneous cancer cells and of the effect on the cytoskeleton indicate that compound *cis* **3b** seems to be a good candidate for further developments and structural modifications. Several analogues are currently being synthesized and assessed for their biological properties: these studies will be reported in due course.

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Confocal laser scanning microscopy studies were performed at the Centre Technologique des Microscopies at University Lyon 1 and cell cycle data were obtained at the Laboratoire de Cytologie Analytique, Fédération de Recherche Santé Lyon Est).

Supplementary data

Supplementary data associated (these data include ^{1}H and ^{13}C NMR spectra of the most important compounds described in this article and anti-proliferative effects of *cis* CA-4, colchicine and *cis* **3b** on HaCaT cells using MTT assay) with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.047.

References and notes

1. Dumontet, C.; Jordan, M. A. *Nat. Rev. Drug Disc.* **2010**, *9*, 790.
2. Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. *Med. Res. Rev.* **1998**, *18*, 259.
3. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209.
4. Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666.
5. 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.
6. (a) Lippert, J. W., III *Bioorg. Med. Chem.* **2007**, *15*, 605; (b) Patterson, D. M.; Rustin, G. J. S. *Clin. Oncol.* **2007**, *19*, 443; (c) McKeage, M. J.; Baguley, B. C. *Cancer* **2010**, *116*, 1859.
7. (a) Pettit, G. R.; Temple, C.; Narayanan, V. L.; Varma, R.; Simpson, M. J.; Boyd, M. R.; Rener, G. A.; Bansal, N. *Anti-Cancer Drug Des.* **1995**, *10*, 299; (b) Siemann, D. W.; Chaplin, D. J.; Walicke, P. A. *Expert Opin. Investig. Drugs* **2009**, *18*, 189.
8. (a) Ohsumi, K.; Nakagawa, R.; Fukuda, Y.; Hatanaka, T.; Morinaga, Y.; Nihei, Y.; Ohishi, K.; Suga, Y.; Akiyama, Y.; Tsuji, T. *J. Med. Chem.* **1998**, *41*, 3022; (b) Delmonte, A.; Sessa, C. *Expert Opin. Investig. Drugs* **2009**, *18*, 1541.
9. (a) Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 3033; (b) 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; (c) Shan, Y.; Zhang, J.; Liu, Z.; Wang, M.; Dong, Y. *Curr. Med. Chem.* **2011**, *18*, 523.
10. Marrelli, M.; Conforti, F.; Statti, G. A.; Cachet, X.; Michel, S.; Tillequin, F.; Menichini, F. *Curr. Med. Chem.* **2011**, *18*, 3035.
11. Theeramunkong, S.; Caldarelli, A.; Massarotti, A.; Aprile, S.; Caprioglio, D.; Zaninetti, R.; Teruggi, A.; Pirali, T.; Grosa, G.; Tron, G. C.; Genazzani, A. A. *J. Med. Chem.* **2011**, *54*, 4977.
12. Shirai, R.; Takayama, H.; Nishikawa, A.; Koiso, Y.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1997.
13. Ohsumi, K.; Hatanaka, T.; Fujita, K.; Nakagawa, R.; Fukuda, Y.; Nihei, Y.; Suga, Y.; Morinaga, Y.; Akiyama, Y.; Tsuji, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3153.
14. Lee, S.; Kim, J. N.; Lee, H. K.; Yoon, K. S.; Shin, K. D.; Kwon, B.-M.; Han, D. C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 977.

15. (a) 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; (b) Bellina, F.; Cauteruccio, S.; Monti, S.; Rossi, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5757.
16. Odlo, K.; Fournier-Dit-Chabert, J.; Ducki, S.; Gani, O. A. B. S. M.; Sylte, I.; Hansen, T. V. *Bioorg. Med. Chem.* **2010**, *18*, 6874.
17. Romagnoli, R.; Baraldi, P. G.; Salvador, M. K.; Preti, D.; Tabrizi, M. A.; Brancale, A.; Fu, X.-H.; Li, J.; Zhang, S.-Z.; Hamel, E.; Bortolozzi, R.; Basso, G.; Viola, G. *J. Med. Chem.* **2012**, *55*, 475.
18. Aprile, S.; Del Grosso, E.; Tron, G. C.; Grosa, G. *Drug Metab. Dispos.* **2007**, *35*, 2252.
19. Beside the design of these *cis*-restricted derivatives, other research groups has replaced the alkene linker by an *exo* methylene group (thus leading to *iso*CA-4 derivatives) or by unsaturated carbocycles. For these two approaches, see respectively: (a) Messaoudi, S.; Tréguier, B.; Hamze, A.; Provot, O.; Peyrat, J.-F.; Rodrigo De Losada, J.; Liu, J.-M.; Bignon, J.; Wdzieczak-Bakala, J.; Thoret, S.; Dubois, J.; Brion, J.-D.; Alami, M. *J. Med. Chem.* **2009**, *52*, 4538; (b) Rasolofonjatovo, E.; Provot, O.; Hamze, A.; Rodrigo, J.; Bignon, J.; Wdzieczak-Bakala, J.; Desravines, D.; Dubois, J.; Brion, J.-D.; Alami, M. *Eur. J. Med. Chem.* **2012**, *52*, 22.
20. Simoni, D.; Romagnoli, R.; Baruchello, R.; Rondanin, R.; Rizzi, M.; Pavani, M. G.; Alloatti, D.; Giannini, G.; Marcellini, M.; Riccioni, T.; Castorina, M.; Guglielmi, M. B.; Bucci, F.; Carminati, P.; Pisano, C. *J. Med. Chem.* **2006**, *49*, 3143.
21. 3,4,5-Trimethoxybenzyltriphenylphosphonium bromide was prepared in two steps from 3,4,5-trimethoxybenzaldehyde, according to this reference: Asakawa, Y.; Tanikawa, K.; Aratani, T. *Phytochemistry* **1976**, *15*, 1057.
22. (*Z*) and (*E*) benzofurane derivatives **3a** were isolated with 26% and 35% yields, respectively, whereas (*Z*) and (*E*) benzothiophenes **3b** were isolated with 8% and 46% yields, respectively.
23. L'Helgoual'ch, J.-M.; Seggio, A.; Chevallier, F.; Yonehara, M.; Jeanneau, E.; Uchiyama, M.; Mongin, F. *J. Org. Chem.* **2008**, *73*, 177.
24. (a) Chinchilla, R.; Nájera, C. *Chem. Soc. Rev.* **2011**, *40*, 5084; (b) Chinchilla, R.; Nájera, C. *Chem. Rev.* **2007**, *107*, 874; (c) Doucet, H.; Hierso, J.-C. *Angew. Chem., Int. Ed.* **2007**, *46*, 834.
25. Kaafarani, B. R.; Wex, B.; Strehmel, B.; Neckers, D. C. *Photochem. Photobiol. Sci.* **2002**, *1*, 942.
26. Fürstner, A.; Nikolakis, K. *Liebigs Ann.* **1996**, 2107.
27. Lawrence, N. J.; Ghani, F. A.; Hepworth, L. A.; Hadfield, J. A.; McGown, A. T.; Pritchard, R. G. *Synthesis* **1999**, *9*, 1656.
28. Giraud, A.; Provot, O.; Hamze, A.; Brion, J.-D.; Alami, M. *Tetrahedron Lett.* **2008**, *49*, 1107.
29. ^{1}H NMR spectrum of the crude product showed a 62/38 *Z*/*E* ratio. Replacement of the triethyl silyl group by a ethoxy dimethyl silyl one did not improve the ratio in favor of the *Z* isomer, as a 57/43 *Z*/*E* ratio was obtained in this case.
30. Li, J.; Hua, R.; Liu, T. *J. Org. Chem.* **2010**, *75*, 2966.
31. The *Z*/*E* ratio was estimated to be 93/7, by ^{1}H NMR on the crude product: traces of the *E* isomer were removed by flash chromatography on silica gel (pre-treated with Et_3N). Despite a careful purification, the stereopure compound (*Z*)-**3a** was isolated with a 58% yield, together with another fraction (34%) which contained a mixture of *Z*/*E* compounds.
32. Mahboobi, S.; Uecker, A.; Sellmer, A.; Cénac, C.; Höcher, H.; Pongratz, H.; Eichhorn, E.; Hufsky, H.; Trümpler, A.; Sicker, M.; Heidel, F.; Fischer, T.; Stocking, C.; Elz, S.; Böhmer, F.-D.; Dove, S. *J. Med. Chem.* **2006**, *49*, 3101.
33. Ketcha, D. M.; Lieurance, B. A.; Homan, D. F. J.; Gribble, G. W. *J. Org. Chem.* **1989**, *54*, 4350.
34. Yasuhara, A.; Sakamoto, T. *Tetrahedron Lett.* **1998**, *39*, 595.
35. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
36. Gaukroger, K.; Hadfield, J. A.; Hepworth, L. A.; Lawrence, N. J.; McGown, A. T. *J. Org. Chem.* **2001**, *66*, 8135.
37. *Z*-Monobromotrimethoxystyrene (91/9 *Z*/*E* mixture) was prepared in two steps from 3,4,5-trimethoxybenzaldehyde, according to this reference: Herz, H.-G.; Queiroz, M. J. R. P.; Maas, G. *Synthesis* **1999**, *6*, 1013.
38. Kassis, P.; Bénéteau, V.; Mérour, J.-Y.; Routier, S. *Synthesis* **2009**, *14*, 2447.
39. Chakrabarty, M.; Kundu, T.; Harigaya, Y. *Synth. Commun.* **2006**, *36*, 2069.
40. Lewin, G.; Aubert, G.; Thoret, S.; Dubois, J.; Cresteil, T. *Bioorg. Med. Chem.* **2012**, *20*, 1231.
41. Previous work reported that *cis* CA-4 was 30 times more potent than its *trans* isomer for the inhibition of tubulin polymerization: Pettit, G. R.; Rhodes, M. R.; Herald, D. L.; Hamel, E.; Schmidt, J. M.; Pettit, R. K. *J. Med. Chem.* **2005**, *48*, 4087.
42. Boukamp, P.; Petrussevska, R. T.; Breitkreutz, D.; Hornung, J.; Markham, A.; Fusenig, N. E. *J. Cell Biol.* **1988**, *106*, 761.
43. Schneider-Manoury, S.; Pehau-Arnaudet, G.; Breitburd, F.; Orth, G. *J. Gen. Virol.* **1990**, *71*, 809.