Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 6269–6285
Bioorganic & Medicinal Chemistry

Inhibitors of tubulin polymerization: Synthesis and biological evaluation of hybrids of vindoline, anhydrovinblastine and vinorelbine with thiocolchicine, podophyllotoxin and baccatin III

Daniele Passarella,[a,*] Alessandra Giardini,[a] Bruno Peretto,[a] Gabriele Fontana,[b] Alessandro Sacchetti,[a] Alessandra Silvani,[a] Cristina Ronchi,[d] Graziella Cappelletti,[c] Daniele Cartelli,[c] Jurgen Borlak[e,†] and Bruno Danieli[a,†]

[a]*Dipartimento di Chimica Organica e Industriale, Via Venezian 21, Università degli Studi di Milano, 20133 Milano, Italy*
[b]*Indena S.p.a., Via Ortles 19, Milano, Italy*
[c]*Dipartimento di Biologia, Università degli Studi di Milano, Via Celoria 26, Milano, Italy*
[d]*D.I.P.A.V., Sezione di Biochimica, Università degli Studi di Milano, Via Celoria 10, Milano, Italy*
[e]*Fraunhofer Institute of Toxicology and Experimental Medicine, Medical School of Hannover, Germany*

Received 15 November 2007; revised 4 April 2008; accepted 11 April 2008

Dedicated to Prof. Joan Bosch (Univ. Barcelona) in the occasion of his 60th birthday

Abstract—A series of novel hybrid compounds obtained by the attachment of anhydrovinblastine, vinorelbine, and vindoline to thiocolchicine, podophyllotoxin, and baccatin III are described. Two types of diacyl spacers are introduced. The influence of the hybrid compounds on tubulin polymerization is reported. The results highlight the importance of the length of the spacer. Immunofluorescence microscopy and flow cytometry measurements that compound with the best in vitro activity could disrupt microtubule networks in cell and prevent the formation of the proper spindle apparatus, thereby causing cell cycle arrest in the G2/M phase. The newly synthesized compounds were tested in the human lung cancer cell line A549.

1. Introduction

Microtubules are complex polymeric structures generated by the circular arrangement of linear polymers (protofilaments) of tubulin, a dimeric protein formed by the combination of two non-identical chains (α- and β-tubulin). As components of the mitotic spindle, microtubules have emerged as a strategic target in anticancer drug discovery. The discovery of substances able to inhibit the formation or the disaggregation of microtubules has always been regarded as a great achievement in the fight against cancer. Among natural products, a few substances have been discovered that are able to interfere with microtubules, and therefore block the cell cycle. Some of these compounds act by inhibiting the aggregation (colchicines, vincristine, and vinblastine), while others inhibit the depolymerization of tubulin (paclitaxel and epothilones). Furthermore, it has been recently established that some agents interacting with tubulin are also able to target the vascular system of tumors[1] causing vasculature recession (induction of apoptosis by a classical anti-angiogenic mechanism) or vasculature 'normalization'.[2] The vasculature normalization favors the cell penetration of anticancer drugs, and apoptosis can be induced by administration of a combination of drugs. The structural diversity of the antitubulin drugs reflects the diversity of the mechanisms of interaction with tubulin.[3] For example, the binary *Vinca* alkaloids vincristine, vinblastine, and the semisynthetic vinorelbine (Fig. 1) induce the destabilization of polymerized tubulin, by binding to a site localized on β-tubulin.[4] Taxanes, and in particular paclitaxel[5] (Fig. 1), lower the dissociation constant at both the *plus* and *minus* end of the microtubules, thereby promoting microtubule assembly and reducing the amount of free tubulin in the cell. Colchicine[6] (Fig. 1)

Keywords: Bivalent inhibitors; Tubulin polymerization; *Vinca* alkaloids.
* Corresponding author. Tel.: +39 02 50314080; fax: +39 02 50314078; e-mail: Daniele.Passarella@unimi.it
† Contributed equally as senior authors.

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.04.025

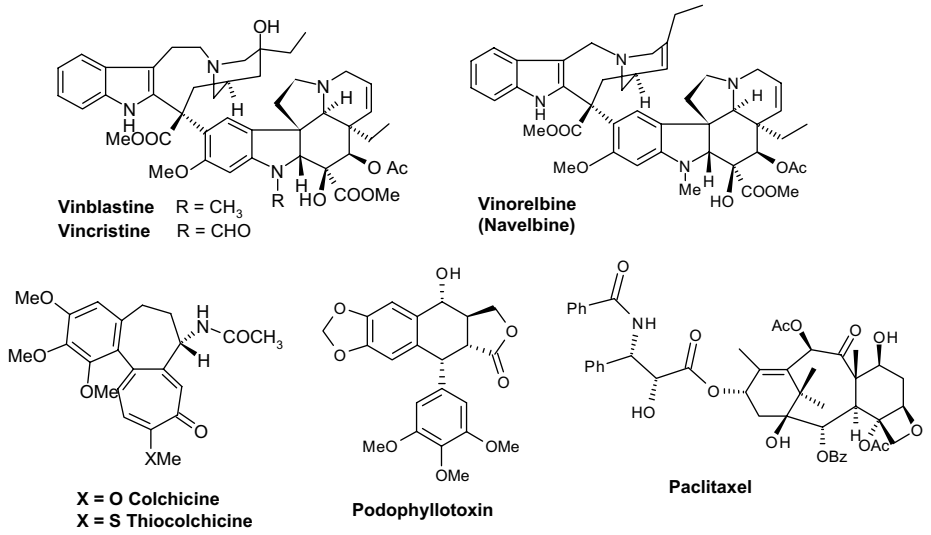


Figure 1. Well-known drugs that disrupt the dynamic tubulin-microtubule equilibrium.

induces various effects on tubulin, the most important of which is the modification of the secondary structure of the protein, followed by inhibition of tubulin polymerization.

Such an effect has been explained through the strong binding to the 'colchicine site' of tubulin that induces an alteration of the secondary structure of β-tubulin at the carboxy terminal.[7] The same mechanism has been reported for thiocolchicine[8] and podophyllotoxin[9] (Fig. 1). Despite the great potential of antitubulin drugs, therapy based on antimitotic agents is still associated with undesired side effects[10] which have a negative impact on patient compliance. The side effects are usually associated with the lack of the selectivity.[11] The discovery of selective, non-toxic, and drug-like active principles is therefore still an urgent demand.

Among the different approaches to the design of new antitubulin agents[12] we explored the possibilities offered by the concept of multivalency,[13] an area which has been reviewed by Whitesides.[14] The theory of multivalency is based on the observation that in Nature the activity and selectivity of some lead compounds are enhanced through the formation of their homo- or heterodimers.[15] As a consequence, the concept of multivalency can be successfully applied as an effective approach for designing ligands, inhibitors, and drugs.

During our research on the synthesis of antitumor compounds,[16] a few years ago we reported the synthesis of taxoid–thiocolchicine hybrids[17] and more recently a dynamic combinatorial library of thiocolchicine–podophyllotoxin derivatives.[18] From a mechanistic point of view, we have shown that these hybrids of antitubulin agents behave as multifunctional tubulin inhibitors. The compounds served as probes to investigate the biology of tubulin and the complex relationships between tubulin inhibition and cytotoxicity. The results obtained encouraged us to develop a broad program directed toward the synthesis of hybrids of naturally occurring antimitotic compounds. Here we report the synthesis of hybrids with anhydrovinblastine and vinorelbine[19] as scaffolds and tiocolchicine, podophyllotoxin and baccatin III as reagents separated by succinyl and decanoyl spacers. The chemistry involved was rather complex. In the case of vinorelbine we could obtain two hybrids by direct attachment of the spacer at position 17 of deacetylvinorelbine, while in the case of the hybrids based on anhydrovinblastine we had first to functionalize vindoline portion at position 17 and then to introduce the 'upper' carboxyvelbanamine part. Thereafter we tested the efficacy of the different hybrids against the polymerization of tubulin. This enabled us to compare not only anhydrovinblastine and vinorelbine but also vindoline. In addition, we studied the anticancer activity of the hybrids in the human lung cancer cell line, A549. For one of new compounds we investigated the effects of inhibition of tubulin polymerization on cell cycle regulation and the structure and distribution of microtubules in cultured cells via immunofluorescence microscopy.

2. Results and discussion

The subsequent Scheme highlights the general strategy that has been used for the synthesis of the hybrid compounds. The synthesis are discussed below.

2.1. Preparation of vindoline-based hybrids (vindoline-C_4-X) (8, 9, and 10, Fig. 3)

17-Deacetylvindoline **1a** (Fig. 2), obtained by careful hydrolysis of vindoline with K_2CO_3 in MeOH, was reacted with succinic anhydride in the presence of pyridine to give the 17-hemisuccinate **1b** that was used as starting material for the preparation of several adducts (*path A*). The presence of the succinic spacer was confirmed by HR-ESI-MS data and by the chemical shift of H-17 that appeared at δ 5.25 with respect to δ 4.17 in 17-deacetylvindoline. The reaction of **1b** with

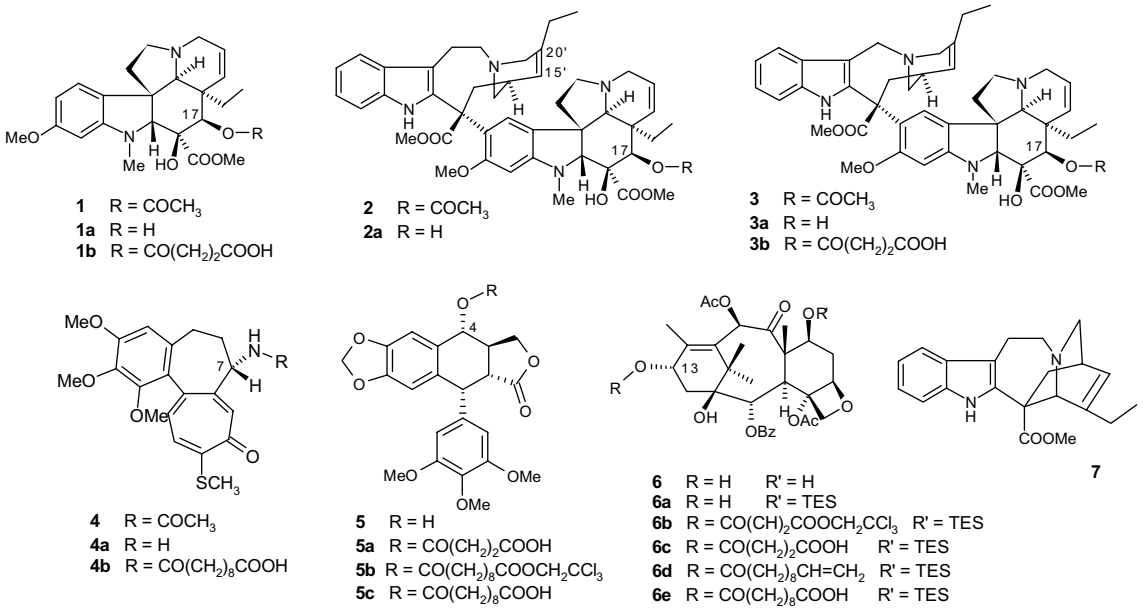


Figure 2. Chemical structure of compounds **1**–**7**.

deacetylthiocolchicine in the presence of DCC and DMAP permitted the formation of the first vindoline–thiocolchicine adduct **8** (Fig. 3). The introduction of the thiocolchicine nucleus was confirmed by the appearance of the ^{1}H NMR spectrum of the typical signals of the thiocolchicine moiety and in particular by the multiplet signal due to H-7 of the thiocolchicine nucleus in the range 4.68–4.59 ppm (thiocolchicine δ 4.35). The same type of condensation reaction was used to generate the vindoline–podophyllotoxin adduct **9**. In this case diagnostic change in the NMR spectrum was the chemical shift of the H-4 of the acylated podophyllotoxin that occurred in the region between δ 6.05 and 5.98, overlapping the dioxymethylene protons, in comparison to δ 4.85–4.77 in podophyllotoxin. Compound **9** was obtained in better yield by reaction of podophyllotoxin with succinic anhydride to give derivative **5a** and subsequent introduction of the vindoline nucleus by acylation (Scheme 1, *path B*). A different approach was necessary for the preparation of vindoline–baccatin adduct **10** as a consequence of the particular reactivity of OH-13 of baccatin III. 7-TES-baccatin **6a**[20] was first reacted with trichloroethylsuccinic acid to give the corresponding derivative **6b** that was deprotected with activated Zn under acidic conditions to give the carboxylic derivative **6c**.[21] The usual condensation reaction with deacetylvindoline **1a** gave the vindoline–baccatin adduct **10** in 61% yield. The chemical shifts of the signals due to H-17 (δ 5.43) and H-13′ (δ 6.20–6.15) confirmed the presence of the diacyl spacer at the expected positions.

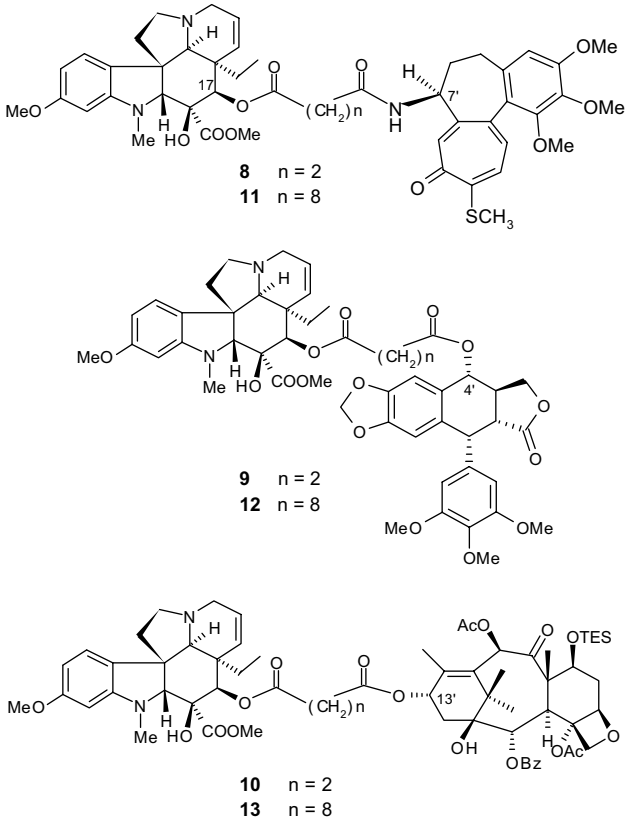
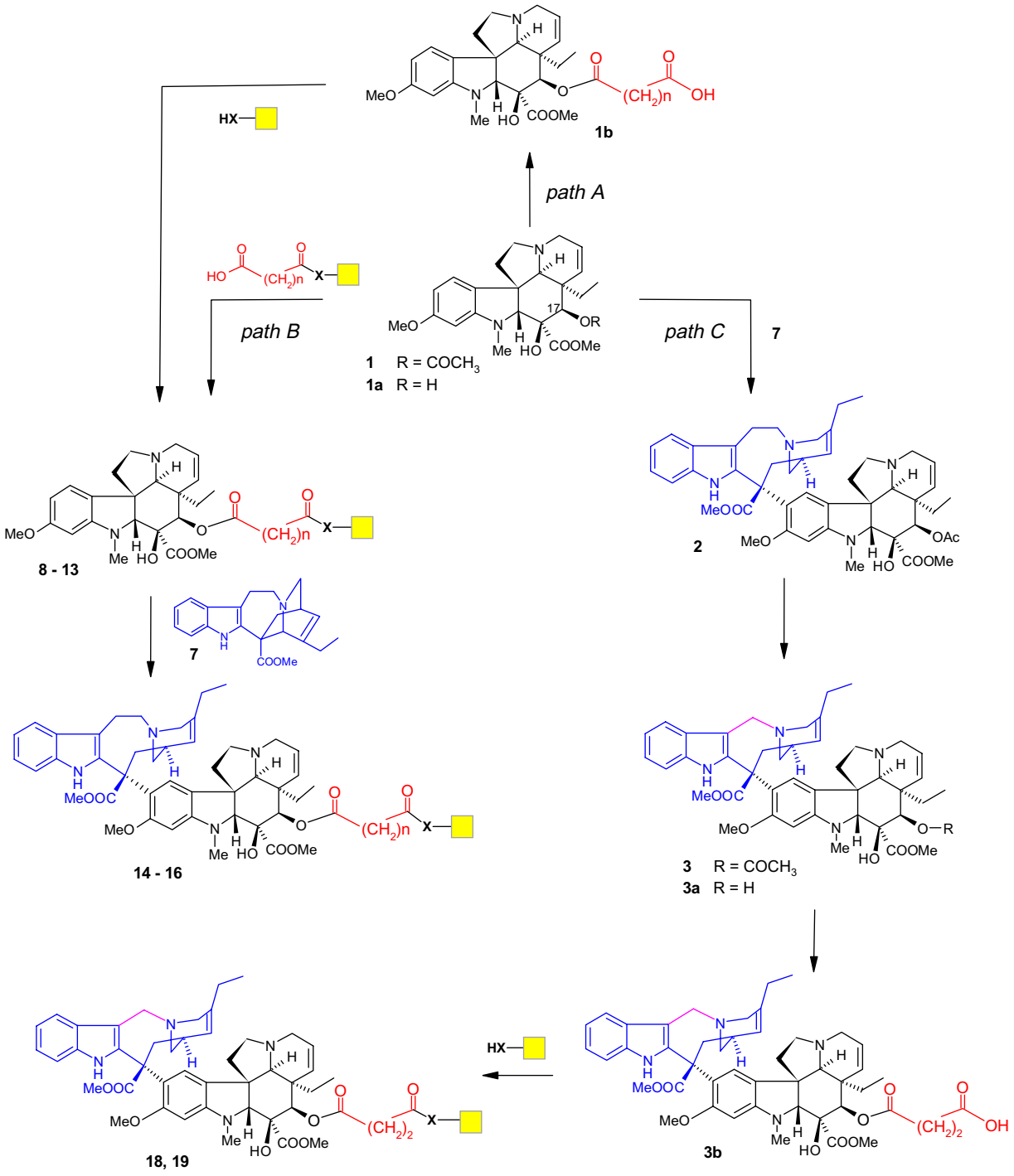


Figure 3. Vindoline-based derivatives **8**–**13**.

2.2. Preparation of vindoline-based hybrids (vindoline-C_{10}-X) (**11**, **12**, and **13**, Fig. 3)

We were unable to prepare in acceptable yield 17-hemisebacoylvindoline from 17-deacetylvindoline and sebacic acid and therefore for the synthesis of the heterodimers with a C_{10} spacer we used the alternative strategy developed before, based on the introduction of the vindoline portion on the preformed hemisebacoyl derivatives of podophillotoxin, *N*-deacetylthiocolchicine, and 7-TES-baccatin (**4b**, **5c**, and **6e**, respectively,



Scheme 1. General strategy for the preparation of hybrid compounds **8**–**19**. For the particular structures see Figures 3–5 and for HX— see Figure 2.

Fig. 2). The hemisebacoyl derivative of podophillotoxin (**5c**) was obtained by condensation of **5** with the monotrichloroethylester of sebacic acid and subsequent hydrolysis of the trichloroethyl ester (**5b**) in the presence of activated Zn. In the case of thiocolchicine this procedure was not possible due to the sensitivity of thiocolchicine to acidic treatment. For this reason we had to accomplish the preparation of the desired adduct by monoamination of the sebacoyl acid with *N*-deacetylthiocolchicine to give **4b**, which was then submitted to a condensation reaction with deacetylvindoline to give **11**. 7-TES-13-sebacoylbaccatin III **6e** was obtained by $NaIO_4$–$RuCl_3$ oxidation[22] of the corresponding 7-TES-13-undecenylbaccatin III **6d**.

2.3. Preparation of anhydrovinblastine-based hybrids (anhydrovinblastine-C_4-X) (14, 15, and 16, Fig. 4)

Conceivably, the access to adducts that incorporate the anhydrovinblastine (AVBL) entity could be obtained

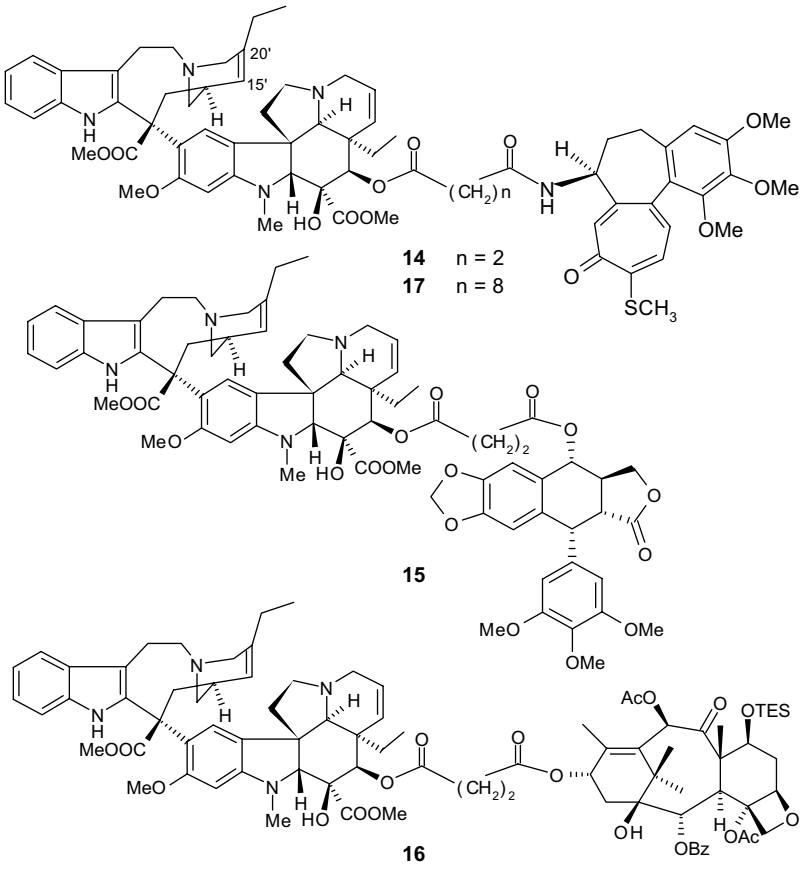


Figure 4. Anhydrovinblastine-based derivatives **14**–**17**.

by acylation of the 17-deacetylanhidrovinblastine. This type of approach is problematic due to the instability of the C20′–C15′ double bond to many experimental conditions. For this reason we chose to introduce the upper portion (the carbomethoxyvelbanamine nucleus) deriving from **7**, on the vindoline-adducts (**8**, **9**) using the biomimetic approach studied by Szantay and Kutney that we successfully applied to the synthesis of vinblastine analogs.[16] The use of $FeCl_3$ in glycine-buffered solution induces an oxidative coupling that proceeds in a stereocontrolled way. The proper stereochemistry at position C16′ of the heterodimers **14** and **15** thus obtained was confirmed by considering the signs for the Cotton effect curves that were as expected for the natural configuration (negative in the range 205–210 and positive in the range 220–235).[23] Application of the same methodology for the preparation of compound **16** was unsuccessful, probably as a result of the low solubility of the substrate **10**. For this reason we turned instead to a Polonowsky–Potier reaction.[24] The use of *m*-CPBA to generate the *N*-oxide of catharanthine and the subsequent reaction with compound **10** in the presence of TFAA generated the expected compound **16** after the addition of $NaBH_4$. Compounds **14**, **15**, and **16** were fully characterized spectroscopically. Despite the interesting chemical result obtained for the introduction of the velbanamine nucleus on complex substrates, we were disappointed to note that compounds **14**, **15**, and **16** were insufficiently stable to be stored for longer than 2 or 3 days, probably as a consequence of the sensitivity of C15′–C20′ double bond.

2.4. Preparation of anhydrovinblastine-based hybrids (anhydrovinblastine-C_{10}-X) (17, Fig. 4)

The reaction of **11** with catharanthine in the presence of $FeCl_3$ in glycine-buffered solution induced the formation of compound **17.** Unfortunately compound **17** suffered from the same instability as compounds **14**–**16**.

2.5. Preparation of vinorelbine-based hybrids (vinorelbine-C_4-X) (18 and 19, Fig. 5)

The semisynthetic alkaloid vinorelbine (Navelbine)[25] appeared an interesting entity to be involved in the formation of bivalent structures with higher stability. We submitted anhydrovinblastine **2** (*path C*)[26] to reaction with NBS and TFA at −60 °C to generate the bromoindolenine on the indole nucleus of the velbanamine upper part. The subsequent rearrangement that resulted in the loss of a carbon atom between N4′ and C7′ was induced by reaction with $AgBF_4$.[27] Vinorelbine was then submitted to reaction with Na_2CO_3 in MeOH to remove the acetyl group from OH-17 to give **3a**. The usual reaction with succinic anhydride gave **3b.** The subsequent reaction with podophyllotoxin and thiocolchicine gave, respectively, compounds **18** and **19**, which were stable and easy to manipulate.

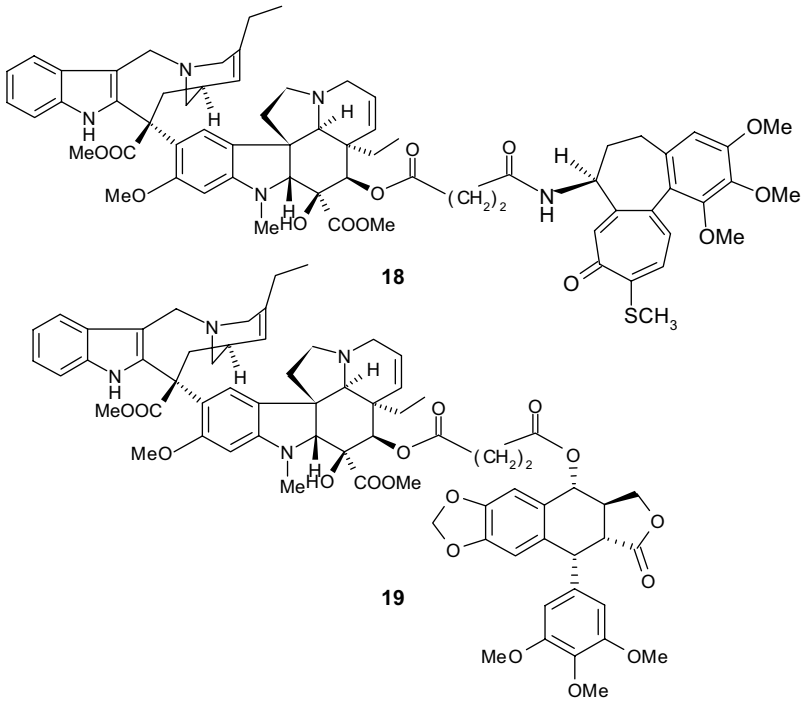


Figure 5. Vinorelbine-based derivatives **18**–**19**.

3. Biological evaluation

To get an insight into the potential biological activity of the novel heterodimers we investigated their ability to affect tubulin polymerization in vitro. In order to assess the effect of the compounds on tubulin assembly, porcine tubulin (purified from brain) was mixed with a standard solution of each sample in the absence of GTP. The solutions were then incubated at 37 °C, and after 15 min GTP was added to allow slow binding drugs to bind to the tubulin. After 30 min the polymerized and the unpolymerized fractions were separated by centrifugation and analyzed by densitometry.

The behavior of the monomers was assumed as baseline (Fig. 6). High red bars mean high content of non-polymeric tubulin, while high blue bars stand for high content of polymeric tubulin. By analyzing the different moieties (Fig. 6) we confirmed that thiocolchicine, podophyllotoxin, and vinorelbine induced more or less a significant shift of tubulin from the polymerized to the unpolymerized pool, whereas vindoline appeared to be unable to affect tubulin polymerization. An unexpected behavior resulted in the case of 7-TES-baccatin when compared with the taxane stabilization of the polymerized form.[28] The test compounds **8**–**13**, **18** and **19** were then analyzed. In the conditions of the assay compounds **14**–**17** were not stable enough to be tested.[29] The results are summarized in Figure 7. These experiments show that a significant inhibition of tubulin polymerization occurred in the presence of compounds **8** and **13**. Interestingly, heterodimers built up with the same moieties connected by different spacers showed different effects on tubulin polymerization. The dimers **8** and **11** contain vindoline and thiocolchicine nucleus but compound **8** inhibited tubulin polymerization whereas **11** was almost ineffective. Analogously, the dimers **13** and **10** contain vindoline and 7-TES-baccatin moieties but the dimer **13** inhibited tubulin polymerization whereas **10** was almost ineffective. We report in Table 1 the ratios of unpolymerized/polymerized tubulin obtained in the presence of the different compounds. By comparing the results, we confirm the significant effect of the dimers **8** and **13** on tubulin polymerization.

Furthermore, the anticancer activities of the new compounds (Fig. 9) were examined in human lung carcinoma A549.[30] All the new compounds exerted

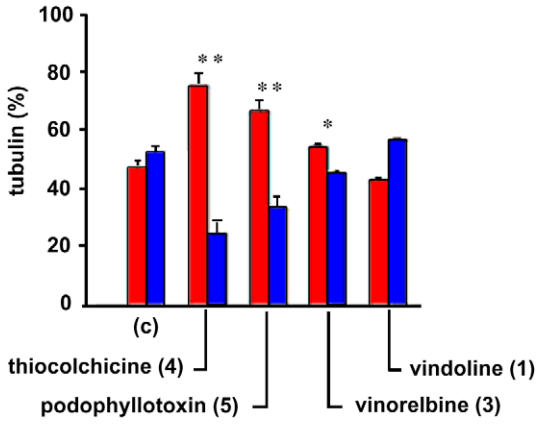


Figure 6. Tubulin polymerization in the presence of 10 μM thiocolchicine (**4**), podophyllotoxin (**5**), vinorelbine (**3**) or vindoline (**1**); (c): control. In red unpolymerized fraction, in blue the polymerized fraction. Bars indicate SEM (standard error of mean). $^{*}P < 0.05$, $^{**}P < 0.001$ versus control, according to Student's unpaired *t*-test.

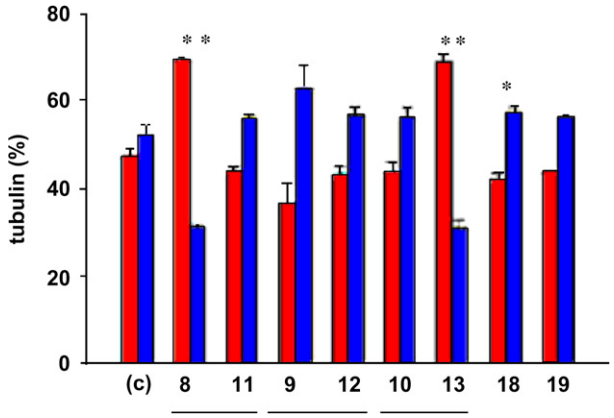


Figure 7. Tubulin polymerization in the presence of 10 μM solution of hybrid compounds; (c): control. In red unpolymerized fraction, in blue the polymerized fraction. Bars indicate SEM (standard error of mean). $^{*}P < 0.05$, $^{**}P < 0.001$ versus control, according to Student's unpaired *t*-test.

antiproliferative activity, albeit at different concentrations. Compound **19** showed the highest cytotoxicity at 10 μM concentration as determined by the MTT assay and when compared with those of vinorelbine and podophyllotoxin. Compound **8** was clearly distinguished from compound **11** despite their structural similarity in accord with the trend of their influence on tubulin polymerization. By contrast, compounds **10** and **13** showed a similar behavior and this was not the case in the tubulin polymerization test.

On the grounds that compound **8** displayed structural simplicity, maintained the ability to inhibit tubulin polymerization, and, interestingly, was more effective as an antiproliferative agent than its moiety thiocolchicine, we moved to obtain a deeper understanding of its behavior in vitro and in cells. We first studied the effect of compound **8** on tubulin polymerization at different concentrations in comparison with thiocolchicine (Fig. 8). The ratio of unpolymerized/polymerized tubulin increases in a dose-dependent manner in the presence of compound **8** and thiocolchicine (see Table 1). In addition, we followed the kinetics of tubulin polymerization in the presence of compound **8** and thiocolchicine (Fig. 10a). We found that both the compounds markedly and similarly interfere with it by lowering the initial rate of assembly rather than the final extent of assembly. Looking at microtubule morphology, we collected the assembled microtubules at the end of polymerization and analyzed them by DIC microscopy (Fig. 10b). Our results show that microtubules assembled in the presence of compound **8** as well as thiocolchicine are shorter than controls.

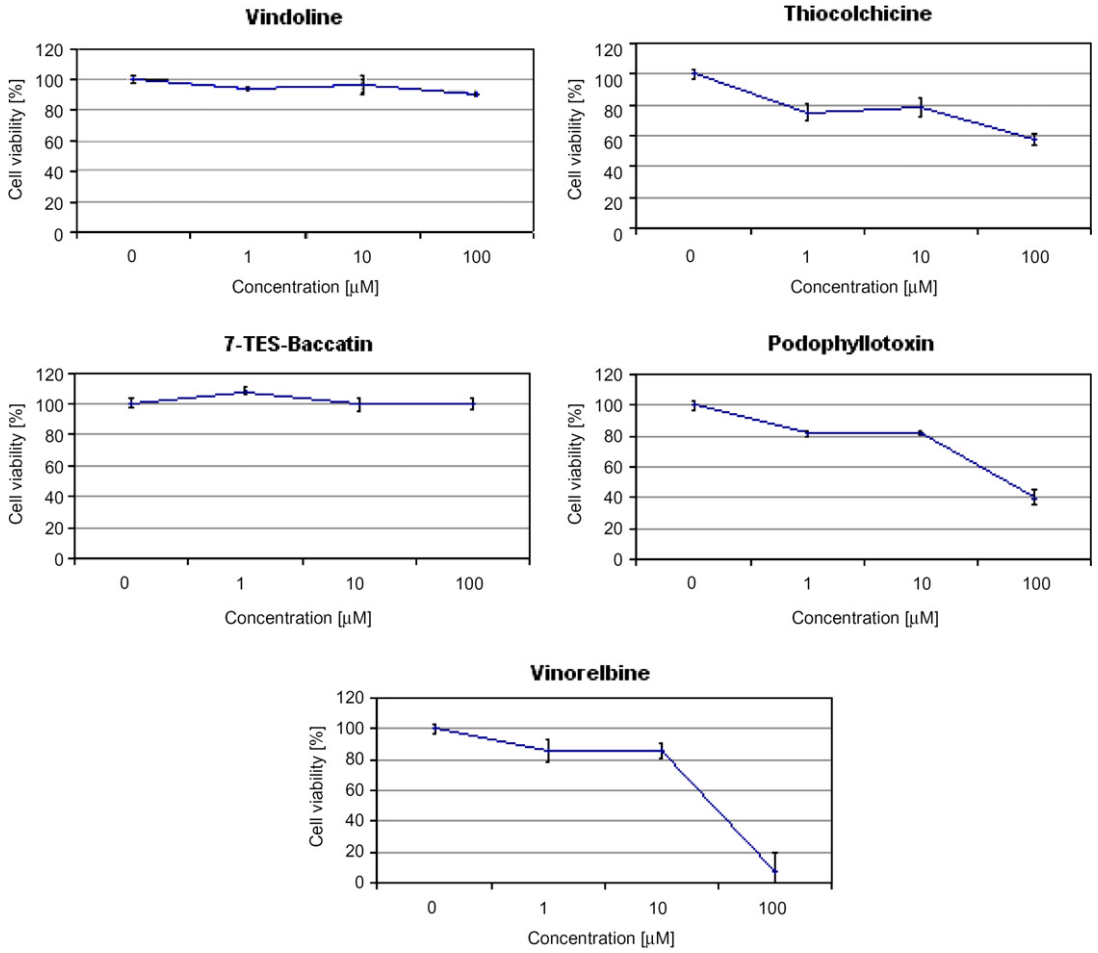


Figure 8. Anticancer activities for vindoline, thiocolchicine, 7-TES-baccatin III, podophyllotoxin, and vinorelbine in human lung carcinoma A549.

Table 1. Ratio of unpolymerized/polymerized tubulin as obtained from data presented in Figures 6 and 7

	Unpolymerized/polymerized tubulin (mean ± SEM)
Control	0.93 ± 0.08
Thiocolchicine	10 μM: 2.55 ± 0.23**
	5 μM: 1.34 ± 0.09**
Podophyllotoxin	1.68 ± 0.12**
Vinorelbine	1.19 ± 0.02*
Vindoline	0.76 ± 0.01 n.s.
8	10 μM: 2.21 ± 0.02**
	5 μM: 1.20 ± 0.03*
11	0.79 ± 0.02 n.s.
9	0.60 ± 0.18 n.s.
12	0.77 ± 0.11 n.s.
10	0.78 ± 0.07 n.s.
13	2.33 ± 0.63**
18	0.74 ± 0.02*
19	0.79 ± 0.01 n.s.

*$P < 0.05$, **$P < 0.001$ versus control according to Student's unpaired *t*-test. Drug concentration 10 μM if not specified.

In order to establish whether the antiproliferative activity of compound **8** is based on cell cycle arrest due to damage to the microtubular cytoskeleton we applied a flow cytometry analysis (Fig. 11). By 24 h, a substantial number of cells (74%) treated with 10 μM compound **8** were in cell cycle arrest at the G2/M phases. This finding was highly significant, when compared to the DMSO vehicle control.

Finally, we investigated microtubule structure and distribution in human lung carcinoma cell line A549 exposed to vindoline, thiocolchicine and compound **8** by indirect immunofluorescence using anti-α-tubulin antibodies (Fig. 12). In control cells (Fig. 12a), we observed a widespread network of long microtubules other than the typical accumulation of microtubules at one side of the nucleus in the region called the microtubule organizing center (MTOC). This conventional microtubule distribution was not significantly changed in the presence of vindoline (Fig. 12b) but underwent dramatic rearrangements in the presence of thiocolchicine (Fig. 12c) and the dimer **8** (Fig. 12d) showing the evident disorganization of the network, fragmented microtubules, and abnormal accumulation in the perinuclear region. The results of the indirect immunofluorescent detection of the microtubular cytoskeleton are consistent with the data coming from the in vitro assay: vindoline is unable to affect the microtubule system in cells as previously observed in tubulin assembly tests, whereas thiocolchicine and the hybrid **8** significantly interfere with tubulin organization in cells as well as with tubulin polymerization in vitro.

4. Conclusions

The preparation of 12 hybrid compounds that contain *Vinca* alkaloids and other antitubulin entities connected by a diacyl spacer has been described. A concise biological test to evaluate the inhibitory effect of these compounds on tubulin polymerization in vitro has been described. The antiproliferative study on A549 showed low activities but almost confirmed the trend noticed in the tubulin polymerization test. Relevant differences in the behavior of compounds that differ in the length of the spacer emerged, in particular compound **8** inhibits the polymerization and **11** is ineffective. Anhydrovinblastine derivatives (**14**–**17**) were not stable and therefore not interesting. On the other hand, vinorelbine derivatives (**18** and **19**) were stable enough to be studied but had low efficacy for the inhibition of tubulin assembly, with an interesting cytotoxicity that suggests a different biological target. Compound **8** was submitted to further studies in order to confirm its interaction with tubulins. Dose-dependence and the kinetics of the inhibition of tubulin polymerization together with the influence on the cell cycle furnished consistent results. The ability of compound **8** to disrupt microtubules in cells has been demonstrated by fluorescence microscopy. Further analyses would be necessary to discover in detail the mechanisms by which this compound affects tubulin polymerization in vitro. In fact, microtubule-targeted drugs affect microtubule system in several different ways, including changing microtubule mass by binding to multiple distinct sites in tubulin dimers or microtubule or by suppressing microtubule dynamics.[31] Finally, the nature and the biological responses of compounds **8** and **19** are the evidence that the creation of conjugates remains a valuable and not trivial strategy to discover new interesting activities.

5. Experimental

All reagents and solvents were of reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries on a Buchi melting point apparatus and are uncorrected. Column chromatography was carried out on flash silica gel (Merck 230–400 mesh). NMR spectra were recorded at 300/400 MHz (^{1}H) and at 75/100 MHz (^{13}C). FAB+ mass spectra were recorded at an ionizing voltage of 6 keV. ESI mass spectra were recorded on FT-ICR instrument.

5.1. Chemistry

5.1.1. Preparation of 17-deacetylvindoline (1a). A mixture of vindoline (**1**, 505 mg, 1.11 mmol), Na_2CO_3 (5.53 mmol) in MeOH (16 ml) was stirred for 4 days at 65 °C. After the evaporation of the solvent the residue was chromatographed on alumina (AcOEt/MeOH/MeOH 85:15:2) to give **1a** (348 mg, 76%). R_f: 0.49 (AcOEt/MeOH 8.5:1.5); $[\alpha]_D$ −22 (MeOH, $c = 0.6$). ^{1}H NMR ($CDCl_3$, 300 MHz) selected signals: 6.85 (1H, d, $J = 8$), 6.28 (1H, dd, $J = 8.2$), 6.05 (1H, d, $J = 2$), 5.85 (1H, dd, $J = 11$, 4.5), 5.70 (1H, d, $J = 11$), 4.08 (1H, s), 3.85 (3H, s), 3.78 (3H, s), 3.70 (1H, s), 3.35–3.49 (2H, m), 2.85 (1H, d, $J = 16$), 2.70 (3H, s), 2.62 (1H, s), 2.48–2.60 (1H, m), 2.20 (2H, dd, $J = 10.5$, 7), 1.38–1.52 (1H, m), 0.91–1.06 (1H, m), 0.67 (3H, t, $J = 7$). ^{13}C NMR ($CDCl_3$, 75 MHz) selected signals: 174.0, 161.8, 154.8, 131.5, 125.8, 124.4, 123.5, 105,0, 96.5, 83.8, 81.5, 74.6, 68.9, 56.0, 53.6, 52.1, 45.3, 43.5, 39.3,

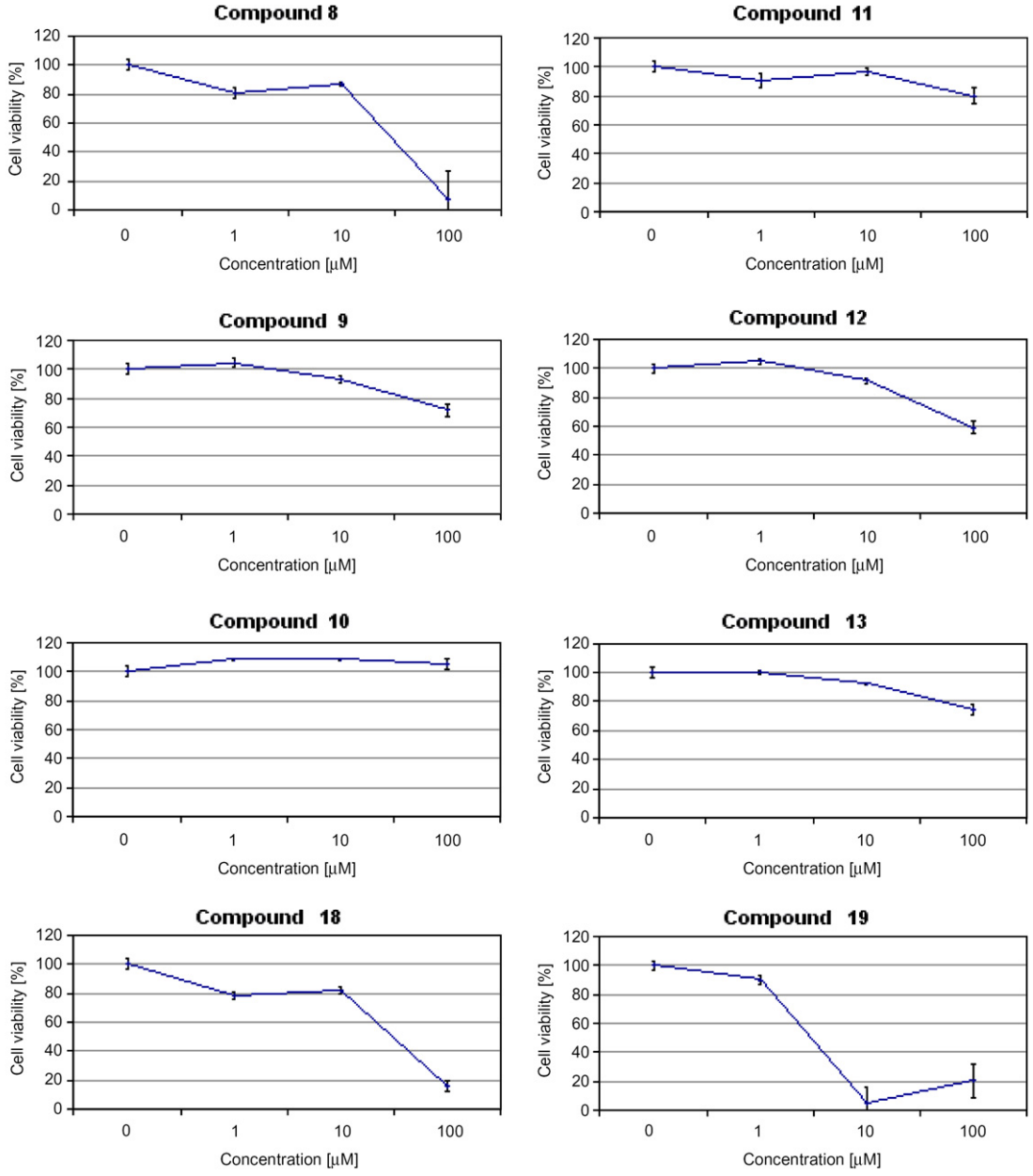


Figure 9. Anticancer activities for compounds **8**–**13**, **18**, and **19** in human lung carcinoma, A549.

33.2, 8.5. ESI positive MS: Anal. Calcd for $C_{23}H_{30}O_5N_2+Na^+$ 437.2047, found 437.2048.

5.1.2. 17-Deacetyl-17-*O*-hemisuccinylvindoline (1b). A mixture of **1a** (190 mg, 0.458 mmol), succinic anhydride (92 mg, 0.917 mmol), and pyridine (8 ml) was stirred for 12 h at room temperature. The solution was concentrated. The addition of CH_2Cl_2, the evaporation, and the treatment of the residue with MeOH gave after subsequent evaporation a residue that was submitted to chromatography (AcOEt/MeOH 8:2) to give compound **1b** (225 mg, 95%). ^{1}H NMR (300 MHz, CD_3COCD_3) selected signals: δ 7.05 (1H, d, J = 12), 6.30 (1H, dd, J = 12, 2), 6.20 (1H, d, J = 2), 5.85 (1H, dd, J = 15, 5), 5.25 (1H, s), 5.20 (1H, d, J = 15), 3.74 (3H, s), 3.69 (3H, s), 3.60 (1H, s), 3.45 (1H, dd, J = 16, 5.5), 3.37–3.26 (1H, m), 2.80 (1H, d, J = 16), 2.68 (1H, s), 2.62 (3H, s), 2.56–2.40 (5H, m), 2.38–2.28 (2H, m), 1.58–1.43 (1H, m), 1.07–0.92 (1H, m), 0.45 (3H, t, J = 7.5). ESI positive MS: Anal. Calcd for $C_{27}H_{34}O_8N_2+Na^+$ 537.2207, found 537.2209.

5.1.3. 17-Deacetyl-17-*O*-(*N*-deacetyl-*N*-succinylthiocolchicinyl)vindoline (8). A mixture of **8** (225 mg, 0.437 mmol), DMAP (53 mg, 0.437 mmol), DCC (180 mg, 0.875 mmol), and deacetylthiocolchicine **4a** (163 mg, 0.437 mmol) was stirred at room temperature for 10 h. The mixture was filtered on Celite and submitted to chromatography (AcOEt/MeOH/NEt_3 94:6:2) to give **8** (75 mg, 20%) as a yellow solid. Mp: 165 °C; R_f: 0.13 (AcOEt/acetone 6:4). ^{1}H NMR (400 MHz, $CDCl_3$) selected signals: δ 7.27 (1H, d, J = 9), 7.24 (1H, s), 7.03

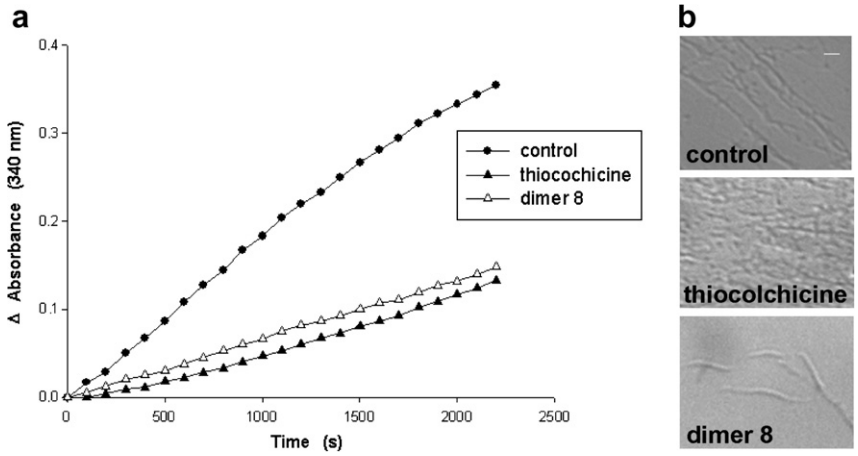


Figure 10. (a) Kinetics of microtubule polymerization in the absence and presence of 10 μM each of thiocolchicine and compound **8**. Tubulin (2.7 mg/ml) was polymerized in the absence and presence of 10 μM of thiocolchicine and **8** and the change in absorbance at 340 nm was monitored. (b) At the end of polymerization, microtubules were pelletted and observed by DIC microscopy (B). Bar = 1 μm.

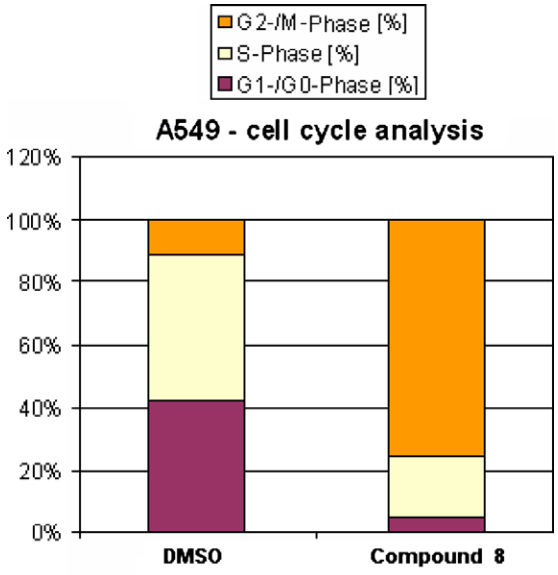


Figure 11. A549 cell cycle analysis for compound **8** (10 μM).

(1H, d, $J = 9$), 6.89 (1H, d, $J = 8.5$), 6.64 (1H, d, $J = 6.5$), 6.55 (1H, s), 6.30 (1H, dd, $J = 8.5$, 2), 6.07 (1H, d, $J = 2$), 5.72 (1H, dd, $J = 8$, 4.5), 5.43 (1H, s), 5.15 (1H, d, $J = 8$), 4.68–4.59 (1H, m), 3.95 (3H, s), 3.91 (3H, s), 3.79 (3H, s), 3.73 (3H, s), 3.65 (3H, s), 3.47–3.45 (1H, m), 3.45–3.42 (1H, m), 2.79 (1H, d, $J = 15$), 2.65 (3H, s), 2.63 (1H, s), 2.57–2.48 (1H, m), 2.49–2.44 (2H, m), 2.42 (3H, s), 1.92–1.82 (2H, m), 1.64–1.57 (1H, m), 1.13–1.10 (1H, m), 0.35 (3H, t, $J = 8.5$). ^{13}C NMR (100 MHz, $CDCl_3$) selected signals: δ 182.8, 173.1, 172.4, 171.2, 161.6, 158.6, 154.1, 153.9, 151.7, 150.9, 142.1, 138.3, 134.8, 134.7, 130.8, 128.9, 126.5, 126.3, 125.5, 124.3, 123.1, 107.7, 104.9, 96.2, 83.8, 79.9, 77.1, 67.5, 61.9, 61.8, 56.5, 55.8, 53.2, 52.7, 52.5, 52.4, 51.5, 44.4, 43.3, 38.6, 37.0, 31.3, 31.2, 30.3, 29.8, 15.5, 8.1. ESI positive MS: Anal. Calcd for $C_{47}H_{55}O_{11}N_3S+Na^+$ 892.3450, found 892.3448.

5.1.4. 4-*O*-Hemisuccinylpodophyllotoxin (5a). A mixture of **5** (780 mg, 1.88 mmol), DMAP (92 mg, 0.75 mmol), imidazole (153 mg, 2.25 mmol), and succinic anhydride (395 mg, 3.95 mmol) was stirred at room temperature for 13 h. The mixture was submitted to chromatography (CH_2Cl_2/MeOH 40: 1) to give **5a** (880 mg, 91%) as a yellow solid. Mp: 165 °C; R_f: 0.23 (CH_2Cl_2/MeOH 40:1). ^{1}H NMR (400 MHz, CD_3COCD_3) selected signals: δ 6.96 (s, 1H), 6.59 (s, 1H), 6.41 (s, 2H), 6.02 (d, 1H, $J = 9.2$ Hz), 4.63 (d, 1H, $J = 4.6$), 4.32 (t, 1H, $J = 9.1$ Hz), 4.15 (t, 1H, $J = 9.1$ Hz), 3.71 (s, 1H), 3.67 (s, 1H), 3.61 (s, 1H), 3.27 (dd, 1H, $J = 10.1$, 4.6 Hz), 2.91–2.80 (m, 1H), 2.71–2.63 (m, 2H), 2.61–2.51 (m, 2H). ESI positive MS: Anal. Calcd for $C_{26}H_{26}O_{11}+Na^+$ 537.1367, found 537.1365.

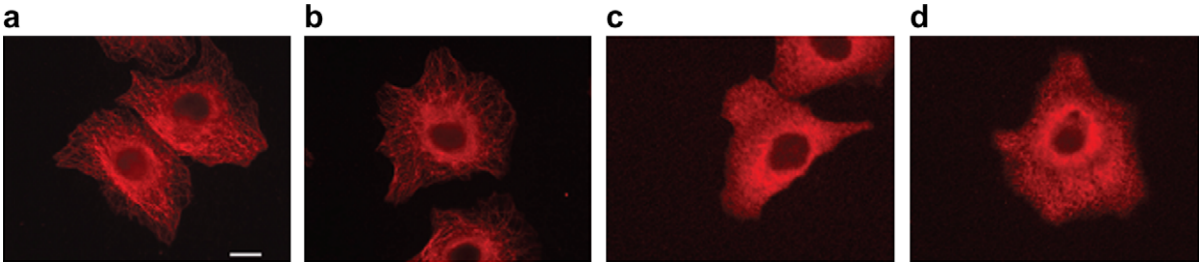


Figure 12. Microtubule organization in human lung carcinoma cell line A549 exposed for 1 h to solvent vehicle alone (a) or 10 μM vindoline **1** (b), thiocolchicine **4** (c), compound **8** (d), as revealed by immunofluorescence localization of α-tubulin. Bar: 5 μm.

5.1.5. 17-Deacetyl-17-*O*-(4-*O*-succinylpodophyllotoxinyl) vindoline (9). A mixture of **5a** (150 mg, 0.362 mmol), DCC (89 mg, 0.43 mmol), DMAP (18 mg, 0.14 mmol), and **1b** (150 mg, 0.362 mmol) and CH_2Cl_2 (10 ml) was stirred for 12 h. The mixture was submitted to chromatography (AcOEt/hexane 20:1) to give **9** (160 mg, 49%) as amorphous yellow solid. R_f: 0.35 (AcOEt). ^{1}H NMR (400 MHz, CD_3COCD_3) selected signals: δ 8.58 (1H, s), 7.08 (1H, d, J = 8.5), 7.05 (1H, s), 6.58 (1H, s), 6.46 (2H, s), 6.31 (1H, dd, J = 8.5, 2.0), 6.18 (1H, d, J = 2.0), 6.05 - 5.98 (3H, m), 5.81 (1H, dd, J = 8.5, 4.0), 5.41 (1H, s), 5.20 (1H, d, J = 8.5), 4.63 (1H,d, J = 4.5), 4.38 (1H, t, J = 9.0), 4.18 (1H, t, J = 9.0), 3.75 (3H, s), 3.71 (6H, s), 3.68 (6H, s), 3.63 (1H, s), 3.53–3.43 (2H, m), 3.25 (1H, dd, J = 13.0, 4.5, H-2′), 2.92–2.83 (1H, m, H-3b), 2.86–2.55 (1H, m, H-5b), 2.81–2.72 (1H, m, H-3′), 2.68 (3H, m, NMe), 2.65 (1H, m, H-21), 2.38–2.26 (m, 2H, CH_2(6)), 1.72 (1H, m, H-19a), 1.21–1.05 (1H, m, H-19b), 0.45 (3H, t, J = 5.5, CH_3(18)). ^{13}C NMR (100 MHz, CD_3COCD_3) selected signals: δ 173.8, 173.8, 171.79(2C), 161.1, 153.6, 152.63(2C), 148.1, 147.6, 137.1, 134.9, 130.4, 124.2, 124.2, 123.1, 109.3, 108.6(2C), 107.3, 104.7, 101.5, 95.6, 83.5, 79.5, 76.8, 73.3, 70.9, 67.3, 59.5, 55.5(2C), 54.6, 52.5, 51.9, 51.2, 51.0, 44.7, 43.9, 43.7, 43.1, 38.8, 38.6, 31.9, 7.2. ESI positive MS: Anal. Calcd for $C_{49}H_{54}O_{15}N_2$+Na^+ 933.3416, found 933.3417.

5.1.6. 13-*O*-Hemisuccinoyl-7-*O*-(triethylsilyl)baccatin III (6c). A mixture of **6a** (1.97 g, 2.81 mmol), DMAP (514 mg, 4.21 mmol,), DCC (1.74 g, 8.43 mmol), and hemytrichloroethylester of succynic acid (2.1 g, 8.43 mmol) in toluene (20 ml) was stirred for 60 h at 90 °C. The mixture was filtered, concentrated, and submitted to chromatography (AcOEt/hexane 1:4) to give **6b** (2.24 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) selected signals: δ 8.07 (2H, d, J = 7.7), 7.62 (1H, t, J = 7.7), 7.50 (2H, t, J = 7.7), 6.44 (1H, s), 6.18 (1H, t, J = 8.7), 5.66 (1H, d, J = 7), 4.94 (1H, d, J = 8.4), 4.76 (2H, sist. AB), 4.47 (1H, dd, J = 10.5, 6.7), 4.30 (1H, d, J = 8.3), 4.14 (1H, d, J = 8.3), 3.83 (1H, d, J = 7), 2.9–2.75 (4H, m), 2.15 (1H, ddd, J = 6.7, 9.6, 15), 2.34 (3H, s), 2.23 (2H, sist. AB), 2.16 (3H, s), 2.03 (3H, s), 1.92–1.83 (1H, m), 1.67 (3H, s), 1.21 (3H, s), 1.16 (3H, s), 0.91 (9H, m), 0.56 (6H, m). A mixture of Zn (1 g) and HCl (2%, 10 ml) was stirred for 5 min. After septum-filtration, the solid was washed with water (pH 7), acetone and ethyl ether. A mixture of activated Zn (2.2 g, 33.82 mmol), **6b** (2.1 g, 2.25 mmol) in AcOH/MeOH (1:1, 20 ml) was stirred for 10 h to convert all the starting material. After Celite filtration the solution was washed with water and extracted with AcOEt. The organic phase was washed with water and evaporated to give **6c** (1.31 g, 73%) as white amorphous solid. ^{1}H NMR (200 MHz, $CDCl_3$) selected signals: δ 8.10 (2H, d, J = 7.7), 7.62 (1H, t, J = 7.7), 7.50 (2H, t, J = 7.7), 6.48 (1H, s), 6.21 (1H, t, J = 8.7), 5.70 (1H, d, J = 7), 4.96 (1H, d, J = 8.4), 4.49 (1H, dd, J = 10.5, 6.7), 4.32 (1H, d, J = 8.3), 4.18 (1H, d, J = 8.3), 3.87 (1H, d, J = 7), 2.90–2.70 (4H, m), 2.60–2.50 (1H, m), 2.38 (3H, s), 2.32–2.22 (2H, m), 2.20 (3H, s), 2.07 (3H, s), 1.95–1.85 (1H, m), 1.71 (3H, s), 1.21 (3H, s), 1.19 (3H, s), 0.91 (9H, t), 0.67–0.56 (6H, m). ESI positive MS: Anal. Calcd for $C_{41}H_{56}O_{14}Si$+Na^+ 823.3332, found 823.3333.

5.1.7. 13-*O*-(17-Deacetyl-17-*O*-succinylvindolinyl)-7-*O*-(triethylsilyl)baccatin III (10). A solution of **6c** (50 mg, 0.062 mmoli), DMAP (3 mg, 0.025 mmol), DCC (15.5 mg, 0.075 mmol), and **1a** (26 mg, 0.063 mmol) in CH_2Cl_2 (3 ml) was stirred for 16 h at room temperature and then filtered on Celite. The evaporation of the solvent gave a mixture that was submitted to chromatography (AcOEt/hexane 6:1 Et_3N 1%) to give **10** (46 mg, 61%) as amorphous solid. $[\alpha]_D$ −51.2 ($CHCl_3$, c = 0.3). ^{1}H NMR (400 MHz, CD_3OD) selected signals: δ 8.11 (2H, d, J = 7.5), 7.65 (1H, t, J = 7.5), 7.53 (2H, t, J = 7.5), 7.03 (1H, d, J = 8.23), 6.51 (1H, s), 6.37 (1H, dd, J_1=8.2, J_2=2.2), 6.17 (2H, m), 6.10–5.75 (1H, m), 5.69 (1H, d, J = 7.1), 5.43 (1H, s), 5.22 (1H, d, J = 10.16), 5.02 (1H, d, J = 7.8), 4.55 (1H, dd, J = 10.4, 6.7), 4.21 (2H, AB-syst), 3.89 (1H, d, J = 7.15), 3.80 e 3.77 (6H, s), 3.62 (1H, s), 3.51.3.44 (1H, m), 3.38 (1H, td, J = 9, 3.8), 2.87 (1H, m), 2.73 (1H, s), 2.7–2.58 (4H, m) 2.67 (3H, s), 2.60–2.5 (2H, m), 2.4–2.2 (4H, m), 2.37 (3H, s), 2.16 (3H, s), 2.04 (3H, s), 1.86 (1H, m), 1.7 (3H, s), 1.60 (1H, m), 1.20 (3H, s), 1.18 (3H, s), 1.15–1.08 (1H, m), 0.96 (9H, t), 0.62 (6H, q, J = 7.5), 0.5 (3H, s). ^{13}C NMR (100 MHz, CD_3OD) selected signals: δ 204.8, 174.13(2C), 173.9, 172.1, 171.1, 167.9, 163.1, 155.4, 142.5, 135.3, 134.8, 131.8(2C),131.4, 130.0(2C), 126.8, 126.0, 124.6, 106.4, 97.3, 85.8, 85.1, 82.3, 81.2, 79.3, 78.4, 77.8, 77.0, 76.5, 74.2, 71.6, 68.5, 60.2, 56.1, 54.4, 53.3, 53.1, 52.4, 45.3, 44.7, 39.4, 38.7, 37.3, 35.1, 32.4, 30.4, 27.1, 23.3, 22.0, 21.1, 15.4, 10.9, 8.4, 7.5, 6.6. ESI positive MS: Anal. Calcd for $C_{64}H_{84}O_{18}N_2Si$+Na^+ 1219.5381, found 1219.5383.

5.1.8. 7-*N*-Deacetyl-*N*-hemisebacoylthiocolchicine (4b). A solution of **4a** (500 mg, 1.34 mmol), sebacoyl acid (271 mg, 1.34 mmol), BOPCl (409 mg, 1.608 mmol), and Et_3N (221 μl, 1.608 mmol) in CH_2Cl_2 (10 ml) was stirred at room temperature for 2 days. After the addiction of HCl (1 N, 10 ml) the organic phase was concentrated and purified by chromatography (AcOEt/MeOH 30: 1) to give **4b** (212 mg, 28%) as amorphous solid. ^{1}H NMR ^{1}H NMR (300 MHz, $CDCl_3$) selected signals: δ 7.58 (1H, s), 7.34 (1H, d, J = 9), 7.10 (1H, d, J = 9), 6.95 (1H, d, J = 5), 6.52 (1H, s), 4.75–4.63 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.62 (3H, s), 2.56–2.40 (1H, m), 2.40 (3H, s), 2.40–2.23 (4H, m), 2.23–2.15 (2H, m), 1.92–1.78 (1H, m), 1.68–1.49 (4H, m), 1.38–1.18 (8H, m); ESI positive MS: Anal. Calcd for $C_{30}H_{39}O_7NS$+Na^+ 580.2339, found 580.2346.

5.1.9. 17-Deacetyl-17-*O*-(*N*-deacetyl-*N*-sebacoylthiocolchicinyl)vindoline (11). A solution of **4b** (230 mg, 0.412 mmol), DMAP (230 mg, 0.412 mmol), DCC (170 mg, 0.825 mmol) and **1a** (170 mg, 0.412 mmol) in CH_2Cl_2 (10 ml) was stirred for 16 h at room temperature. After Celite filtration, the mixture was submitted to chromatography (AcOEt/Et_3N 0.1%) to give **11** (212 mg, 54%) as amorphous solid. ^{1}H NMR (400 MHz, CD_3OD) selected signals: δ 7.38–7.31 (1H, m), 7.19 (1H, s), 7.02 (1H, d, J = 8), 6.71 (1H, s), 6.35

(1H, dd, $J = 8$, 2), 6.18 (1H, d, $J = 2$), 5.85 (1H, dd, $J = 10$, 4), 5.39 (1H, s), 5.15 (1H, d, $J = 10$), 4.55–4.48 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.77 (3H, s), 3.76 (3H, s), 3.64 (3H, s), 3.62 (1H, s), 3.48 (1H, dd, $J = 16$, 5), 3.42–3.35 (1H, m), 2.85 (1H, d, $J = 16$), 2.73 (1H, s), 2.65 (3H, s), 2.62–2.56 (1H, m), 2.47 (3H, s), 2.45–2.13 (8H, m), 2.32–2.25 (2H, m), 2.00–1.90 (1H, m), 1.65–1.54 (4H, m), 1.27–1.37 (8H, m), 1.18–1.09 (1H, m), 0.50 (3H, t, $J = 5.5$). ^{13}C NMR (100 MHz, CD_3OD) selected signals: δ 182.4, 174.3, 173.7, 172.1, 161.5, 158.5, 154.0, 153.8, 152.1, 150.9, 141.5, 138.7, 135.0, 134.7, 130.0, 127.6, 127.2, 125.6, 125.2, 124.3, 122.8, 107.6, 104.7, 95.8, 83.4, 79.7, 76.1, 66.9, 60.6, 60.2, 55.4, 54.5, 52.7, 52.0(2C), 51.4, 50.6, 43.5, 43.0, 37.6, 35.9, 35.4, 33.7, 30.6, 29.2, 28.8(4C), 28.7, 25.3, 13.6, 6.6. ESI positive MS: Anal. Calcd for $C_{53}H_{67}O_{11}N_3S+Na^+$ 976.4389, found 976.4387.

5.1.10. 4-*O*-Hemisebacoylpodophyllotoxin (5c). A mixture of **5** (159 mg, 0.384 mmol), DMAP (19 mg, 0.154 mmol), DCC (95 mg, 0.461 mmol), and hemytrichloroethylester of sebacic acid (155 mg, 0.461 mmol) in CH_2Cl_2 (16 ml) was stirred for 13 h at room temperature. The mixture was submitted to chromatography (AcOEt/hexane 1:3) to give **5b** (244 mg, 87%) as a white amorphous solid. A mixture of Zn (1 g) and HCl (2%, 10 ml) was stirred for 5 min. After septum-filtration the solid was washed with water, acetone, and ethyl ether. A mixture of activated Zn (306 mg, 4.69 mmol), and **5b** (229 mg, 0.312) in AcOH/MeOH (1:1, 5 ml) was stirred for 10 h. After Celite filtration the solution was washed with water and extracted with AcOEt. The evaporation of the solvent gave **5c** (176 mg, 94%) as white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) selected signals: δ 7.07 (1H, s), 6.57 (1H, s), 6.45 (2H, s), 6.06–5.97 (3H, m), 4.66 (1H, d, $J = 4.5$), 4.38 (1H, t, $J = 9.0$), 4.19 (1H, t, $J = 9.0$), 3.27 (1H, dd, $J = 13.0$, 4.5), 2.81–2.72 (1H, m), 2.40–2.23 (2H, m), 2.23–2.15 (2H, m), 1.66–1.47 (4H, m), 1.35–1.15 (8H, m). ESI positive MS: Anal. Calcd for $C_{32}H_{38}O_{11}+Na^+$ 621.2306, found 621.2307.

5.1.11. 17-Deacetyl-17-*O*-(4-*O*-sebacoylpodophyllotoxinyl)vindoline (12). A mixture of **5c** (168 mg, 0.281 mmol), DCC (69 mg, 0.337 mmol), DMAP (14 mg, 0.112 mmol), and**1b** (116 mg, 0.281 mmol) and CH_2Cl_2 (10 ml) was stirred for 12 h. The mixture was submitted to chromatography (AcOEt/hexane 20:1) to give **12** (130 mg, 46%) as amorphous yellow solid. R_f: 0.35 (AcOEt). ^{1}H NMR (400 MHz, CD_3COCD_3) selected signals: δ 8.58 (1H, s), 7.10 (1H, d, $J = 8.5$), 6.92 (1H, s), 6.58 (1H, s), 6.42 (2H, s), 6.32 (1H, dd, $J = 8.5$, 2.0), 6.18 (1H, d, $J = 2.0$), 6.04 (2H, s), 5.98 (1H, d, $J = 9$), 5.81 (1H, dd, $J = 8.5$, 4.0), 5.39 (1H, s), 5.16 (1H, br d, J = 8.5), 4.63 (1H,d, $J = 4.5$), 4.38 (1H, dd, $J = 9.0$, 9.1), 4.18 (1H, dd, $J = 9.0$, 9.1), 3.75 (3H, s), 3.71 (6H, s), 3.68 (6H, s), 3.64 (1H, s), 3.48 (1H, dd, $J = 14$, 4), 3.39 (1H, td, $J = 4$, 8), 3.25 (1H, dd, $J = 14$, 4.6), 2.92–0.45 (23H, m), 2.68 (3H, m, NMe); ESI positive MS: Anal. Calcd for $C_{55}H_{66}O_{15}N_2+Na^+$ 1017.4355, found 1017.4361.

5.1.12. 13-*O*-Hemisebacoyl-7-*O*-(triethylsilyl)baccatin III (6e). A solution of **6a** (900 mg, 1.29 mmol), undecenoyl acid (1.56 ml, 7.74 mmol), DCC (1.59 g, 7.74 mmol), and DMAP (0.645 mmol) in toluene (10 ml) was stirred at room temperature for 14 h. The mixture was filtered and the solvent evaporated. The crude mixture was submitted to chromatography (AcOEt/hexane 1:9) to give **6d** (970 mg, 87%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) selected signals: δ 8.10 (2H, d, $J = 7.5$), 7.61 (1H, t, $J = 7.5$), 7.46 (2H, t, $J = 7.5$), 6.47 (1H, s), 6.20–6.10 (1H, m), 5.89–5.72 (1H, m), 5.66 (1H, d, $J = 7$), 5.01–4.93 (3H, m), 4.46 (1H, dd, $J = 10$, 7), 4.30 (1H, d, $J = 8.3$), 4.13 (1H, d, $J = 8.3$), 3.83 (1H, d, $J = 7$), 2.56–2.33 (3H, m), 2.31 (3H, s), 2.20 (2H, d, $J = 8.9$), 2.16 (3H, s), 2.02 (3H, s), 1.92–1.84 (1H, m), 1.8–1.55 (4H, m) 1.67 (3H, s), 1.43–1.20 (8H, m), 1.21 (3H, s), 1.17 (3H, s), 0.91 (9H, s), 0.59 (6H, s). **6d** (918 mg, 1.06 mmol) was dissolved in $CH_3CN/CCl_4/H_2O$ (1:1:2) (20 ml) and treated with $NaHCO_3$ (890 mg, 10.6 mmol), and $NaIO_4$ (1.93 g, 9.01 mmol). After 5 min $RuCl_3$ (33 mg, 0.159 mmol) was added and the mixture was stirred for 10 days at room temperature in the dark. Every 2 days a quote of $RuCl_3$ (22 mg, 0.106 mmol) was added. The mixture was diluted with AcOEt and treated with $H_2SO_4$2N. The organic phase was washed with a saturated solution of Na_2SO_3 (100 ml) and ice. The mixture was submitted to chromatography (AcOEt/hexane 1:4) to give **6e** (766 mg, 82%) as white amorphous solid. ^{1}H NMR ($CDCl_3$, 300 MHz) selected signals: δ 8.1 (2H, d, $J = 7.5$), 7.6 (1H, t, $J = 7.5$), 7.46 (2H, t, $J = 7.5$), 6.45 (1H, s), 6.16 (1H, br t, J = 8.8), 5.67 (1H, d, $J = 7$), 4.93 (1H, d, $J = 10$), 4.47 (1H, dd, $J = 10.2$, 7), 4.29 (1H, d, $J = 8.3$), 4.14 (1H, d, $J = 8.3$), 3.82 (1H, d, $J = 7$), 2.59–2.33 (5H, m), 2.31 (3H, s), 2.20 (2H, d, $J = 8.9$), 2.16 (3H, s), 2.02 (3H, s), 1.95–1.80 (1H, m), 1.78–1.58 (4H, m) 1.67 (3H, s), 1.44–1.30 (8H, m), 1.21 (3H, s), 1.17 (3H, s), 0.91 (9H, s), 0.59 (6H, s). ESI positive MS: Anal. Calcd for $C_{47}H_{68}O_{14}Si+Na^+$ 907.4271, found 907.4272.

5.1.13. 13-*O*-(17-Deacetyl-17-*O*-sebacoylvindolinyl)-7-*O*-(triethylsilyl)baccatin III (13). A solution of **6e** (40 mg, 0.045 mmoli), DMAP (2.2 mg, 0.018 mmol), DCC (11.2 mg, 0.054 mmoli), and **1a** in CH_2Cl_2 (3 ml) was stirred for 16 h at room temperature and then filtered on Celite. The evaporation of the solvent gave a mixture that was submitted to chromatography (AcOEt/hexane 2:1, 0.5% Et_3N) to give **13** (39 mg, 69%). $[\alpha]_D^{25}$ +133 ($CHCl_3$, $c = 0.3$). ^{1}H NMR (400 MHz, CD_3OD) selected signals: δ 8.11 (2H, d, $J = 7$), 7.65 (1H, t, $J = 7.5$), 7.53 (2H, t, $J = 7.5$), 7.03 (1H, d, $J = 8.2$), 6.51 (1H, s), 6.35 (1H, dd, $J = 8.2$, 2.2), 6.17 (2H, m), 5.87 (1H, ddd, $J = 6.6$, 4.7, 1.7), 5.69 (1H, d, $J = 7.1$), 5.41 (1H, s), 5.16 (1H, d, $J = 10.2$), 5.02 (1H, d, $J = 8$), 4.55 (1H, dd, $J = 10.5$, 6.7), 4.21 (2H, AB-syst, $J = 8.4$), 3.89 (1H, d, $J = 7.2$), 3.78 e 3.77 (6H, s), 3.62 (1H, s), 3.49 (1H, m), 3.4 (1H, m), 2.89 (1H, m), 2.75 (1H, s), 2.66 (3H, s), 2.61–2.57 (2H, m), 2.35–2.27 (8H, m), 2.36 (3H, s), 2.17 (3H, s), 2.03 (3H, s), 1.95–1.80 (1H, m), 1.7 (3H, s), 1.62 (5H, m, 1), 1.45 - 1.30 (8H, m), 1.22 (3H, s), 1.18 (3H, s), 0.96 (9H, t), 0.62 (6H, q, $J = 7.5$), 0.5 (3H, s). ^{13}C NMR (100 MHz, CD_3OD) se-

lected signals: δ 204.8, 173.7, 173.4, 172.5, 170.2, 169.4, 166.2, 161.4, 153.8, 140.6, 133.8, 133.2, 130.0, 129.7, 128.3, 125.1, 124.3, 122.9, 104.7, 95.7, 84.1, 83.4, 80.7, 79.6, 77.5, 76.1, 76.1, 75.3, 74.7, 72.5, 69.5, 66.8, 58.5, 54.4, 52.7, 51.5, 51.5, 50.6, 46,8, 43.5, 43.2, 43.0, 37.7, 37.0, 35.6, 33.9, 33.7, 33.4, 30.7, 28.7, 25.5, 24.6, 21.6, 20.4, 19.4, 13.7, 9.2, 6.7, 5.8, 4.9. ESI positive MS: Anal. Calcd for $C_{70}H_{96}O_{18}N_2Si+Na^+$ 1303.6320, found 1303.6319.

5.1.14. 17-Deacetyl-17-*O*-(*N*-deacetyl-*N*-succinylthiocolchicinyl)anhydrovinblastine (14). A solution of catharanthine·HCl (55.5 mg, 0.149 mmol), $FeCl_36H_2O$ (270 mg, 0.747 mmol), glycine buffer (4 ml), and HCl (4 ml, 0.1 N) was stirred at room temperature for 2.5 h. **8** (130 mg, 0.149 mmol). After 2.5 h $NaBH_4$ (6.1 mg, 0.160 mmol) was dissolved in NH_4OH (0.74 ml). The solution was extracted with CH_2Cl_2. After chromatographic purification (AcOEt/MeOH/Et_3N 20:2:0.4) **14** (40 mg, 22%) was obtained as a yellow amorphous solid. ^1H NMR (300 MHz, CD_3COCD_3) selected signals: δ 9.18 (1H, br s), 8.48 (1H, s), 7.80 (1H, d, *J* = 6.5), 7.45 (1H, d, *J* = 7), 7.40 (1H, d, *J* = 7), 7.20 (1H, s), 7.20 (1H, s), 7.12 (1H, s), 7.11 (1H, t, *J* = 7), 7.01 (1H, t, *J* = 7), 6.78 (1H, s), 6.32 (1H, s), 5.51 (1H, dd, *J* = 8, 4.5), 5.35–5.45 (1H, m), 5.30 (1H, s), 5.25 (1H, d, *J* = 8), 4.61–4.50 (1H, m), 3.88 (3H, s), 3.83 (3H, s), 3.80 (3H, s), 3.65 (3H, s), 3.62 (3H, s), 3.50 (3H, s), 2.68 (3H, s), 2.41 (3H, s), 0.99 (1H, t, *J* = 7.5), 0.62 (3H, t, *J* = 5.5). ^{13}C NMR (100 MHz, CD_3COCD_3) selected signals: δ 184.1, 177.2, 173.9(2C), 173.5, 160.2, 159.9, 155.5, 154.8, 153.7, 152.6, 143.0, 140.3, 137.0, 136.8, 136.5, 132.0, 131.9, 130.6, 129.3, 129.0, 127.3, 125.6, 125.4, 125.1, 123.8, 123.7(2C), 120.3, 119.3, 117.7, 112.2, 109.1(2C), 95.6, 84.6, 81.6, 78.0, 66.7, 62.3, 62.0, 56.9, 56.9, 56.2, 55.9, 54.9, 54.1, 53.3, 53.1, 52.3, 51.7, 51.1, 47.0, 46.1, 44.4, 39.1, 37.5, 36.1, 34.1, 32.1, 31.3, 30.1, 30.0, 29.3, 25.1, 15.4, 12.9, 9.3. FABMS: 1205 (M^+). ESI positive MS: Anal. Calcd for $C_{68}H_{79}O_{13}N_5S+Na^+$ 1228.5287, found 1228.5288.

5.1.15. 17-Deacetyl-17-*O*-(4-*O*-succinylpodophyllotoxinyl)anhydrovinblastine (15). The use of the same procedure described for the preparation of **14** and the addition of compound **9** as electrophile gave **15** (28%) (chromatographic purification CH_2Cl_2/MeOH 30:1, 24%). ^1H NMR (500 MHz, CD_3COCD_3) selected signals: δ 7.47 (1H, d, *J* = 7.5), 7.38 (1H, d, *J* = 7.5), 7.08 (1H, t, *J* = 7.5), 7.04 (1H, s), 7.01 (1H, t, *J* = 7.5), 6.84 (1H, s), 6.62 (1H, s), 6.48 (2H, s), 6.38 (1H, s), 6.05 (1H, d, *J* = 9.1), 6.04 (2H, AB-syst), 5.77 (1H, dd, *J* = 8.5, 4.0), 5.45–5.39 (1H, m), 5.38 (1H, s), 5.37 (1H, d, *J* = 8.5), 4.65 (1H, d, *J* = 4.5), 4.42 (1H, dd, *J* = 8.7, 7.2), 4.22 (1H, dd, *J* = 10.5, 8.7), 3.73 (3H, s), 3.72 (3H, s), 3.72 (3H, s), 3.68 (6H, s), 3.68 (3H, s), 3.40–3.30 (1H, m), 3.57–3.49 (1H, m), 3.38–3.20 (2H, m), 3.37–3.21 (1H, m), 3.35–3.29 (1H, m), 2.75–2.68 (1H, m), 3.20–3.12 (1H, m), 2.48–2.32 (1H, m), 3.20 (1H, s), 3.10–3.00 (1H, m), 3.37–3.21 (1H, m), 2.90–2.76 (1H, m), 2.90–2.75 (4H, m), 2.82 (1H, s), 2.71 (3H, s), 2.55 (1H, br d, J = 12.5), 3.45–3.38 (1H, m), 2.45–2.35 (1H, m), 3.37–3.21 (1H, m), 2.00–1.90 (2H, m), 2.00–1.89 (1H, m), 2.19–2.10 (1H, m), 1.48–1.35 (1H, m), 1.72–1.59 (1H, m), 1.26–1.21 (1H, m), 1.00 (3H, t, *J* = 7.4), 0.77 (3H, t, *J* = 7.3). ^{13}C NMR (125 MHz, CD_3COCD_3) selected signals: δ 173.5, 173.1,173.0, 171.9, 161.2, 156.1, 152.7(2C), 147.9, 147.5, 137.5, 136.5, 135.6, 135.0, 132.8, 130.9, 130.6, 129.4, 129.1, 125.9, 125.0, 124.3, 124.2, 121.7, 121.2, 118.5, 118.4, 117.3, 111.2, 109.3, 108.7, 107.3, 101.6, 94.1, 83.3, 79.3, 76.9, 73.4, 70.9, 65.2, 59.5, 55.6(3C), 54.2, 53.1, 53.1, 51.1(2C), 50.6, 50.2(2C), 50.2, 46.0, 44.7, 43.7, 42.6, 38.9, 37.7, 34.1, 33.5, 30.7, 28.9–28.7, 27.1, 25.4, 8.2, 7.8. MSFAB (M^++1) 1247. ESI positive MS: Anal. Calcd for $C_{70}H_{78}O_{17}N_4+Na^+$ 1269.5254, found 1269.5257.

5.1.16. 13-*O*-(17-Deacetyl-17-*O*-succinylanhydrovinblastinyl)-7-*O*-(triethylsilyl)baccatin III (16). A solution of catharanthine (143 mg, 0.425 mmol) in CH_2Cl_2 was treated with *m*-CPBA (110 mg, 0.64 mmol in CH_2Cl_2) at 0 °C. The solution was maintained at the same temperature for 10 min and poured in $NaHCO_3$ (20 ml, 10%). The extraction with CH_2Cl_2 gave the *N*-oxide-catharanthine (141 mg) which was immediately dissolved in CH_2Cl_2 (2 ml) and compound **10** (186 mg, 0.155 mmol) was added. TFAA (165 μl, 1.17 mmol) was added at −78 °C. After 1 h the solvent was removed and the mixture dissolved in MeOH (8 ml). $NaBH_4$ (64 mg, 1.7 mmol) was added at 0 °C. After 20 min the solution was poured in water and extracted with CH_2Cl_2. The evaporation of the solvent gave a mixture that was submitted to chromatography (AcOEt/MeOH 7:1) to give **16** (53 mg, 22 %). ^1H NMR (400 MHz, CD_3COCD_3) selected signals: δ 8.14 (2H, d, *J* = 7.5), 7.68 (1H, t, *J* = 7.5), 7.57 (2H, t, *J* = 7.5), 7.46 (1H, d, *J* = 7.8), 7.38 (1H, d, *J* = 7.8), 7.08 (1H, t, *J* = 7.8), 7.01 (1H, t, *J* = 7.8), 6.82 (1H, s), 6.52 (1H, s), 6.37 (1H, s), 6.25 (1H, t, *J* = 8.6), 5.81 (1H, dd, *J* = 10.2, 3.9), 5.72 (1H, d, *J* = 7), 5.41 (1H, br d, J = 5.3), 5.36 (1H, d, *J* = 10.2), 5.00 (1H, d, *J* = 8.5), 4.59 (1H, dd, *J* = 10, 6.7), 4.18 (2H, br s), 3.93 (1H, d, *J* = 7), 3.68 (1H, s), 2.78 (1H, s), 2.74 (3H, s), 2.47–2.32 (2H, m), 0.97 (9H, t, *J* = 9), 0.64 (6H, q, *J* = 9). ^{13}C NMR (100 MHz, CD_3COCD_3) selected signals: δ 133.2, 130.6, 130.0, 128.6, 124.3, 124.1(2C), 121.6, 118.5, 117.9, 111.1, 94.2, 77.7, 76.8, 75.9, 75.1, 74.8, 72.5, 69.6, 58.3, 54.3, 52.8, 51.3, 50.2, 46.9, 37.9, 37.3, 36.1, 26.0, 22.1, 20.6, 19.9, 14.1, 9.6, 6.2, 5.0. ESI positive MS: Anal. Calcd for $C_{85}H_{108}O_{20}N_4Si+Na^+$ 1555.7218, found 1555.7221.

5.1.17. 17-Deacetyl-17-*O*-(*N*-deacetyl-*N*-sebacoylthiocolchicinyl)anhydrovinblastine (17). The use of the same procedure described for the preparation of **14** and the addition of compound **11** as electrophile gave **17** (Chrom. AcOEt/MeOH/Et_3N 20:2:0.4) (24 %). ^1H NMR ^1H NMR (300 MHz, CD_3COCD_3) selected signals: δ 9.15 (1H, br s), 8.70–8.40 (1H, large), 7.75 (1H, d, *J* = 6.5), 7.47 (1H, d, *J* = 7), 7.38 (1H, d, *J* = 7), 7.18 (2H, s), 7.15 (1H, s), 7.11 (1H, t, *J* = 7), 7.00 (1H, t, *J* = 7), 6.31 (1H, s), 6.78 (1H, s), 6.32 (1H, s), 5.85 (1H, dd, *J* = 8, 4.5), 5.41–5.32 (1H, m), 5.31 (1H, s), 5.28 (1H, d, *J* = 8), 4.61–4.48 (1H, m), 3.88 (3H, s), 3.83 (3H, s), 3.80 (3H, s), 3.65 (3H, s), 3.62 (3H, s), 3.50 (3H, s), 2.68 (3H, s), 2.41 (3H, s), 3.90–0.70 (32H, m). ^{13}C NMR (100 MHz, CD_3COCD_3) selected signals: δ 181.5, 177.2, 172.0(2C), 171.5, 160.2, 157.9, 153.5,

152.9, 151.1, 150.8, 141.7, 137.8, 134.6, 134.5, 133.8, 132.0, 131.9, 130.6, 129.3, 129.0, 127.3, 125.6, 125.4, 125.1, 123.8, 123.7(2C), 120.3, 119.3, 117.7, 111.1, 109.1, 107.7, 95.6, 84.6, 81.6, 78.0, 66.7, 62.3, 62.0, 56.9, 56.9, 56.2, 55.9, 54.9, 54.1, 53.3, 53.1, 52.3, 51.7, 51.1, 47.0, 46.1, 44.4, 39.1, 37.5, 36.1, 35.4, 34.1, 33.7, 32.1, 31.3, 30.1, 30.0, 29.3, 28.8(4C), 28.7, 25.3, 25.1, 15.4, 12.9, 9.3; ESI positive MS: Anal. Calcd for $C_{74}H_{91}O_{13}N_5S+H^+$ 1290.6407, found 1290.6419.

5.1.18. Vinorelbine (3). AVBL (196 mg, 0.248 mmol) was dissolved in CH_2Cl_2 (1 ml). NBS (49.5 mg, 0.278 mmol) and CF_3COOH (27.5 μl, 0.357 mmol) in CH_2Cl_2 (1 ml) were added at −60 °C and the solution was stirred for 2 h at the same temperature. CH_3COONH_4 (51.68 mg, 0.67 mmol in 400 μl of H_2O) and $AgBF_4$ (53.86 mg, 0.277 mmol in THF/H_2O 1:1) were added at room temperature. After 20 h $NaHCO_3$ (1.6 ml, 10%) was added and the solution was extracted with CH_2Cl_2. The crude mixture was submitted to chromatography (AcOEt/MeOH/Et_3N 98.25:0.75:1) to give **3** (101 mg, 53%) as a amorphous solid. ^1H NMR (500 MHz, CD_3COCD_3) selected signals: δ 9.72 (1H, s), 8.45 (1H, s), 7.76 (1H, d, $J = 10$), 7.45 (1H, d, $J = 10$), 7.13 (1H, t, $J = 10$), 7.09 (1H, t, $J = 10$), 6.58 (1H, s), 6.36 (1H, s), 5.81–5.78 (2H, m), 5.30 (1H, br d, J = 10), 5.28 (1H, s), 4.53–4.44 (1H, m), 4.40 (1H, d, $J = 12.86$), 3.87 (3H, s), 3.78–3.74 (1H, m), 3.70 (3H, s), 3.63 (3H, s), 3.60 (1H, s), 3.54 (1H, br d, J = 14.01), 3.33–3.27 (1H, m), 3.28 (1H, dd, $J_1 = 13.92$, $J_2 = 4.23$), 3.20 (1H, dt, $J_1 = 9.7$, $J_2 = 5.6$), 2.95 (1H, dd, $J_1 = 15.76$, $J_2 = 7.32$), 2.74 (3H, s), 2.73 (1H, s), 2.35 (1H, dd, $J_1 = 13.92$, $J_2 = 4.23$), 2.66 (1H, d, $J = 15.76$), 2.41–2.38 (1H, m), 2.35 (1H, dt, $J_1 = 9.7$, $J_2 = 5.6$), 2.11–2.05 (3H, m), 1.99 (3H, s), 1.95–1.89 (1H, m), 1.63–1.56 (2H, m), 1.39–1.29 (1H, m), 1.10 (3H, t, $J = 7.46$), 0.68 (3H, t, $J = 7.34$). ^{13}C NMR (100 MHz, CD_3COCD_3) selected signals: δ 174.1, 170.0, 171.5, 158.4, 153.1, 135.3, 130.6, 128.8, 124.1, 123.8, 123.6, 123.1, 122,0, 120.1, 120.1, 118.4, 111.3, 93.7, 83.0, 79.7, 76.4, 64.6, 55.4, 54.9, 53.2, 51.7,51.0, 50.2, 49.7, 47.2, 44.3, 44.6, 42.7, 37.7, 36.6, 30.6, 27.5, 20.2, 11.7, 7.6. ESI positive MS: Anal. Calcd for $C_{45}H_{54}O_8N_4+Na^+$ 801.3834, found 801.3835.

5.1.19. 17-*O*-Deacetylvinorelbine (3a). A solution of **3** (265 mg, 0.341 mmol) and Na_2CO_3 in MeOH (10 ml) was stirred at 65 °C for 14 days. Evaporation of the solvent and chromatography (alumina, AcOEt/MeOH/Et_3N 10:1:0.1) **3a** (149 mg, 59%). $[\alpha]_D^{25}$ +98.2 ($CHCl_3$, $c = 0.61$). ^1H NMR (400 MHz, CD_3OD) selected signals: δ 7.73 (1H, d, $J = 7.12$), 7.34 (1H, dd, $J_1 = 7.12$, $J = 1.2$), 7.18 (1H, td, $J = 7.1$, 1.2), 7.14 (1H, td, $J = 7.1$, 1.2), 6.29 (2H, s), 5.83 (1H, dd, $J = 10$, 3.7), 5.78 (1H, d, $J = 4.9$), 5.64 (1H, d, $J = 10$), 4.42–4.36 (2H, m), 4.03 (1H, br d, J = 15.3), 3.96 (1H, s), 3.88–3.79 (1H, m), 3.86 (3H, s), 3.82 (3H, s), 3.75 (3H, s), 3.53 (1H, s), 3.43 (1H, d, $J = 14$), 3.31–3.26 (1H, m), 3.20 (1H, dt, $J = 10.3$, 3. 9), 3.06 (1H, dd, $J = 15.5$, 7.64), 2.76 (3H, s), 2.67–2.59 (2H, m), 2.52 (1H, s), 2.37 (1H, dd, $J = 15.5$, 4.), 2.28 (1H, dt, $J = 10.3$, 4.5), 2.14–1.99 (3H, m), 1.79–1.69 (1H, m), 1.66–1.56 (2H, m), 1.35–1.22 (1H, m), 1.11 (3H, t, $J = 7.5$), 0.81 (3H, t, $J = 7.3$). ^{13}C NMR (100 MHz, CD_3OD) selected signals: δ 175.2, 172.9,158.6, 153.0, 134.9, 134.7, 134.1, 130.5, 128.5, 123.8, 123.4, 122.6, 122.3, 119.5, 119.3, 117.6, 110.8, 93.2, 82.6, 81.1, 74.0, 65.5, 54.9, 54.9, 53.1, 51.8, 51.4, 50.3, 49.5, 46.5, 44.4, 44.3, 42.6, 37.4, 35.8, 31.8, 28.7, 27.4, 11.2, 7.3. ESI positive MS: Anal. Calcd for $C_{43}H_{52}O_7N_4+Na^+$ 759.3728, found 759.3728.

5.1.20. 17-Deacetyl-17-*O*-(*N*-deacetyl-*N*-succinylthiocolchicinyl)vinorelbine (18). A mixture of **3** (100 mg, 0.136 mmol), DMAP (7 mg, 0.054 mmol), imidazole (11 mg, 0.163 mmol), and succinic anhydride (20 mg, 0.204 mmol) in CH_2Cl_2 (8 ml) was stirred for 12 h at room temperature. The solution was washed with water. The evaporation of the solvent gave a crude compound (**3b**, 110 mg, 97%) that was directly used for the succeeding preparation. A solution of **3b** (55 mg, 0.066 mmol), DCC (33 mg, 0.161 mmol), DMAP (7 mg, 0.054 mmol), and **4a** (55 mg, 0.147 mmol) in CH_2Cl_2 (15 ml) was stirred for 24 h. Evaporation of the solvent and chromatography (AcOEt/MeOH/Et_3N 10:1:0.2) gave **18** (24 mg, 30%) as an amorphous solid. $[\alpha]_D^{25}$ −66.3 ($CHCl_3$, $c = 0.8$). ^1H NMR (400 MHz, CD_3COCD_3) selected signals: δ 9.64 (1H, s), 8.42 (1H, s), 7.83 (1H, d, $J = 6.9$), 7.69 (1H, d, $J = 7.8$), 7.43 (1H, d, $J = 7.8$), 7.22 (2H, AB-syst), 7.16 (1H, s), 7.15 (1H, td, $J = 7.8$. 1.2), 7.09 (1H, t, $J = 7.8$), 6.79 (1H, s), 6.52 (1H, s), 6.33 (1H, s), 5.75 (1H, br s), 5.49 (1H, dd, $J = 10$, 4), 5.25 (1H, s), 5.30–5.19 (1H, m), 4.60–4.50 (1H, m), 4.41–4.28 (2H, m), 3.89 (3H, s), 3.88 (3H, s), 3.85 (3H, s), 3.74–3.60 (1H, m), 3.65 (6H, s), 3.62 (3H, s), 3.56 (1H, s), 3.46 (1H, d, $J = 15$), 3.25–3.13 (3H, m), 2.90 (1H, dd, $J = 15.4$, 7.4), 2.75–2.66 (1H, m), 2.71 (3H, s), 2.67 (1H, s), 2.66–2.49 (5H, m), 2.49–2.40 (2H, m), 2.44 (3H, s), 2.40–2.26 (2H, m), 2.27–2.18 (1H, m), 2.10–1.98 (3H, m), 1.98–1.89 (1H, m), 1.89–1.79 (1H, m), 1.54–1.43 (2H, m), 1.29–1.19 (1H, m), 1.08 (3H, t, $J = 7.5$), 0.56 (3H, t, $J = 7.2$). ^{13}C NMR (100 MHz, CD_3COCD_3) selected signals: δ 182.6, 175.3, 172.8, 172.5, 171.4, 159.6, 159.0, 154.9; 154.0, 152.2, 151.7, 142.9, 137.3, 136.3, 135.6, 135.3, 134.8, 131.9, 130.0, 129.3, 127.2, 126.8, 125.1, 124.8, 124.6, 124.1, 122.9, 121.5, 120.2, 119.2, 112.3, 108.9; 94.8, 84.1, 80.8, 77.4, 66.0, 61.8, 61.4, 56.6, 56.4, 56.1, 55.9, 54.2, 53.1, 52.7, 52.0, 51.4, 50.9, 48.5, 46.0, 45.6, 43.9, 38.7, 38.0, 37.3, 31.5, 31.3, 30.8, 30.6–29.3(2C), 28.8, 15.1, 12.9, 8.6. ESI positive MS: Anal. Calcd for $C_{67}H_{77}O_{13}N_5S+Na^+$ 1214.5131, found 1214.5132.

5.1.21. 17-Deacetyl-17-*O*-(4-*O*-succinylpodophyllotoxinyl) vinorelbine (19). A solution of crude **3b** (55 mg, 0.066 mmol), DCC (33 mg, 0.161 mmol), DMAP (7 mg, 0.054 mmol), and **5** (56 mg, 0.132 mmol) in CH_2Cl_2 (15 ml) was stirred for 24 h. Evaporation of the solvent and chromatography (CH_2Cl_2/MeOH 15:1) gave **19** (25 mg, 31%) as an amorphous solid. ^1H NMR (400 MHz, CD_3COCD_3) selected signals: δ 9.75 (1H, s), 7.81 (1H, d, $J = 7.8$), 7.45 (1H, d, $J = 7.8$), 7.14 (1H, t, $J = 7.8$), 7.09 (1H, t, $J = 7.8$), 7.01 (1H, s), 6.58 (1H, s), 6.56 (1H, s), 6.45 (2H, s), 6.33 (1H, s), 6.06–5.99 (3H, m), 5.83(1H, d, $J = 5.3$), 5.74 (1H, dd, $J = 10.2$, 3.9), 5.34 (1H, d, $J = 10.2$), 5.29 (1H, s), 4.68 (1H, A portion of AB-syst), 4.65 (1H, d, $J = 4.6$), 4.49 (1H, B portion of AB-syst), 4.40 (1H, dd, $J = 8.7$, 7.2), 4.21 (1H, t,

$J = 8.7$), 3.92–3.82 (1H, m), 3.85 (3H, s), 3.70 (6H, s), 3.69–3.61 (1H, m) 3.68 (3H, s), 3.65 (3H, s), 3.48–3.38 (1H, m), 3.28 (1H, dd, $J = 10.2$, 4.7), 3.24–3.14 (2H, m), 3.04–2.82 (2H, m), 2.82–2.60 (6H, m), 2.74 (4H, s), 2.60–2.52 (1H, m), 2.41–2.32 (1H, m), 2.15–2.07 (2H, m), 2.07–2.02 (1H, m), 1.98–1.89 (1H, m), 1.82–1.70 (1H, m), 1.65–1.53 (1H, m), 1.40–1.29 (1H, m), 1.11 (3H, t, $J = 7.4$), 0.67 (3H, t, $J = 7.3$). ^{13}C NMR (100 MHz, CD_3COCD_3) selected signals: δ 174.5, 174.1, 173.9, 172.8, 172.7, 159.5, 154.2, 153.8(2C), 148.1, 147.6, 136.5, 136.2, 131.5, 130.2, 125.2, 124.6(2C), 123.4, 120.6, 119.6, 112.2, 110.3, 110.1(2C), 108.2, 102.5, 94.9, 84.1, 80.6, 78.0, 74.5, 72.0, 66.1, 60.6, 56.7(2C), 56.4, 54.2(2C), 52.8, 52.0, 51.3, 51.0, 47.9, 45.8, 45.4, 45.0, 44.8, 43.9, 40.0, 38.6, 36.9, 31.7, 30.4, 29.2, 28.3, 12.5, 8.5. ESI positive MS: Anal. Calcd for $C_{69}H_{76}O_{17}N_4+Na^+$ 1255.5098, found 1255.5099.

5.2. Biology

5.2.1. Tubulin assembly assay. Tubulin was purified from porcine brain purchased from a local slaughterhouse, conserved before use in ice-cold Pipes buffer (100 mM K-Pipes, pH 6.9, 2 mM EGTA, and 1 mM $MgCl_2$) and used as soon as possible. Pure tubulin was obtained by three cycles of warm-cold polymerization–depolymerization followed by anion-exchange chromatography (MonoQ column, Pharmacia) to separate tubulin from MAPs.[32] Protein concentration was determined by MicroBCA assay kit (Pierce). Stock solutions of thiocolchicine, podophyllotoxin, TES-baccatin, vinorelbine, vindoline, and all the obtained hydrids were prepared by dissolving the powders at a concentration of 5 mM in DMSO. To assess their effects on tubulin assembly, porcine tubulin (2.7 mg/ml) was mixed with different compounds (final concentration 10 μM) or equal volume of the solvent (final 0.2% DMSO) at room temperature in an assembly buffer minus GTP (100 mM K-Pipes, pH 6.9, 2 mM EGTA, 1 mM $MgCl_2$, and 10% glycerol). The reaction mixtures were incubated at 37 °C for 15 min to allow slow binding drugs to bind to the tubulin. The reaction mixtures were then chilled on ice, GTP (final concentration, 1 mM) was added, and they were incubated for 30 min at 37 °C. At the end of polymerization, unpolymerized and polymerized fractions of tubulin were separated by centrifugation at 30,000*g* for 30 min at 30 °C. The collected microtubules were resuspended in SDS–PAGE sample buffer (2% w/v SDS, 10% v/v glycerol, 5% v/v b-mercaptoethanol, 0.001% w/v bromophenol blue, and 62.5 mM Tris, pH 6.8) and the unpolymerized tubulin was diluted 3:1 with 4× SDS–PAGE sample buffer. Equal proportions of each fraction were resolved by a 7.5% SDS-gel and stained with Coomassie blue. Densitometric analyses of stained gels were performed by using the ImageMaster VDS Software (Pharmacia Biotech), and the obtained data were elaborated using SigmaPlot 8.0 program (Systat Software Inc., Point Richmond, CA, USA). At least three independent experiments were performed with each compound. Differences between the effects of the different compounds were evaluated by a Student's *t*-test for unpaired data with a confidence level of 95%. To further analyze the effects of selected compounds, the kinetics of tubulin polymerization was followed turbidimetrically at 340 nm in Ultraspec 300 spectrophotometer (Pharmacia) equipped with temperature controller. Reaction mixture contained 2.7 mg/ml tubulin, different compounds (final concentration 10 μM) or equal volume of the solvent (final 0.2% DMSO), in assembly buffer (80 mM K-Pipes pH 6.9, 2 mM EGTA, 1 mM $MgCl_2$, and 10% glycerol). After addition of GTP (final concentration 1 mM), reaction mixture was transferred to cuvettes in spectrophotometer and the polymerization reaction was followed at 37 °C for 40 min.

5.2.2. DIC microscopy. Microtubules were collected by centrifugation at 30,000 g for 30 min at 30 °C, fixed with 10% glycerol, 0.5% glutaraldehyde in BRB 80, and put onto coverslips. Image acquisition was performed using a Zeiss Axiovert 200 equipped with differential interference contrast (DIC) optics, an 63× oil objective, and a digital image recording system (Axiocam HRM Rev. 2 camera driven by Axiovision software rel. 4.4, Zeiss).

5.2.3. Cytotoxicity by MTS assay. A549 cells were harvested and plated in 96-well flat-bottomed microplates at a density of 10^3 cells/well. Assays were performed in quintuplicates. Cells were allowed to attach for 24 h. Vindoline, vinorelbine, podophylotoxin, thiocholchicine, 7-TES-baccatin III, **8**–**13**, **18**, and **19** were prepared in medium at three different concentrations (1 μM, 10 μM, and 100 μM) and were added to the plates at a volume of 100 μl/well. After 24 h of incubation 20 μl of the CellTiter 96® and AQ_{ueous} One Solution Reagent (Promega Corporation, Madison, WI, USA) were added to each well and the plates were incubated for 1 h at 37 °C. The CellTiter 96® AQ_{ueous} One Solution Reagent contains a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine ethosulfate; PES). PES has a high chemical stability, which allows it to form a stable solution with MTS. The absorbance was read at 490nm on a plate spectrophotometer (Victor$^3{}_{TM}$ 1420 Multilabel Counter, Perkin Elmer Instruments, Shelton, USA). Cell cytotoxicity was expressed as the percentage of the controls.

5.2.4. Cell cycle analysis. The effects of DMSO and **8** on the cell cycle were studied using flow cytometry analysis. Cells were plated in six-well sterile plastic plates at a density of $10^5 - 2\times10^5$cells/well and were allowed to attach for 24 h. After drug treatment cells were incubated for 24 h at 37 °C and in 5% CO_2 atmosphere. Cells were collected by trypsinization and DNA staining was performed with the CycleTEST PLUS DNA Reagent Kit (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). According to the manufacturers' instructions cells were washed with a buffer solution containing sodium citrate, sucrose, and dimethyl sulfoxide (DMSO). Then cells were incubated with a sequence of three steps: (a) 10 min at room temperature with Solution A containing trypsin in a spermine tetrahydrochloride detergent buffer (to digest cell membranes and cytoskeleton); (b) 10 min at room temperature with

Solution B containing a trypsin inhibitor and ribonuclease A in citrate-stabilizing buffer with spermine tetrahydrochloride (to inhibit the trypsin activity and to digest RNA); (c) 15 min in the refrigerator with Solution C containing propidium iodide and spermine tetrahydrochloride in citrate-stabilizing buffer. Analysis was performed using a FACScan (Becton Dickinson GmbH Immunzytometrische Systeme, Heidelberg, Germany) and data analysis was carried out with CELLQuest software, while cell cycle distribution was determined using Modifit software (Verity Software House, Inc.).

5.2.5. Immunofluorescence analyses. Microtubule organization in cell was revealed by indirect immunofluorescence (IFI) analyses. Human lung carcinoma cell line A549 (CCL-185; American Type Culture Collection, Rockville, MD, USA) was grown in minimal essential medium with Earle's (E-MEM), supplemented with 10% fetal bovine serum (Hyclone Europe, Oud-Beijerland, Holland), 2 mM L-glutamine, 100 U/ml penicillin, and non-essential amino acids. Cells were maintained at 37 °C in a humidified atmosphere at 5% CO_2. Experiments were carried out with cells plated on glass coverslips at a density of 1.5×10^4 cells/cm^2 and grown for 24 h in control medium following an incubation of 1 h in the presence of 10 μM drugs or solvent vehicle alone (DMSO). At the end of the treatments, cells were fixed and stained as previously described.[33] Briefly, A549 cells were fixed and permeabilized for 10 min with methanol at −20 °C, washed with PBS, and blocked in PBS + 1% bovine serum albumin (BSA) for 15 min at room temperature. To localize tubulin, the cells were incubated with monoclonal anti-α-tubulin antibody (clone B-5-1-2, Sigma–Aldrich), 1:500 in PBS for 1 h at 37 °C. As secondary antibodies we used goat anti-mouse Alexa Fluor™ 594 (Molecular Probes), 1:1000 in PBS + 5% BSA for 45 min at 37 °C. The coverslips were mounted in Mowiol® (Calbiochem)–DABCO (Sigma–Aldrich) and examined with a Zeiss Axiovert 200 microscope equipped with a 63× Neofluor lens. Images were acquired with an Axiocam camera (Zeiss) and PC running Axiovision software (Zeiss).

Acknowledgments

The authors are grateful for the financial support provided by Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) COFIN 2001—2003 Program 'Sostanze Naturali ed Analoghi Sintetici con Attività Antitumorale'. A.G. expresses her gratitude to INDENA S.p.A. (Milano) for the PhD fellowship. This research has been developed under the umbrella of CM 0602 COST Action 'Inhibitors of Angiogenesis: design, synthesis and biological exploitation'.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.04.025.

References and notes

1. Thorpe, P. E.; Chaplin, D. J.; Blakey, D. C. *Cancer Res.* **2003**, *63*, 1144.
2. Jain, R. K. *Science* **2005**, *307*, 58.
3. (a) Jordan, M. A. *Curr. Med. Chem. Anticancer Agents* **2002**, *2*, 1; (b) Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253; (c) Heald, R.; Nogales, E. *J. Cell Sci.* **2002**, *115*, 3.
4. (a) Rai, S. S.; Wolff, J. *J. Biol. Chem.* **1996**, *271*, 14707; (b) Gigant, B.; Wang, C.; Ravelli, R. B. G.; Roussi, F.; Steinmetz, M. O.; Curmi, P. A.; Sobel, A.; Knowssow, M. *Nature* **2005**, *435*, 519.
5. (a) Wilson, L.; Miller, H. P.; Farrell, K. W.; Snyder, K. B.; Thompson, W. C.; Purich, D. L. *Biochemistry* **1985**, *24*, 5254; (b) Schiff, P. B.; Horwitz, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1561; (c) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665; (d) Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5312.
6. Uppuluri, S.; Knipling, L.; Sackett, D. L.; Wolff, J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11598.
7. (a)*The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1984; vol. 23, (b) Sackett, D. L.; Varma, J. K. *Biochemistry* **1993**, *32*, 13560.
8. Bai, R.; Covell, D. G.; Pei, X.-F.; Ewelli, J. B.; Nguyeni, N. Y.; Brossi, A.; Hamel, E. *J. Biol. Chem.* **2000**, *275*, 40443.
9. (a) Cowan, C. R.; Cande, W. Z. *J. Cell Sci.* **2002**, *115*, 3747; (b) Tseng, C. J.; Wang, Y. J.; Liang, Y. C.; Jeng, J. H.; Lee, W. S.; Lin, J. K.; Chen, C. H.; Liu, I. C.; Ho, Y. S. *Toxicology* **2002**, *175*, 123.
10. Attard, G.; Greystoke, A.; Kaye, S.; De Bono, J. *Pathol. Biol.* **2006**, *54*, 72.
11. For recent examples of synthesis of tubulin polymerization inhibitors see: (a) Zhang, Q.; Peng, Y.; Wang, X. I.; Keenan, S. M.; Arora, S.; Welsh, W. J. *J. Med. Chem.* **2007**, *50*, 749; (b) Zuse, A.; Schmidt, P.; Baasner, S.; Böhm, K. J.; Müller, K.; Gerlach, M.; Günther, E. G.; Unger, E.; Prinz, H. *J. Med. Chem.* **2006**, *49*, 7816.
12. (a) Langer, M.; Kratz, F.; Rothen-Rutishauser, B.; Wunderli-Alemnspach, H.; Beck-Sickinger, A. G. *J. Med. Chem.* **2001**, *44*, 1341; (b) Safavy, A.; Bonner, J. A.; Waksal, H. W.; Buchsbaum, D. J.; Gillespie, G. Y.; Khazaeli, M. B.; Arani, R.; Chen, D. T.; Carpenter, M.; Raisch, K. P. *Bioconjugate Chem.* **2003**, *14*, 302.
13. *Synthetic Multivalent Molecules*; Choi, Seok-Ki, Ed.; Wiley Interscience: USA, 2004.
14. Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2754.
15. See as examples (a) Camps, P.; Formosa, X.; Munoz-Torrero, D.; Petrignet, J.; Badia, A.; Clos, M. V. *J. Med. Chem.* **2005**, *48*, 1701; (b) Feng, S.; Wang, Z.; He, X.; Zheng, S.; Xia, Y.; Jiang, H.; Tang, X.; Bai, D. *J. Med. Chem.* **2005**, *48*, 655; (c) Bombuwala, K.; Kinstle, T.; Popik, V.; Uppal, S. O.; Olesen, J. B.; Vina, J.; Heckman, C. A. *Beilstein J. Org. Chem.* **2006**, *2*, 13; For a review see: (d) Berube, G. *Curr. Med. Chem.* **2006**, *13*, 131.
16. Danieli, B.; Lesma, G.; Martinelli, M.; Passarella, D.; Silvani, A.; Pyuskyulev, B.; Tam, Mai Ngoc. *J. Org. Chem.* **1998**, *63*, 8586.
17. Danieli, B.; Giardini, A.; Lesma, G.; Passarella, D.; Silvani, A.; Appendino, G.; Noncovich, A.; Fontana, G.; Bombardalli, E.; Sterner, O. *Chem. Biodiv.* **2004**, *1*, 327.
18. Danieli, B.; Giardini, A.; Lesma, G.; Passarella, D.; Peretto, B.; Sacchetti, S.; Silvani, A.; Pratesi, G.; Zumino, F. *J. Org. Chem.* **2006**, *71*, 2848.
19. For a review see: *Antitumor Bisindole Alkaloids from Catharanthus roseus (L.). The Alkaloids*; Brossi, A.,

Suffness, M., Eds.; Academic Press: San Diego, 1990; Vol. 37.
20. Baloglu, E.; Miller, M. L.; Cavanagh, E. E.; Marien, T. P.; Roller, E. E.; Chari, R. V. J. A. *Synlett* **2005**, *5*, 817.
21. Bombardelli, E.; Pontiroli, A. PCT Int. Appl. WO 2001068089 A1 20010920 CAN 135:242385 AN 2001: 693085.
22. Asselin, A. A.; Humber, L. G.; Dobson, T. A.; Komlossy, J.; Martel, R. R. *J. Med. Chem.* **1976**, *19*, 787.
23. Parish, C. A.; Dong, J.-G.; Bornmann, W. G.; Chang, J.; Nakanishi, K.; Berova, N. *Tetrahedron* **1998**, *54*, 15739.
24. Langlois, N.; Gueritte, F.; Langlois, Y.; Potier, P. *J. Am. Chem. Soc.* **1976**, *98*, 7017.
25. (a) Potier, P. *Semin. Oncol.* **1989**, *16*, 2; (b) Gregory, R. K.; Smith, I. E. *Br. J. Cancer* **2000**, *82*, 1907.
26. Szàntay, C., Jr.; Balàzas, M.; Bolcskei, H.; Szantay, C. *Tetrahedron* **1991**, *47*, 1265.
27. (a) Moyano, N.; Iturraspe, J.; Nunez, J. L. *Eur. Pat. Appl.* **2001**, EP 1118616 A1 20010725 CAN 135:107498 AN 2001:541636; (b) Andriamialisoa, R. Z.; Langlois, N.; Langlois, Y.; Potier, D. G. *Tetrahedron* **1980**, *36*, 3053; (c) Mengeney, P.; Andriamialisoa, R. Z.; Lallemand, J. Y.; Langlois, N.; Langlois, Y.; Potier, D. G. *Tetrahedron* **1979**, *35*, 2175.
28. In our hands 7-TES-baccatin III resulted to inhibit tubulin polymerization. We have found in the literature the description of the cytotoxic mechanism mediated by baccatin III (Pengsuparp, T.; Kingstone, D. G. I.; Neidigh, K. A.; Cordell, G. A.; Pezzuto, J. M. *Chem. Biol. Interact.* **1996**, *101*, 103,) but no data regarding the biological activity of 7-TES-baccatin III toward tubulin polymerization. Specific studies are in progress.
29. The stability of all the compounds have been evaluated in the conditions of biological assays. Compounds **8–13**, **18** and **19** resulted stable and no products deriving from hydrolysis of the spacer have been detected.
30. The use of 24 h incubation time and not a longer time period (as frequently done for the assessment of antiproliferative effects of cytotoxic compounds) was preferred in order to appreciate any early variation from the behavior of the well-known building blocks.
31. For a review see: Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253.
32. Hyman, A.; Drechsel, D.; Kellogg, D.; Salser, S.; Sawin, K.; Steffen, P.; Wordeman, L.; Mitchison, T. *Methods Enzymol.* **1991**, *196*, 478.
33. (a) Hamel, E. *Cell Biochem. Biophys.* **2003**, *38*, 1; (b) Cappelletti, G.; Maggioni, M. G.; Maci, R. *J. Neurosci. Res.* **1999**, *56*, 28.