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# Caulerpenyne–colchicine hybrid: Synthesis and biological evaluation

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**Abstract**—The synthesis of an analog of caulerpenyne having a trimethoxyaryl moiety was achieved in 11% overall yield over 11 steps. Its biological activity has been evaluated as inhibitor of in vitro tubulin polymerization or angiogenesis.


## 1. Introduction

Marine algae of the order Caulerpales are known for their chemical defense against predators by producing secondary metabolites. The majority of these compounds are sesquiterpenoids and diterpenoids, often acyclic. The terminal 1,4-diacetoxybutadiene moiety is a common functional group to most of these metabolites and uniquely found in this group of marine algae. Nowadays, more than 30 toxins with this moiety have been isolated from the Udoteaceae and Caulerpaceae families such as caulerpenyne, flexiline, dihydrorhipocephaline, and crispatenine (Fig. 1).[1] The 1,4-Diacetoxybutadiene moiety represents an acetylated bis-enol form of the 1,4-dialdehyde constellation, to which a high degree of biological activity is generally attributed what we have recently confirmed.[2]

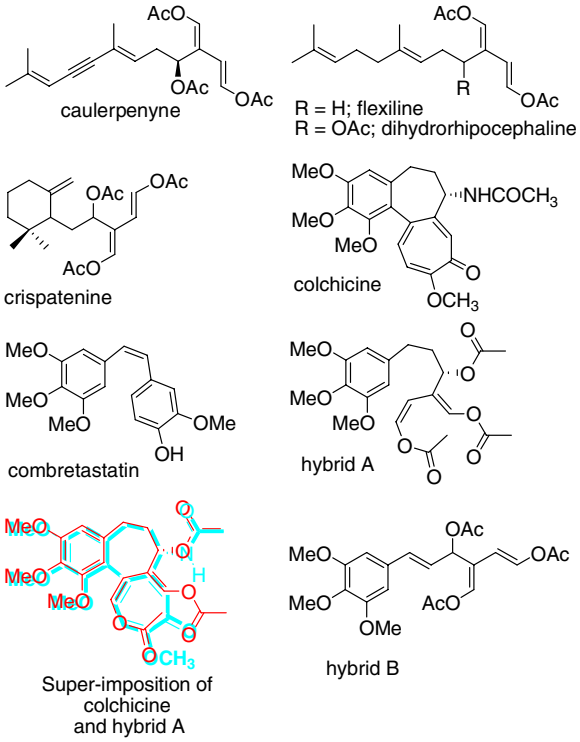


**Figure 1.**

*Keywords*: Caulerpenyne; Colchicine; Biological evaluation; Tubulin polymerization; Cell cytotoxicity; In vitro angiogenesis.
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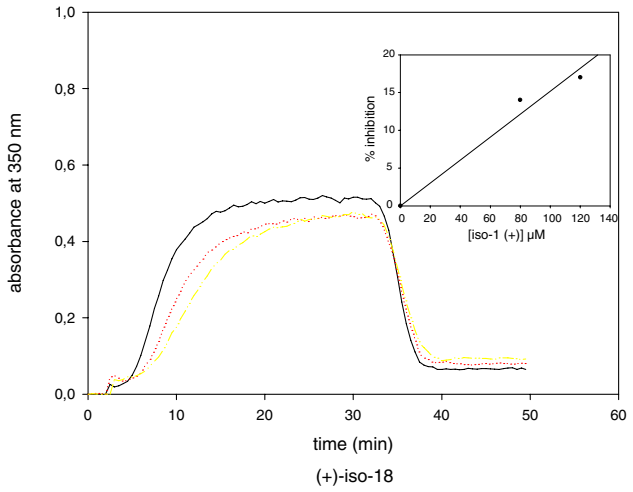



**Figure 2.** Effect of (+)-*iso*-**18** on tubulin assembly in microtubules.

Indeed, some metabolites containing this moiety have been implicated in chemical defense against grazing fishes and invertebrates in herbivore-rich tropical waters and this has, for example, been proposed to explain the proliferation from Italy to Spain of *Caulerpa taxifolia*, a tropical green seaweed accidentally introduced in the Mediterranean sea. From *C. taxifolia* were isolated nine mono- and sesquiterpenes such as caulerpenyne[3] (CYN) which represents the main toxin of this algae and well known for its important biological activity.[4] We have also demonstrated that CYN has an antiproliferative activity on tumor cell line SK-N-SH, modifies their microtubule network, and inhibits the brain purified tubulin and microtubule proteins polymerization with $IC_{50}$ in a range of 20–50 µM.[5]

Tubulin, the major structural component of microtubules, participates actively in mitotic spindle formation and chromosomal organization during cell division. Thus, this protein is a target of anti-mitotic drugs called anti-tubulin agents. Since only a few molecules like taxoids or Vinca-alkaloids are used for cancer chemotherapy, a lot of energy is being spent in finding new antimitotic drugs. One of the approaches consists in the synthesis and analysis of numerous analogs of colchicine (Fig. 1), a well-known natural cytotoxic product which inhibits the polymerization of microtubule with an $IC_{50}$ of 1.7 ± 0.09 µM.[6] However, the toxicity of colchicine has hampered its use in cancer chemotherapy so far. During the search of new colchicine analogs derivatives or not from the natural product, a series of compounds, which interact with the colchicine-binding site of tubulin, were discovered. For example, Combretastatin A-4 phosphate, the most promising antitumor agent, recently passed phase II clinical trials.

In a similar way, to find a new potential molecule which can inhibit the microtubule formation process, we thought about a compound possessing the more biologically active fragment of caulerpenyne (diacetoxybutadiene moiety) and the cycle A (trimethoxybenzene moiety recognized by tubuline) of colchicine instead of the terminal unsaturated alkyl chain of CYN.

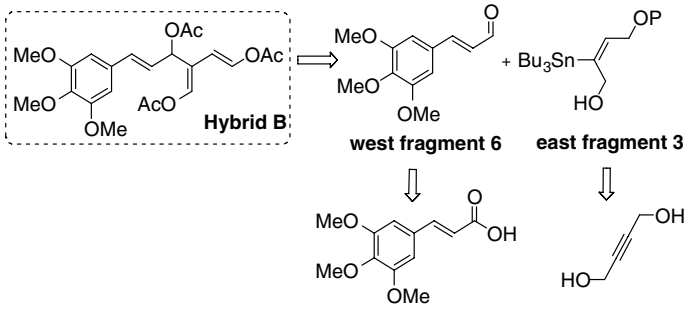
Herein, we report our investigation on the synthesis and the biological evaluation of two colchicine–caulerpenyne hybrids **A** and **B** (Fig. 1). Our interest for the synthesis of hybrid **A** was justified by a perfect superimposition with colchicine in which all heteroatoms or basic functions overlap. Moreover, there is an obvious flexibility of the alkyl chain compared to the rigidity of the seven-membered ring of the colchicine. A good reason for the synthesis of hybrid **B** is that it includes a bis-allylic acetate function which could be easily replaced by a thio- or amino-moiety during binding with the amino-acids of the tubulin. Moreover, numerous analogs furnishing a library of colchicine analogs could be easily prepared by allylic substitution.

## 2. Results and discussion

### 2.1. Synthesis

For a ready access, synthesis of hybrid **B** was investigated first. Our strategy for synthesizing hybrid **B** from the commercially available cinnamic acid and but-2-yn-1,4-diol is outlined in Scheme 1 and was derived to the one we applied in our recent synthesis of caulerpenyne and dihydrorhipocephaline.[2,7] Hybrid **B** was obtained by a coupling reaction between the vinylstannanes (east segment) (tin–lithium exchange) derived from butynediol and trimethoxycinnamaldehyde (west segment).

Synthesis of the vinyl segment **3** began with palladium complex catalyzed hydrostannation[8] of but-2-yn-1,4-diol 1 to give (*E*)-vinyltin reagent **2** in which the more accessible alcohol function was selectively protected as

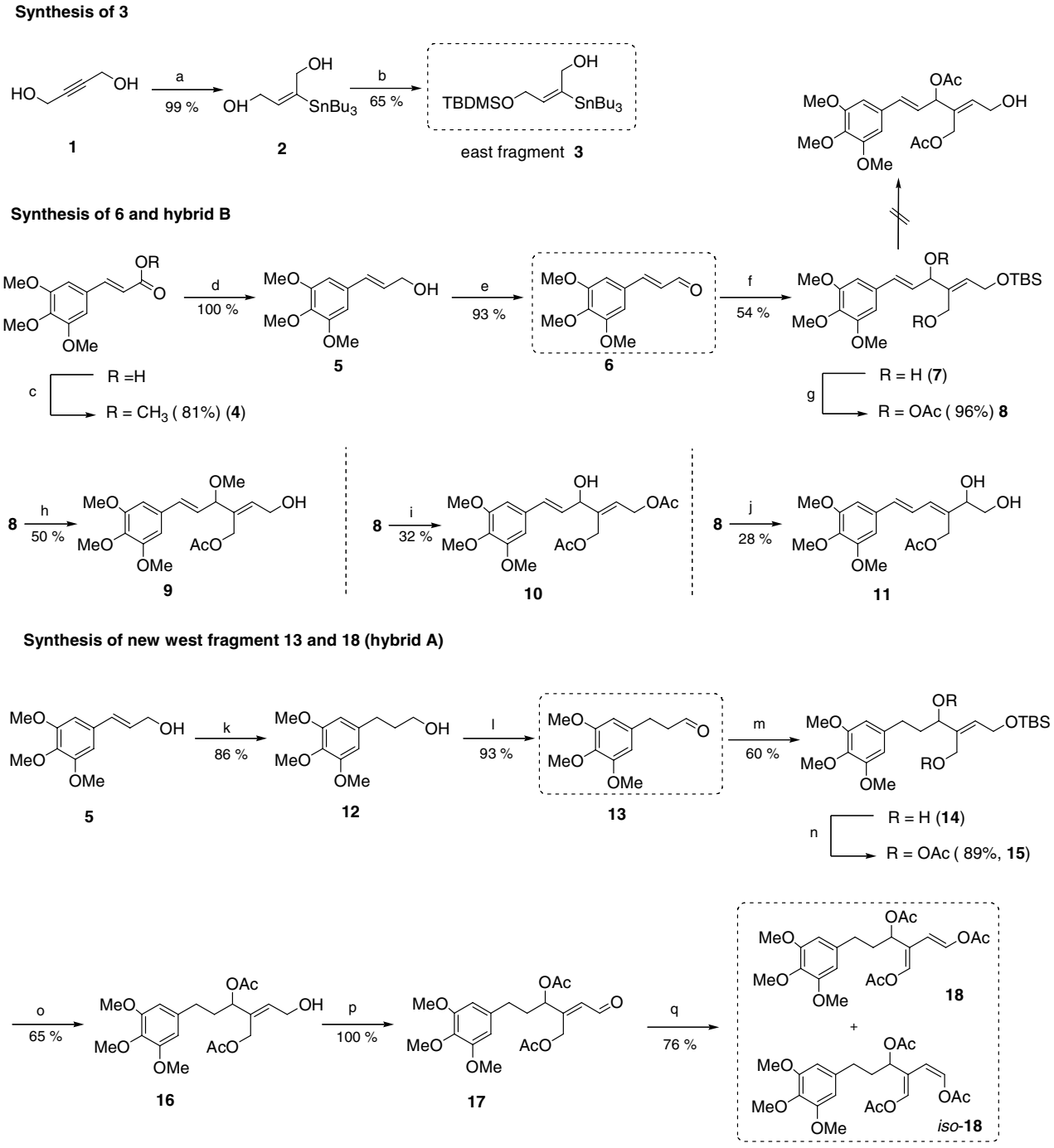



**Scheme 1.**

*tert*-butyldimethylsilyl ether in 64% yield over two steps.[9]

Synthesis of west fragment **6** was obtained over three steps and is resumed in Scheme 2. Trimethoxycinnamic acid was converted into the corresponding ester **4** in 81% yield.[10] Reduction of **4** using 2 equiv of Dibal-H in DCM at −78 °C furnished the alcohol **5** in quantitative yield. Finally, oxidation of the allylic alcohol function with $MnO_2$ gave the desired west fragment **6** in 93% yield.[11] (75% yield over three steps).

The assembly of the fragments **3** and trimethoxycinnamaldehyde **6** and the construction of carbon skeleton of the hybrid **B** are described in Scheme 2. The coupling reaction between west segment **6** and the carbanion generated by tin–lithium exchange reaction[9a] on the east segment **3** gave diol **7** in fair yield (54%). At this stage,




a) $Bu_3SnH$, $PdCl_2(PPh_3)_2$, THF; b) TBDMSCl, imidazole, DMF, 0°C; c) MeOH, $H_2SO_4$, Δ; d) Dibal-H, $CH_2Cl_2$, - 78 °C; e) $MnO_2$, EtOAc; f) *(i)* MeLi.LiBr, - 35 °C; *(ii)* **3**, - 35 °C; g) $Ac_2O$, DMAP, Pyridine; h) MeOH, $NH_4F$; i) TBAF, THF; j) AcOH, THF, $H_2O$ k) nickel acetate tetrahydrated, $NaBH_4$, EtOH; l) Dess-Martin Periodinane, $CH_2Cl_2$; m) *(i)* MeLi.LiBr, - 35 °C; *(ii)* **3**, - 35 °C; n) $Ac_2O$, DMAP, Pyridine; o) HF.Pyridine, THF; p) Dess-Martin Periodinane, $CH_2Cl_2$; q) $Ac_2O$, DMAP, $NEt_3$, 80 °C

**Scheme 2.**

the two hydroxyl groups of **7** were protected as acetates using acetic anhydride and a catalytic amount of DMAP in pyridine to give crude clean bis-acetate **8** (96%). The next step is the desilylation[12] of **8** by the complex HF-pyridine to provide the corresponding alcohol. Unfortunately the desired alcohol was never observed, only unidentified degradation products were obtained. Several conditions were tested but no one gave expected results. Using ammonium fluoride in THF only starting material was recovered at the end of reaction. The same reaction conducted in *tert*-butanol furnished several unidentified products. Ammonium fluoride in MeOH underwent to the deprotection of the silyl ether but the secondary acetate was replaced by methoxy group furnishing **9** in 50% yield. Migration of the acetate function from the secondary to the primary alcohol was observed using anhydrous TBAF in THF giving **10** in 32% yield. Same results were already described for the synthesis of caulerpenyne.[7a] The last trial was to use a mixture of acetic acid in aqueous THF. One more time these conditions gave poor results and diol **11** was obtained in only 28% yield. All these results show that the bis-allylic acetate between the double bond is very sensitive to the reaction conditions used and can undergo to the corresponding allylic carbocation which can react with various nucleophiles.

Due to the very low yields obtained, we turned our attention to the hybrid **A** in which the central double bond was replaced by a single carbon–carbon bond. From the allylic alcohol **5**, the double bond was reduced using nickel acetate, sodium borohydride, and hydrogen to give the desired compound **12** in 86% yield. Oxidation of the primary alcohol using Dess–Martin periodinane[13] furnished the new west fragment **13** in 93% yield (65% yield over four steps from trimethoxycinnamic acid).

As above the assembly between west segment **13** and the carbanion generated by tin–lithium exchange reaction on the east segment **3** gave diol **14** in 60% yield. Protection of diol **14** into diacetates using standard conditions afforded **15** in 89% yield. This time, deprotection of the silyl ether function with HF-pyridine furnished the desired alcohol **16** in 65% yield. The alcohol function was then oxidized with Dess–Martin periodinane to give the sensitive aldehyde **17** in quantitative yield. To achieve the synthesis of the colchicine–caulerpenyne hybrid, the last step was the construction of the diacetoxybutadiene moiety. We used the conditions developed by our group[7b] ($NEt_3$, DMAP, $Ac_2O$ at 80 °C) and our target was obtained in 76% yield as a 1:1 mixture of two racemic diastereomers **18** and **18′**. Separation of each enantiomer was realized by semi-preparative chiral HPLC to give (+)-**18**, (−)-**18**, (+)-*iso*-**18**, and (−)-*iso*-**18**.[14]

## 2.2. Biology

**2.2.1. Cell cytotoxicity.** Since CYN and colchicine are cytotoxic in several tumor cell lines,[4] we determined the cytotoxicity of the different isomers of **18** and **18′** on HaCaTs cells (keratynocytes, immortalized skin cells). Results are consigned in Table 1 and Figure 3. Figure 3 shows the percentage of living cells, determined by the MTT assay, after treatment with isomers of **18** and *iso*-**18** (1–10 μM) during 72 h. No significant effects were observed for concentrations lower than 1 μM. After 72 h of treatment, the $IC_{50}$ were $2.8 \pm 0.2$, $2.0 \pm 0.5$, $4.0 \pm 0.1$, and $1.4 \pm 0.1$ μM for (+)-**18**, (−)-**18**, (+)-*iso*-**18**, and (−)-*iso*-**18**, respectively. We conclude that these CYN–colchicine hybrids are less active than colchicine but induced a concentration-dependent inhibition of HaCaTs cells which is of the same order as the $IC_{50}$ observed for CYN on the same cells.[15]

**2.2.2. Tubulin polymerization.** Because colchicine and CYN inhibit the microtubule formation in vitro, it was interesting to determine the effect of the four isomers on this biological activity. The in vitro purified tubulin polymerization process was investigated by turbidimetry (Fig. 2). Increase in absorption at 350 nm results in an increase of microtubule mass. Tubulin was incubated for 35 min at 37 °C without (control) or with various concentrations of (+)-**18**, (−)-**18**, (+)-*iso*-**18**, and (−)-*iso*-**18** in a $Mg^{2+}$-free polymerization buffer. After 35 min, the polymerization process was induced by adding 10 mM $Mg^{2+}$. In the presence of drugs, we observed only slight decrease in turbidity and concentration up to 100 μM was necessary to reduce the polymerization by 20%. The $IC_{50}$ of all isomers of **18** and **18′** are higher than 100 μM, whereas the $IC_{50}$ of natural CYN is around 20 μM and colchicine 2 μM. On the contrary to what we excepted, this molecule, which possesses both colchicine and CYN moiety known to be involved in the biological activity on tubulin, was not a powerful microtubule formation inhibitor in vitro.

**2.2.3. In vitro angiogenesis.** Angiogenesis (the formation of new blood vessels from pre-existing ones) is a key process in both tumor growth and metastasis. This can be inhibited by conventional anti cancer drugs such as anti-tubulin agent. Thus, the effect of one CYN hybrid was investigated on the in vitro angiogenesis by measuring the inhibition of capillary network formation on Matrigel™ with HMEC-1 cells (Human Microvascular Endothelial Cell line). At low concentration (up to 1 μM), there was no significant inhibition of angiogenesis (Fig. 4). High concentrations (10 μM) were necessary to observe more than 50% inhibition. In comparison, paclitaxel inhibited in vitro angiogenesis with a concentration near by 1 nM, a 10,000-fold lower concentration.[16]

**Table 1.** Cytotoxicity on HaCaTs cells and inhibition of polymerization of tubulin of (+)-18, (−)-18, (+)-*iso*-18, and (−)-*iso*-18

| Products | Cytotoxicity $IC_{50}$ (μM) | Tubulin polymerization $IC_{50}$ (μM) |
|---|---|---|
| (+)-**18** | $2.8 \pm 0.2$ | >100 |
| (−)-**18** | $2.0 \pm 0.5$ | >100 |
| (+)-*iso*-**18** | $4.0 \pm 0.1$ | >100 |
| (−)-*iso*-**18** | $1.4 \pm 0.1$ | >100 |

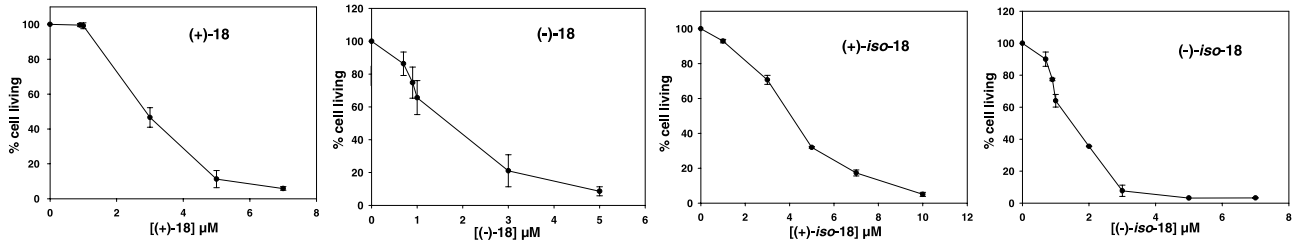



**Figure 3.** Cytotoxicity on HaCaTs cells of (+)-**18**, (−)-**18**, (+)-*iso*-**18**, and (−)-*iso*-**18**.

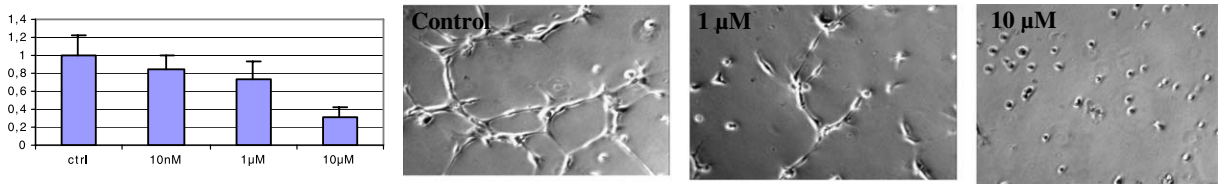



**Figure 4.** Percentage of total capillary tube length in function of (+)-**18** concentrations.

## 3. Conclusion

We synthesized a new hybrid compound of caulerpenyne and colchicine in 11 steps with 11% yield. Since the different isomers of this hybrid were not powerful inhibitor of in vitro tubulin polymerization process and angiogenesis, they showed an acceptable cytotoxicity on HaCaTs cells (around 2.5 µM).

## 4. Experimental

### 4.1. Generality

All reactions sensitive to oxygen and moisture were carried out in oven-dried glassware under a slight positive pressure of argon unless otherwise noted. $^{1}$H NMR and $^{13}$C NMR spectra were determined on a Bruker, AC300.

Chemical shifts for $^{1}$H NMR were reported in parts per million (ppm) downfield from tetramethylsilane as the internal standard and coupling constants are in hertz (Hz). Chemical shifts for $^{13}$C NMR were reported in ppm relative to the central line of a triplet at 77.16 ppm for deuteriochloroform. Reagents and solvents were of commercial grades and were used as supplied. Dichloromethane, benzene, and toluene were distilled from calcium hydride and stored over molecular sieves 4 Å. THF and diethyl ether were distilled from sodium benzophenone prior to use. *N*,*N*-Dimethylformamide was purchased anhydrous and stored over molecular sieves 4 Å under argon. Mass spectra (MS) were performed with a Sciex API III Plus triple quadrupole system with a pneumatically assisted electrospray interface (Sciex, Thornhill, Canada). The analytical chiral HPLC experiments were performed on a unit composed of a Merck D-7000 system manager, Merck-Lachrom L-7100 pump, Merck-Lachrom L-7360 oven, Merck-Lachrom L-7400 UV-detector, and on-line Jasco CD-1595 circular dichroism.

Hexane and ethanol, HPLC grade, were degassed and filtered on a 0.45 µm membrane before use. The column used is Chiralcel OD-H (250 × 4.6 mm) from ChiralTechnologies Europe (Illkirch, France). Retention times $t_R$ in minutes, retention factors $k_i = (t_{R_i} - t_{R_0})/t_{R_0}$, and enantioselectivity factor $\alpha = k_2/k_1$ are given. $t_{R_0}$ was determined by injection of tri-tertio-butyl benzene. The sign given by the on-line circular dichroism is the sign of the product in the solvent used for the chromatographic separation. The two analyses were performed at 25 °C, with hexane/ethanol (95/5) as mobile phase and 1 mL/min as flow-rate, with UV and CD at 254 nm. **18** $t_{R_1}(-) = 14.58$, $t_{R_2}(+) = 17.87$, $k_1(-) = 3.78$, $k_2(+) = 4.86$, $\alpha = 1.29$. *iso*-**18**: $t_{R_1}(-) = 12.84$, $t_{R_2}(+) = 16.52$, $k_1(-) = 3.21$, $k_2(+) = 4.42$, $\alpha = 1.38$. Semi-preparative separations were performed on an unit composed of Merck D-7000 system manager, Merck-Hitachi L-6000 pump, Rheodyne valve with a 500 µL loop, and a Merck-Hitachi L-4000 UV-detector. For **18** and *iso*-**18**, Chiralcel OD-H (250 × 10 mm) was used with hexane/ethanol (95:5) as mobile phase, 1 mL/min as flow-rate, and UV at 254 nm. HMEC-1 were obtained from the Cell Culture Laboratory in the Hôpital de la Conception (Assistance publique des Hôpitaux de Marseille, France). They were routinely maintained in culture at 37 °C and 5% $CO_2$. HMEC-1 were grown in MDCB-131 medium (Life Technologies) containing 10% heat-inactivated fetal bovine serum, 2 mmol/L glutamine, 1% penicillin and streptomycin, 1 µg/ml hydrocortisone (Pharmacia & Upjohn, St-Quentin-Yvelines, France), and 10 ng/ml epithelial growth factor (R&D Systems, Minneapolis, MN). HaCaTs were obtained from ATCC. They were routinely maintained in culture at 37 °C and 5% $CO_2$. HaCaTs were grown in DMEM (Dulbecco's modified Eagle's medium) containing 10% fetal bovine serum, 1% penicillin and streptomycin, and 2% glutamine. The drugs were prepared in DMSO and maintained frozen at −20 °C.

### 4.2. (*E*)-2-Tributylstannylbut-2-ene-1,4-diol (2)[8]

To a THF solution (15 mL) of but-2-yn-1,4-diol (2.58 g, 30 mmol) and $PdCl_2(PPh_3)_2$ (420 mg, 2 mol%) was added dropwise a THF solution (20 mL) of tributyltin hydride (9.68 mL, 36 mmol) over a period of 1 h. The originally light yellow solution abruptly turned orange-brown. After stirring over 15 min, THF was evaporated under vacuum. The crude product was then purified by flash chromatography (light petroleum/$Et_2O$, 6:4–2:8) to give **2** in 99% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.84–0.93 (m, 15H; $3\times CH_3$, $3\times CH_2$), 1.23–1.35 (m, 6H; $3\times CH_2$), 1.43–1.53 (m, 6H; $3\times CH_2$), 1.64 (br t, $J$ = 5.6 Hz, 1H; OH), 1.73 (br t, $J$ = 5.2 Hz, 1H; OH), 4.18 (br t, $J$ = 5.4 Hz, 2H; $CH_2$), 4.36 (br d, $J$ = 3.7 Hz, $^3J_{Sn,H}$ = 37 Hz, 2H; $CH_2$), 5.77 (br t, $J$ = 5.4 Hz, $^3J_{Sn,H}$ = 67 Hz, 1H; CH).

### 4.3. (*E*)-4-*tert*-Butyldimethylsilyloxy-2-tributylstannyl but-2-en-1-ol (3)[9a]

To a solution of **2** (3.8 g, 10 mmol) in DMF (100 mL) at 0 °C were added imidazole (0.68 g, 10 mmol) and *tert*-butyldimethylsilylchloride (1.5 g, 10 mmol). The solution was stirred at 0 °C for 6 h and then crushed ice (2.5 g) was added. The solution was diluted with $Et_2O$ (200 mL), washed with saturated aqueous $NH_4Cl$ solution, dried over $MgSO_4$, and evaporated. The crude product was then purified by flash chromatography (light petroleum/$Et_2O$, 10:0–9:1) to give **3** in 65% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.06 (s, 6H; $2\times CH_3$), 0.84–0.92 (m, 15H; $3\times CH_3$, $3\times CH_2$), 0.88 (s, 9H, $3\times CH_3$), 1.23–1.53 (m, 12H; $6\times CH_2$), 1.80 (br t, $J$ = 5.5 Hz, 1H; OH), 4.20 (br d, $J$ = 5.4 Hz, $^4J_{Sn,H}$ = 15 Hz, 2H; $CH_2$), 4.32 (br d, $J$ = 5.5 Hz, $^3J_{Sn,H}$ = 37 Hz, 2H; $CH_2$), 5.68 (br t, $J$ = 5.4 Hz, $^3J_{Sn,H}$ = 69 Hz, 1H; CH).

### 4.4. 3,4,5-Trimethoxymethylcinnamat (4)

Compound **4** was prepared according to a modified procedure of Peterson et al.[10] A solution of cinnamic acid (5 g, 21 mmol) and $H_2SO_4$ (five drops) in MeOH (42 ml) was warmed to reflux overnight. The solution was then cooled to room temperature and the product **4** precipitated. The solid was filtered off, dried under vacuum to give **4** in 81% yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 3.79 (s, 3H), 3.87 (br s, 9H), 6.34 (d, 1H, $J$ = 16.1 Hz), 6.74 (s, 1H), 7.59 (d, 1H, $J$ = 16.1 Hz).

### 4.5. (*E*)-3-(3,4,5-Trimethoxy-phenyl)-prop-2-en-1-ol (5)[17]

To a solution of **4** (3 g, 11.89 mmol) in $CH_2Cl_2$ (80 ml) at −78 °C, under argon, was added dropwise Dibal-H (17.45 ml, 26.16 mmol, 1.5 M in toluene). The mixture was stirred for 1 h and half at −78 °C and MeOH (15 ml) was added. The solution was warmed to room temperature. A saturated aqueous potassium sodium tartrate solution was added and the mixture was stirred for 2 h. The aqueous layer was extracted with ethyl acetate and the organic layers were washed with a saturated aqueous NaCl solution, dried over $MgSO_4$, and concentrated. The crude product was then purified by flash chromatography (light petroleum/$Et_2O$, 2:8) to give **5** in quantitative yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 3.82 (s, 3H), 3.84 (s, 6H), 4.29 (dd, 2H, $J$ = 5.7, 1.3 Hz), 6.25 (dt, 1H, $J$ = 5.7, 15.9 Hz), 6.50 (d, 1H, $J$ = 15.9 Hz), 6.58 (s, 2H).

### 4.6. (*E*)-3-(3,4,5-Trimethoxy-phenyl)-prop-2-enal (6)[18]

To a solution of $MnO_2$ (7.17 g, 82.5 mmol) in ethyl acetate (120 ml) was added a solution of **5** (1.85 g, 8.25 mmol) in ethyl acetate (40 ml). The solution was stirred overnight, filtered over Celite®, and concentrated. The crude product **6** was obtained in 93% yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 3.90 (s, 9H), 6.64 (dd, 1H, $J$ = 15.9, 7.8 Hz), 6.79 (s, 2H), 7.4 (d, 1H, $J$ = 15.9 Hz), 9.69 (d, 1H, $J$ = 7.8 Hz).

### 4.7. 2-[2-(1,1,2,2-Tetramethyl-propoxy)-ethylidene]-5-(3,4,5- trimethoxy-phenyl)-pent-4-ene-1,3-diol (7)

To a solution of **3** (2 g, 4.07 mmol) in THF (50 ml) was added, at −35 °C and under argon, dropwise MeLi·LiBr (1.5 M in $Et_2O$, 5.7 ml, 8.547 mmol). The solution was kept at −35 °C for 2 h and then **6** (995 mg, 4.477 mmol) was added. The solution was kept at −35 °C for 24 h and then quenched with a $NH_4Cl$ solid (650 mg). The mixture was filtered, concentrated, and then purified by flash chromatography (light petroleum/$Et_2O$, 7:3 → 3:7 → 0/1) to give **7** in 54% yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.10 (s, 6H), 0.91 (s, 9H), 2.37 (br s, OH), 2.52 (br s, OH), 3.84 (s, 3H), 3.87 (s, 6H), 4.27 (m, 2H), 4.33 (d, 2H, $J$ = 5.9 Hz), 4.90 (m, 1H), 5.83 (t, 1H, $J$ = 5.9 Hz), 6.18 (dd, 1H, $J$ = 15.9, 6.04 Hz), 6.59 (d, 1H, $J$ = 15.9 Hz), 6.61 (s, 2H); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ −5.2 ($2\times CH_3$), 18.3 (C), 25.9 ($3\times CH_3$), 56.1 ($2\times CH_3$), 58.5 ($CH_2$), 59.6 ($CH_2$), 60.9 ($CH_3$), 76.3 (CH), 103.6 ($2\times CH$), 129.4 (CH), 129.6 (CH), 130.7 (CH), 132.4 (C), 137.8 (C), 140.9 (C), 153.3 ($2\times C$); MS: $m/z$ (ESI+) 442 $[M+NH_4]^+$.

### 4.8. Acetic acid 2-acetoxymethyl-4-(1,1,2,2-tetramethyl-propoxy)-1-[2-(3,4,5-trimethoxy- phenyl)-vinyl]-but-2-enyl ester (8)

A solution of **7** (420 mg, 0.990 mmol), acetic anhydride (0.374 ml, 3.959 mmol), and DMAP (6 mg) in pyridine (5 ml) under argon was stirred overnight. The mixture was quenched with a saturated aqueous $NaHCO_3$ solution and the aqueous layer was extracted with ethyl ether. The combined organic layers were washed with saturated aqueous $CuSO_4$ solution and water, dried over $MgSO_4$, and concentrated under vacuum to give **8** in 96% yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.08 (s, 6H), 0.90 (s, 9H), 2.03 (s, 3H), 2.10 (s, 3H), 3.84 (s, 3H), 3.87 (s, 6H), 4.37 (d, 2H, $J$ = 5.8 Hz), 4.63 (d, 1H, $J$ = 12.5 Hz), 4.70 (d, 1H, $J$ = 12.5 Hz), 5.90 (d, 1H, $J$ = 7.2 Hz), 5.97 (t, 1H, $J$ = 5.8 Hz), 6.05 (dd, 1H, $J$ = 15.7, 7.2 Hz), 6.57 (d, 1H, $J$ = 15.7 Hz), 6.60 (s, 2H); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ −5.1 ($2\times CH_3$), 18.4 (C), 21.0 ($3\times CH_3$), 21.3 ($CH_3$), 26.0 ($CH_3$), 56.2 ($2\times CH_3$), 59.6 ($CH_2$), 59.8 ($CH_2$), 61.0 ($CH_3$), 75.5 (CH), 103.9 ($2\times CH$), 125.1 (CH), 131.9 (C), 132.4 (C), 133.5 (CH), 134.5 (CH), 138.4 (C), 153.4 ($2\times C$), 169.8 (C), 170.8 (C); MS: $m/z$ (ESI+) 526 $[M+NH_4]^+$.

### 4.9. 3-(3,4,5-Trimethoxy-phenyl)-propan-1-ol (12)[19]

In an Erlenmeyer, were placed nickel acetate tetrahydrated (4.06 g, 16.3 mmol) and 95% ethanol (110 ml). The solution was purged with $H_2$, then a solution of $NaBH_4$ (16 mmol, 1 M in absolute ethanol, 16 ml) was added. The black mixture was stirred for 30 min and then a solution of **5** (3.66 g, 16.3 mmol) in 95% ethanol (110 ml) was added. The mixture was stirred overnight, filtered on Celite®, and concentrated. Diethyl ether was added and the organic layer was washed with a saturated aqueous NaCl solution, dried over $MgSO_4$, and concentrated to give the crude product **12** in 86% yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.9 (m, 3H), 2.65 (m, 2H), 3.69 (t, 2H, $J$ = 6.4 Hz), 3.82 (s, 3H), 3.84 (s, 6H), 6.42 (s, 2H).

### 4.10. 3-(3,4,5-Trimethoxy-phenyl)-propan-1-al (13)[19]

To a solution of **12** (3.1 g, 13.7 mmol) in $CH_2Cl_2$ (380 ml), under argon, at 0 °C, was added Dess–Martin periodinane (6.97 g, 16.4 mmol). The reaction mixture was stirred at room temperature and monitored by TLC. After disappearance of the starting material, the mixture was poured into a saturated aqueous solution of $Na_2S_2O_3$/$NaHCO_3$ (700 ml, 1:1) and shaken vigorously for 5 min. The aqueous layer was extracted with diethyl ether. The combined organic layers were washed with a saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash chromatography (light petroleum/$Et_2O$, 6:4) to give **13** in 93% yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 2.75–2.92 (m, 4 H), 3.81 (s, 3H), 3.84 (s, 6H), 6.40 (s, 2H), 9.82 (s, 1H).

### 4.11. 2-[2-(1,1,2,2-Tetramethyl-propoxy)-ethylidene]-5-(3,4,5- trimethoxy-phenyl)-pentane-1,3-diol (14)

To a solution of **3** (150 mg, 0.305 mmol) in THF (5 ml), under argon, at −35 °C, was added dropwise MeLi·LiBr (1.5 M in $Et_2O$, 0.427 ml, 0.641 mmol). The solution was kept at −35 °C for 2 h and then **13** (75 mg, 0.336 mmol) was added. The solution was kept at −35 °C for 3 h and quenched with a $NH_4Cl$ solid (50 mg). The mixture was filtered on Celite®, concentrated, and purified by flash chromatography (light petroleum/$Et_2O$, 1:1 → 3:7 → 0:1) to give **14** in 60% yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.08 (s, 6H), 0.89 (s, 9H), 1.89–196 (m, 2H), 2.53–2.74 (m, 4H), 3.81 (s, 3H), 3.83 (s, 6H), 4.18 (br t, 1H, $J$ = 6.6 Hz), 4.23 (s, 2H), 4.28 (d, 2H, $J$ = 6.0 Hz), 5.70 (t, 1H, $J$ = 6.0 Hz), 6.41 (s, 2H). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ −5.1 (2 × $CH_3$), 18.4 (C), 26.0 (3 × $CH_3$), 32.7 ($CH_2$), 37.4 ($CH_2$), 56.2 (2 × $CH_3$), 58.5 ($CH_2$), 59.6 ($CH_2$), 60.9 ($CH_3$), 75.9 (CH), 105.4 (2 × CH), 129.0 (CH), 136.2 (C), 137.7 (C), 142.6 (C), 153.2 (2 × C); MS: $m/z$ (ESI+) 444 $[M+NH_4]^+$.

### 4.12. Acetic acid 2-acetoxymethyl-4-(1,1,2,2-tetramethyl-propoxy)-1-[2-(3,4,5-trimethoxy- phenyl)-ethyl]-but-2-enyl ester (15)

A solution of **14** (71 mg, 0.166 mmol), acetic anhydride (0.0633 ml, 0.666 mmol), and DMAP (1 mg) in pyridine (1 ml), under argon, was stirred overnight and quenched with a saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with ethyl ether. The combined organic layers were washed with saturated aqueous $CuSO_4$ solution and water, dried over $MgSO_4$, and concentrated under vacuum to give crude **15** in 89% yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.04 (s, 6H), 0.87 (s, 9H), 1.90–2.03 (m, 2H), 2.00 (s, 3H), 2.03 (s, 3H), 2.49–2.58 (m, 2H), 3.79 (s, 3H), 3.82 (s, 6H), 4.30 (d, 2H, $J$ = 5.9 Hz), 4.61 (s, 2H), 5.27 (br t, 1H, $J$ = 5.9 Hz), 5.84 (t, 1H, $J$ = 5.9 Hz), 6.35 (s, 2H). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ −5.1 (2 × $CH_3$), 18.3 (C), 20.9 ($CH_3$), 21.2 ($CH_3$), 25.9 (3 × $CH_3$), 32.3 ($CH_2$), 35.1 ($CH_2$), 56.1 (2 × $CH_3$), 59.5 ($CH_2$), 59.6 ($CH_2$), 60.9 ($CH_3$), 75.3 (CH), 105.35 (2 × CH), 132.7 (C), 134.7 (CH), 136.3 (C), 136.9 (C), 153.2 (2 × C), 170.1 (C), 170.6 (C); MS: $m/z$ (ESI+) 528 $[M+NH_4]^+$.

### 4.13. Acetic acid 2-acetoxymethyl-4-hydroxy-1-[2-(3,4,5-trimethoxy-phenyl)-ethyl]-but-2- enyl ester (16)

To a solution of **15** (76 mg, 0.149 mmol) in THF (2.3 ml) was added an excess of HF·Pyridine (0.136 ml, 1.161 mmol). The mixture was stirred at room temperature and monitored by TLC. After disappearance of the starting material, the solution was concentrated and then purified by flash chromatography (light petroleum/$Et_2O$, 2:8 → 0:1) to give **16** in 65% yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.87–2.06 (m, 2H), 2.02 (s, 3H), 2.05 (s, 3H), 2.30 (br s, OH), 2.47–2.64 (m, 2H), 3.80 (s, 3H), 3.83 (s, 6H), 4.26 (br d, 2H, $J$ = 6.4 Hz), 4.62 (d, 1H, $J$ = 12.5 Hz), 4.71 (d, 1H, $J$ = 12.5 Hz), 5.26 (dd, 1H, $J$ = 8.0, 5.4 Hz), 5.93 (t, 1H, $J$ = 6.4 Hz), 6.36 (s, 2H). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 21.0 ($CH_3$), 21.2 ($CH_3$), 32.3 ($CH_2$), 35.2 ($CH_2$), 56.1 (2 × $CH_3$), 58.5 ($CH_2$), 59.4 ($CH_2$), 60.9 ($CH_3$), 75.2 (CH), 105.4 (2 × CH), 133.1 (CH), 134.6 (C), 136.4 (C), 136.8 (C), 153.3 (2 × C), 170.3 (C), 171.0 (C); MS: $m/z$ (ESI+) 414 $[M+NH_4]^+$.

### 4.14. Acetic acid 2-acetoxymethyl-4-oxo-1-[2-(3,4,5-trimethoxy-phenyl)-ethyl]-but-2-enyl ester (17)

To a solution of **16** (38 mg, 0.096 mmol) in $CH_2Cl_2$ (2.6 ml) under argon at 0 °C was added Dess–Martin periodinane (49 mg, 0.115 mmol). The mixture was added at room temperature and monitored by TLC. After disappearance of the starting material, the mixture was poured into a separating funnel containing a saturated aqueous $Na_2S_2O_3$/ $NaHCO_3$ solution (5 ml, 1:1) and shaken vigorously for 5 min. The aqueous layer was extracted with diethyl ether (3 × 5 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and concentrated under vacuum to give crude product **17** in a quantitative yield. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.97–2.10 (m, 2H), 2.04 (s, 3H), 2.10 (s, 3H), 2.51–2.71 (m, 2H), 3.80 (s, 3H), 3.83 (s, 6H), 4.98 (d, 1H, $J$ = 13.9 Hz), 5.08 (d, 1H, $J$ = 13.9 Hz), 5.33 (br t, 1H, $J$ = 6.3 Hz), 6.11 (d, 1H, $J$ = 7.4 Hz), 6.35 (s, 2H), 10.08 (d, 1H, $J$ = 7.4 Hz). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 20.7 ($CH_3$), 21.0 ($CH_3$), 32.2 ($CH_2$), 35.1 ($CH_2$), 56.2 (2 × $CH_3$), 59.3 ($CH_2$), 60.9 ($CH_3$), 73.3 (CH), 105.4 (2 × CH),

128.7 (CH), 136.2 (C), 136.5 (C), 153.4 (2 × C), 154.9 (C), 170.0 (C), 170.2 (C), 190.5 (CH); MS: *m/z* (ESI+) 412 $[M+NH_4]^+$.

### 4.15. Acetic acid 4-acetoxy-2-acetoxymethylene-1-[2-(3,4,5-trimethoxy-phenyl)-ethyl]-but-3- enyl ester (18)

In a dry Schlenk tube, a solution of **17** (38 mg, 0.096 mmol), DMAP (12 mg, 0.096 mmol), $NEt_3$ (3 ml), and acetic anhydride (0.027 ml, 0.289 mmol) was stirred under argon at 80 °C and monitored by TLC. After disappearance of the starting material, the mixture was concentrated under vacuum. The crude product was then purified by flash chromatography (light petroleum/$Et_2O$, 1:1) to give two isomers (1/1 *E*,*Z*/*Z*,*Z*) **18** and *iso*-**18** in 76% yield. $^1H$ NMR of **18** ($CDCl_3$, 300 MHz) $\delta$ 1.88–2.30 (m, 4H), 2.08 (s, 3H), 2.09 (s, 3H), 2.15 (s, 3H), 2.53–2.60 (m, 2H), 3.81 (s, 3H), 3.85 (s, 6H), 5.83 (d, 1H, $J$ = 12.6 Hz), 5.92 (t, 1H, $J$ = 7.3 Hz), 6.39 (s, 2H), 7.23 (s, 1H), 7.63 (d, 1H, $J$ = 12.6 Hz). $^1H$ NMR of *iso*-**18** ($CDCl_3$, 300 MHz) $\delta$ 1.63–1.89 (m, 2H), 1.99–2.28 (m, 2H), 2.06 (s, 3H), 2.12 (s, 3H), 2.21 (s, 3H), 2.48–2.63 (m, 2H), 3.81 (s, 3H), 3.85 (s, 6H), 5.22 (d, 1H, $J$ = 7.2 Hz), 5.94 (t, 1H, $J$ = 7.2 Hz), 6.39 (s, 2H), 7.25 (d, 1H, $J$ = 7.2 Hz), 7.84 (s, 1H); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 20.6 ($CH_3$), 20.7 ($CH_3$), 20.8 ($CH_3$), 20.9 ($CH_3$), 21.1 ($CH_3$), 21.2 ($CH_3$), 31.8 ($CH_2$), 32.0 ($CH_2$), 34.1 ($CH_2$), 34.4 ($CH_2$), 56.2 (4 × $CH_3$), 60.9 (2 × $CH_3$), 69.0 (2 × CH), 103.7 (CH), 105.4 (4 × CH), 109.3 (CH), 117.1 (C), 119.1 (C), 134.4 (CH), 135.3 (CH), 136.4 (2 × C), 136.7 (C), 136.8 (C), 137.2 (CH), 137.9 (CH), 153.3 (4 × C), 167.27 (C), 167.3 (C), 167.4 (C), 167.9 (C), 170.2 (2 × C); MS: *m/z* (ESI+) 454 $[M+NH_4]^+$.

### 4.16. Growth inhibition assays

HaCaTs cells were seeded in 96-well plates and were incubated for 24 h before adding different concentrations of drug. After a 72-h drug treatment, cells were incubated for 2 h with 0.5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) which allows us to measure growth inhibition. The stain was eluted with 100 μL DMSO and absorbance was measured at 550 nm (MR-7000, Dynatech, Denkendorf, Germany). Percentage of living cells was expressed as the percentage of viable cells divided by the total number of cells.

### 4.17. Tubulin polymerization

Tubulin was purified from lamb brain soluble extract by ammonium sulfate fractionation and ion exchange chromatography.[20] Tubulin was stored in liquid nitrogen and prepared for use as described.[21] Tubulin concentration was determined spectrometrically in 6 M guanidine HCl ($E_{275nm}$ = 1.09 L $g^{-1}$ $cm^{-1}$). The buffer used to polymerize tubulin is 20 mM sodium phosphate buffer, 1 mM EGTA, 3.4 M glycerol, and 0.1 mM GTP, pH 6.95. After 35 min incubation at 37 °C with drug, the polymerization was started with an addition of 10 mM $MgCl_2$. The mass of polymer formed was monitored by turbidimetry at 350 nm in a Beckman DU 7400 spectrophotometer.

### 4.18. In vitro Capillary network formation

A 24-well culture plate was coated with 250 μL of 5.25 mg/mL Matrigel™ (BD Sciences) at 4 °C. Matrigel™ was then allowed to solidify for 1 h at 37 °C before cell seeding. One hour later, the drug was added for 4 h at 37 °C and photographs were taken after incubation using Metamorph software (Universal Imaging Corp., Downingtown, PA) and a DM-IRBE microscope (Leica, Bensheim, Germany) coupled with a digital camera (CCD camera coolsnap FX, Princeton Instruments, Trenton, NJ). The formation of capillary networks was quantitatively evaluated by measuring the total capillary tube length in 10 view fields per well using Metaview software. Sigma Stat software (Jandel Scientific, San Rafael, CA) was used to perform statistical analysis. Data were expressed as means ± SD.

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