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Lignopurines: A new family of hybrids between cyclolignans and purines. Synthesis and biological evaluation

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1. Introduction

Hybrid formation is a classic strategy in drug design based in combining different bioactive fragments or molecules to get the corresponding "hybrids" or "conjugates" [1]. It is based in the fact that many natural products present hybrid structures and their activities are the resultant of combining those from the individual fragments [2,3]. In many cases, conjugates are able to improve their precursors' properties, to overcome resistance associated to individual fragments or to show different activities or even different mechanisms of action to that of their precursors [1-3]. In some cases the presence of an endogenous metabolite, as a part of the conjugate drug, serves to transport the other active part towards the biological target where the metabolite is well recognized [3].

The promising biological properties observed in several cases increased the interest of the scientific community in this strategy of drug design with the aim of generating new activities or modulating the already existing ones [2-8]. It also provides a very high

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ABSTRACT

A new family of hybrids between cyclolignans related to podophyllic aldehyde, a non-lactonic cyclolignan, and purines were prepared and evaluated against several human tumour cell lines. Both fragments, cyclolignan and purine, were linked through aliphatic and aromatic chains. The influence on the cytotoxicity of the purine substitution and the nature of the linker is analyzed. The new family was slightly less cytotoxic than the parent podophyllic aldehyde, although the selectivity is maintained or even improved and among the linkers used, the presence of an aromatic ring gave the most potent and selective derivatives within the new series tested. Cell cycle and confocal studies demonstrate that these derivatives interfere with the tubulin polymerization and arrest cells at the G_2/M phase, in the same way than the parent compounds podophyllotoxin and podophyllic aldehyde do.

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diversity of structures due to the large possibilities of combination. The use of hybridization as a way to obtain new drugs is especially interesting in research related to chemotherapy, where natural products play a relevant role in the development of new drugs [9].

Among those natural products, our research group has been interested in the chemomodulation of the antineoplastic activity of cyclolignans related to podophyllotoxin 1 (Fig. 1), the main component of Podophyllum resin, whose pharmacological properties (antiviral, cytotoxic, antirheumatic, cathartic, etc.) have been well recognized for centuries [10]. In this sense, our group has been engaged in the design and synthesis of new and better podophyllotoxin derivatives. We have prepared a large number of podophyllotoxin analogues through chemical modification of most rings and positions of the lignan skeleton [11,12] and very recently some of us have reported on the importance of attaching bulky substituents at position 7α [13]. Along these studies, we also prepared a potent and selective derivative that lacked the lactone ring, the podophyllic aldehyde **2** [14] (Fig. 1). The lactone ring is a structural feature that was previously considered essential for the antineoplastic activity of podophyllotoxin analogues. This compound showed an important cytotoxicity and selectivity against HT-29 human colon carcinoma and certain breast and brain cancer cell lines [14a] and became our lead compound for further structural modifications, including those presented here.

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Fig. 1. Structures of cyclolignans in clinical use (A) and structure of selective podophyllic aldehyde (B).

It is worth to point out that a few semisynthetic derivatives of podophyllotoxin are in clinical use for the treatment of different malignancies, including lung and testicular carcinoma, lymphoma or nonlymphocytic leukaemia [15,16]. Those derivatives are etoposide, teniposide and etopophos (Fig. 1), which could be considered themselves as hybrids of podophyllotoxin and modified glucose, being examples of conjugates that show a different mechanism of action to that of their precursors. While podophyllotoxin inhibits the assembly of tubulin into microtubules, avoiding the formation of the achromatic spindle and arresting cell division in metaphase, etoposide and analogues inhibit DNA topoisomerase II, preventing re-ligation of the double-stranded breaks. Another podophyllotoxin derivative is tafluposide. currently undergoing clinical trials, which is a dual Topo I and Topo II inhibitor [17]. Tafluposide has the hydroxyl groups in the sugar moiety of etopophos acylated with the pentafluorophenoxyacetyl group and it is another example of a different mechanism of action to that of their precursors.

Additionally, podophyllotoxin and its derivatives have also been hybridized with different cytotoxic agents in an attempt to generate compounds, which can act simultaneously on different biological targets such as topoisomerases and microtubules [18]. Thus, conjugates of epipodophyllotoxin (Fig. 2) with camptothecin [19] and paclitaxel [20] have been described by Cheng and Lee. The first ones showed both anti Topo-I and Topo-II activities and those hybridized with paclitaxel were more potent than the parent compounds. Passarella and col. have hybridized podophyllotoxin with thiocolchicine [21] and different vinca alkaloids [22].

We have also found in literature studies where different analogues of podophyllotoxin have been conjugated with artesunate [23] and lexitropsines [24]. Bisepipodophyllotoxins [25] with interesting cytotoxicity have been described in literature as well. However, and despite the significant role of nitrogenated bases in many biological processes and the important biological activities described either for pyrimidine or purine nucleosides, only a few synthetic hybrids of cyclolignans and pyrimidines have been reported [26] and we have not found any example of structures conjugating podophyllotoxin analogues with purines. However, there are several examples of natural conjugates between purine and terpenoids, such as asmarines, agelasines and agelasimines that showed a very interesting antineoplastic cytotoxicity [27]. This fact and the potential interest of this family of compounds as antitumour agents lead us to consider the development of "lignopurines" as a new type of bioactive podophyllotoxin derivatives.

In our previous work [14,28] we described that podophyllic aldehyde **2** itself, its imino derivatives at C-9 and other aldehydes containing longer and structurally varied ester chains or bioisosteric amides at C-9' can inhibit tubulin polymerization by interacting with the microtubule network. Those compounds were also able to induce delayed cellular apoptosis after 48 h of treatment [14a] and these biological properties confirmed that the γ -lactone ring is not an essential feature for cytotoxicity, whereas the presence of an electrophilic function at C-7 and/or C-9 seems important for the antineoplastic potency observed for this type of cyclolignans.

We now describe the synthesis and biological evaluation of lignopurines, a novel family of hybrids between podophyllic aldehyde derivatives and different substituted purines. Both precursors are attached through the position C-9' of the cyclolignan and the nitrogen N-9(N-7) of the purine using aliphatic and aromatic linkers. The cytotoxicity evaluation and cell-cycle arrest studies for these derivatives are presented here as well.

2. Results and discussion

2.1. Chemistry

The general structure for the new lignopurines presented in this work is illustrated in the retrosynthetic Scheme 1 by the conjugates ("hybrid model") formed by the junction of a cyclolignan and a purine through different spacers. The numbering for the cyclolignan component (Fig. 1) used in this work is in accordance with IUPAC rules for lignans [29].

The lignopurine objective could be obtained through two main synthetic strategies, depending on whether the linker is firstly attached to the purine system (Scheme 1, route A) or to the lignan derivative (Scheme 1, route B). Thus, route A involves the alkylation of the purine with the linker and a later condensation with dihydroxyacid **3**, generating the aldehyde group in the last step by oxidation under Swern conditions. Route B implies an initial esterification of the lignan with the corresponding linker and a final condensation with the purine. The cyclolignan fragment was picropodophyllic acid, **3**, which is the precursor of the lead compound podophyllic aldehyde **2**. Compound **3** can be easily prepared from podophyllotoxin **1** [14b] and the later was isolated from *Podophyllum* resin as previously described by us [30].

The purines used for these transformations were purine itself and 6-chloropurine, which were commercially available. To study the effect that the presence of a nucleophile at C-6 has on the cytotoxicity of the conjugates, the chlorine in 6-chloropurine was replaced by a propylamine group. The substitution [31] was performed by treatment of 6-chloropurine with propylamine yielding N⁶-propyladenine in a 60% yield after chromatography. Since bifunctionalized hydrocarbon chains are reported in the literature as the most frequently used spacers [2,32] and to take advantage of our experience in cyclolignan derivatisation as esters [28], we decided to use dibromides as linker agents (Scheme 2). Two different alkylene dibromides (1,3-dibromopropane and 1,6dibromohexane) and one phenylene analogue (α, α' -dibromo-pxylene) were selected for the preparation of the hybrids in order to study the effect that the size of the spacer would have on the cytotoxic activity of the conjugates. All esterification reactions



Fig. 2. A) Hybrids of epipodophyllotoxin with camptothecin (left) and paclitaxel (right). B) Hybrids of podophyllotoxin with thiocolchicine (left) and vinorelbine (right).

described in this work were carried out using the minimum volume of dry DMF. This aspect was critical because dilution leads to re-lactonization with formation of the 8'-epimeric lactone, pic-ropodophyllin [28].

2.1.1. Route A: condensation of the lignans with previously alkylated purines

As mentioned before, route A involves the reaction of dihydroxyacid **3** (easily obtained from podophyllotoxin **1** isolated from *Podophyllum* resin), with purines that had been previously alkylated with different linkers.

The linkers were attached to the purines by alkylation at room temperature using potassium carbonate as a base [33] and dimethylformamide as the solvent. In general, N-9 alkylated purines **4**–**9** were generated as the main reaction products, being the N-7 alkylated derivatives **7a** and **9a**, when detected, the minor reaction product (Scheme 2). The alkylated purines were used to prepare the esters of **3** (Scheme 3). The reactions were performed in DMF, at room temperature and in the presence of potassium carbonate [28]. When the purines with the shorter linker (**4** and **5**) were used, a complex reaction crude was obtained where the expected products **10** and **11**, which would lead to aldehydes **16** and **17**, were not found. In the case of the purines with longer linkers, the hybrid dihydroxyesters **12–15** were obtained in variable yields and traces of picropodophyllin were always detected. When mixtures of N-9 and N-7 alkyl purines **7** and **9** were used for the condensation process, the corresponding regioisomer derivatives could be identified in the reaction products.

Due to their lability and lactonization tendency, conjugated dihydroxyesters **12–15** were not purified and the crude products were directly transformed into the corresponding aldehydes **18–21** by oxidation under Swern conditions. Again, when mixtures of N-9 and N-7 alkyl purines were used, both regioisomeric aldehydes could be identified in the reaction products. Only in the case of **19**,



Scheme 1. Retrosynthetic scheme for the two main strategies to get the cyclolignan conjugates.



Scheme 2. Preparation of the alkylated purines.



Scheme 3. Condensation of the lignans with previously alkylated purines following route A.

both regioisomers (**19** and **19a**) were isolated and characterized by two-dimensional NMR experiments.

2.1.2. Route B: treatment of the lignan with the linker and final condensation with the purine

In view of the aldehydes **16** and **17** were not obtained by previous route A, further studies were devoted to explore an alternative way to condensate purines and lignan derivatives. Thus, route B implied an initial reaction of the lignan carboxylate with the corresponding dibromide and a final condensation with the purine. In this case, the aldehyde formation could be carried out either before or after the condensation process. If the purine is attached before the formation of the unsaturated aldehyde, the dihydroxyesters could be conjugated with the purine either as a free diol or

protected, as its acetonide, in order to avoid re-lactonization of the cyclolignan. These possibilities are shown in Scheme 4.

According with this planning, the linkers were attached to the cyclolignans through ester-type bonds. Reaction of dihydroxyacid **3** with different dibromides at room temperature [28], in small volumes of dimethylformamide and using potassium carbonate as a base gave esters **22–24** (Scheme 5), which were the starting points for the different possibilities of route B.

2.1.2.1. B_1 . Generation of the aldehyde prior to condensation with the purine. The bromide esters **22–24** were oxidized under Swern conditions to generate the corresponding aldehydes **25–27** in good yields. All of them were purified and characterized by ¹H and ¹³C NMR. Different reaction conditions were studied for the



Scheme 4. Retrosynthetic analysis for the preparation of the cyclolignan hybrids by route B.



Scheme 5. Preparation of the cyclolignans hybrids following route B.

condensation process between those esters and the purines, and various solvents (DMF, acetonitrile) and bases (K_2CO_3 , NaH) were explored. Reaction times were generally long (15 h to 3 d) and, as happened before, the best results were obtained when reactions were carried out in DMF, at room temperature and using potassium carbonate as the base. However, partial aromatization of the cyclolignan skeleton was observed and mixtures of the hybrid aldehydes (16, 17, 19 and 20) and their derivatives with aromatized C-ring (28, 29, 30 and 31) were always found. Both aldehydes co-eluted in chromatography, so their separation was difficult and only small amounts of the aromatic aldehydes 28 and 29 could be isolated.

Working in this way, when the mixture of both aldehydes **16** and **28** was kept under the same reaction conditions used for the condensation, transformation of **16** into **28** was observed. In all these cases, only N-9 alkylated derivates were found in the reaction crudes (Scheme 5).

2.1.2.2. B₂. Condensation of dihydroxyesters with purines. Due to the issues arose from the aromatization of the aldehydes we explored the formation of hybrids before the Swern oxidation, generating the α , β -unsaturated aldehydes at the end of the route. Thus, dihydroxyesters **22** and **23** were condensed with 6-chloropurine in DMF



Scheme 6. Preparation of hybrids containing nitrogen at C-9.

Table 1		
Cytotoxicity of podophyllic ald	dehyde derivatives ($GI_{50} \pm SD/\mu M$)	١.

Compound	MB-231	HT-29	A-549
1 ^a	_	0.024	0.012
2	0.2 ± 0.0082	0.039 ± 0.0048	0.06 ± 0.003
16	7.7 ± 0.5	0.71 ± 0.026	0.79 ± 0.026
17	$\textbf{2.4} \pm \textbf{0.17}$	0.91 ± 0.02	0.75 ± 0.079
18	$\textbf{2.4} \pm \textbf{0.18}$	0.53 ± 0.036	0.31 ± 0.031
19	3.1 ± 0.081	0.77 ± 0.069	$\textbf{0.4} \pm \textbf{0.052}$
19a	3.7 ± 0.25	1.7 ± 0.15	1.2 ± 0.0083
20	$\textbf{0.81} \pm 0.031$	$\textbf{0.051} \pm 0.0046$	$\textbf{0.029} \pm 0.0014$
21	5.1 ± 1.9	1.2 ± 0.16	1.2 ± 0.1
22	0.16 ± 0.025	0.14 ± 0.0057	0.14 ± 0.014
23	0.35 ± 0.02	0.16 ± 0.0091	0.25 ± 0.015
25	0.13 ± 0.031	0.14 ± 0.02	0.19 ± 0.014
26	1.8 ± 0.048	0.74 ± 0.026	0.66 ± 0.075
27	$\textbf{0.7} \pm 0.17$	$\textbf{0.077} \pm 0.0077$	$\textbf{0.085} \pm 0.0043$
29	5 ± 0.26	2.1 ± 0.016	2 ± 0.07
36	$\textbf{0.74} \pm 0.074$	$\textbf{0.09} \pm 0.02$	$\textbf{0.059} \pm 0.0048$
37	3.5 ± 0.1	2.2 ± 0.023	2.3 ± 0.18
38	1.3 ± 0.015	0.63 ± 0.011	0.58 ± 0.018
Etoposide	$\textbf{7.2} \pm \textbf{8.5}$	9.4 ± 0.087	$\textbf{2.2} \pm \textbf{0.81}$

 GI_{50} values represent the mean \pm SD of three independent experiments. ^a Data for compound **1** was taken from our previous research [28]. Data in bold

correspond to the most potent compounds in the series.

and in the presence of potassium carbonate, but no reaction took place in any case and mixtures of starting materials and pic-ropodophyllin were found (Scheme 5).

2.1.2.3. B_3 . Condensation of dihydroxyesters, protected as acetonides, with purines. The re-lactonization of the dihydroxyesters during the condensation process and the fact that hybrids containing trimethylene chain as a spacer had not been generated yet by any previous attempt, forced us to explore a new methodology for the preparation of these compounds. Thus, we decided to generate a protected derivative of bromoester **22** that would be the substrate for the condensation with the corresponding purines (Scheme 5). The acetonide formation could be performed before or after the reaction of the acid with 1,3-dibromopropane. The protection of the dihydroxyacid **3** by treatment with 2,2-dimethoxypropane and p-toluenesulfonic acid [34] followed by reaction with 1,3-dibromopropane provided acetonide 32 in only 33% yield, while the protection of the already synthesized bromoester 22 in similar conditions, generated the acetonide 32 in 86% yield. This acetonide **32** was condensed with purine and 6-chloropurine in the same conditions described above to afford derivatives 33 and 34 respectively. Hybrids were then deprotected by reaction with p-toluenesulfonic acid in water/acetone [35] solution to get the diol-esters 10 and 11 and these compounds were finally oxidized under Swern conditions. Thus, and despite the need of a longer and more complex route, aldehydes 16 and 17 were synthesized and purified in acceptable yields and completely characterized through their NMR spectra.

2.1.3. Hybrids containing nitrogen attached to position C-9 of the cyclolignan

Once the new family of lignopurines was prepared, and with the aim of studying the effect that substitution of oxygen by nitrogen at C-9 position of the cyclolignan had on the cytotoxicity of the previously obtained conjugates, we prepared new derivatives containing a nitrogen atom at this position. The synthetic methodology chosen for these transformations was the reductive amination of the aldehydes [28], and propylamine was selected for these reactions as it can be eliminated in vacuo and transformations can be easily carried out in dichloromethane solution. Thus, aldehydes 19 and **20** were treated with propylamine and anhydrous magnesium sulphate at room temperature during 3 d, generating the corresponding imines 35 and 36, in quantitative yields, that were finally reduced with sodium borohydride in methanol (Scheme 6). Crude products were purified by column chromatography, leading to amines 37 and 38 in low yields, despite the presence of triethylamine in the chromatographic eluents, due to their liability and tendency to form the corresponding γ -lactams [28].



Fig. 3. Effects of compounds **16**–**19**, **19a**, **20**, **21** and **29** on cell cycle in HeLa cells. (A) Cells were incubated with 1 μ M of compound **18** for 24 h and stained with propidium iodide (PI). Their DNA content was analyzed by fluorescence flow cytometry. The positions of G₀/G₁ and G₂/M peaks are indicated by arrows. (B) Quantitative measurements of G₂/M arrest following 24 h treatment of HeLa cells with 1 μ M of the indicated compounds. Control untreated cells were run in parallel. Data shown are means \pm S.D. or representative of three independent experiments.

2.2. Cytotoxicity studies

Most of the compounds prepared in this work were evaluated *in vitro* in order to determine their cytotoxic activity using a colorimetric cell growth inhibition assay [36]. Thus, cytotoxicity was measured against the human tumour cell lines: MB-231 (breast carcinoma), A-549 (lung carcinoma) and HT-29 (colon carcinoma), and the results are shown in the Table 1, expressed as GI₅₀ values (drug concentration in μ M causing a 50% growth inhibition). The GI₅₀ values found for the parent podophyllotoxin **1**, the "lead compound" **2**, and the semisynthetic derivative in clinical use, etoposide, have also been included as references.

All the compounds tested, considered as analogues of the podophyllic aldehyde **2**, were cytotoxic. Some of them resulted several times more potent against HT-29 and A-459 carcinomas than against MB-231 carcinoma cell lines, showing GI₅₀ values in the μ M range or below and being, in general, more cytotoxic than etoposide.

Considering the different functionalities at positions C-9 and C-9' of the lignan, the dihydroxyesters **22** and **23** showed GI_{50} values in the μ M range or below, and compared to **2**, no significant differences between the three lines tested were observed. Among the aldehyde-ester derivatives **25–27**, it can be observed that they

maintained the antineoplastic cytotoxicity, being aldehyde **27** a few times more potent against HT-29 and A-549 than against MB-231 cells.

The new family of hybrids **16–21** was slightly less cytotoxic than the parent compound **2**, although the selectivity is maintained or even improved as in the case of **20**, lignopurine containing an aromatic link and being the most potent and selective derivative. It showed lower GI_{50} values than the podophyllic aldehyde and also it was several times more potent against A-459 and HT-29 cell lines than MB-231 cells.

Regarding the purine fragment, the hybrids containing either purine or 6-chloropurine showed similar GI₅₀ values, so the presence or absence of the chlorine at the purine C-6 position has no significant influence on the cytotoxicity of the resulting conjugates (**16** and **18** vs. **17** and **19**). However, the substitution of the chlorine by a propylamino group seems to decrease the potency of the compound (**21** vs. **19**). In those cases where the separation of the N-9 and N-7 regioisomers was possible, it has been observed that compounds containing N-7 alkyl purines were less cytotoxic than N-9 alkyl derivatives (**19a** vs. **19**).

About the nature of the linker, it can be observed that there are no significant differences between L_1 and L_2 , however those analogues with the aromatic linker L_3 were the most potent and



Fig. 4. Effects of compounds **16–19**, **19a**, **20**, **21** and **29** on the microtubule network of HeLa cells. Cells were incubated for 24 h in the absence (control) or in the presence of 1 µM of the indicated compounds and the fixed and processed for immunofluorescence of microtubules (green fluorescence) and nuclei (blue fluorescence) as described in Experimental section. Bar, 10 µm. The photomicrographs shown are representative of at least three independent experiments performed.

selective derivatives (**20**, **27** and **36**). This is in accordance with our previous work [28] in which we also found that an aromatic ring in the alkyl fragment of the ester function increased the cytotoxicity.

Transformation of the lignan aldehyde group at C-9 position into nitrogen derivatives led to a decrease of the cytotoxicity values against A-549 and HT-29 cell lines (**37** and **38** *vs.* **19** and **20**), although the analogue **38**, which has the aromatic linker, kept the GI₅₀ in the same range than the hybrids with linear linkers. The only derivative with an imine group at this position, **36**, maintained the cytotoxicity of its precursor aldehyde in the same level as described previously [28].

2.3. Cell cycle analysis

To gain further insight into the mode of action of these compounds, we analyzed the effects of compounds **16–19**, **19a**, **20**, **21** and **29** on cell cycle by flow cytometry in human cervical cancer HeLa cells, which were also sensitive to the podophyllic aldehyde derivatives at the low micromolar range (data not shown). Fig. 3 shows that these compounds (1 μ M) induced an almost total G₂/M arrest after 24 h incubation. Fig. 3A shows a representative histogram of the cell cycle profile shown after HeLa cell incubation with compound **18**. Similar cell cycle profiles were obtained after incubation with the other podophyllotoxin derivatives as shown in Fig. 3B, where the percentages of cells showing G₂/M arrest upon incubation with the indicated podophyllotoxin derivatives were determined.

After this initial cell cycle arrest, the onset of apoptosis was observed following 72 h incubation (data not shown). Because these compounds are podophyllotoxin derivatives, it could be feasible that the G_2/M cell cycle arrest might be due to the disruption of the microtubule network [37]. This notion was supported by analyzing the effect of the above compounds on the microtubule network by confocal microscopy. We found that compounds **16–19**, **19a**, **20**, **21** and **29** totally disrupted microtubules in human cancer HeLa cells when compared to untreated cells (Fig. 4). Thus, the strong G_2/M arrest following treatment with the above podophyllotoxin derivatives seems to be mediated by microtubule disruption, which eventually leads to apoptosis through a series of mechanisms that are not yet completely understood [37].

3. Conclusions

A new family of hybrids, named lignopurines, have been prepared by the junction of the non-lactonic podophyllic aldehyde and purines. Both fragments were linked through aliphatic and aromatic chains and evaluated against several tumour cell lines. The cyclolignan derivatives reported here retain the antineoplastic cytotoxicity of their chemical precursors, podophyllotoxin 1 and podophyllic aldehyde 2. The most potent compounds have a doubly benzylic aromatic fragment as the linker, and within them, 20 and 36 actually correspond to desired lignopurine hybrids, while derivative 27 is an intermediate in their synthesis bearing a brominated fragment, which could contribute to its cytotoxicity by an additional alkylating mechanism. The lignopurines synthesized also retain the antimitotic mode of action of the parent compounds, based on the inhibition of tubulin polymerization, as it is demonstrated for compounds 16-19 and 29 (Figs. 3 and 4). These data suggest that the approach described here to generate novel podophyllotoxin derivatives keeps the features of the parent drug, acting on microtubules and cell cycle arrest in tumour cells. Thus incorporating new structural fragments that could be bioactive by themselves, as the purine moiety, could lead to better anticancer agents.

4. Experimental section

4.1. Chemistry

NMR spectra were recorded on a Bruker AC 200 at 200 MHz for ¹H and 50.3 MHz for ¹³C in deuterochloroform with TMS as internal standard. Chemical shift (δ) values are expressed in ppm followed by multiplicity and coupling constants (*J*) in Hz. The complete NMR signals are given for the first compound of each series described here, and only characteristic signals are indicated for the remainder. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter in chloroform solution and UV spectra on a Hitachi 100-60 spectrophotometer in ethanol solution. IR spectra were obtained on a Nicolet Impact 410 spectrophotometer and wavenumbers are given in cm⁻¹. HRMS were run in a VG-TS-250 spectrometer working at 70 eV. Elemental analyses (C, H, N) were obtained with a LECO CHNS-932 and were within $\pm 0.4\%$ of the theoretical values. Solvents and reagents were purified by standard procedures as necessary and the chlorinated solvents, including deuterochloroform, were filtered through sodium bicarbonate prior its use, in order to eliminate acid traces. Dimethylformamide was dried over molecular sieves and treated with K₂CO₃ for the same reason.

4.1.1. General method for the synthesis of alkylated purines 4-9

A solution of purine and K_2CO_3 in dry DMF was stirred at room temperature for 15 min. The alkylating agent was then added and the mixture stirred at room temperature for 1 h. The crude product was diluted with EtOAc and filtered, and the solution was washed with sat aq NaCl, dried over Na₂SO₄ and filtered. The solvent was removed under vacuum to give the alkylated purines 9-(3-bromopropyl) purine **4**, 9-(3-bromopropyl)-6-chloropurine **5**, 9-(6-bromohexyl)) purine **6**, 9-(6-bromohexyl)-6-chloropurine **7**, 7-(6-bromohexyl)-6chloropurine **7a**, 9-(α' -bromo-*p*-xylenyl)-6-chloropurine **8**, 9-(6bromohexyl)-N₆-propyladenine **9**. Detailed experimental procedure is described in Supplementary data.

4.1.2. General method for the preparation of hybrid aldehydes **18–21** using route A

A mixture of picropodophyllic acid **3** and K_2CO_3 was dissolved in dry DMF and stirred at room temperature for 30 min. Then the alkylated purine was added and the mixture stirred at room temperature for 1–16 h. The crude product was diluted with EtOAc and filtered, and the organic phases were washed with sat aq NaCl, dried over Na₂SO₄ and filtered. The solvent was removed under vacuum to give the corresponding dihydroxyesters (**12–15**) that were transformed, without further purification, into the corresponding aldehydes **18–21** by oxidation under Swern conditions.

Swern oxidation: To a precooled (-55 °C) and stirred solution of oxalyl chloride (3 equiv) in dry CH₂Cl₂ (5 mL) was added dropwise a solution of DMSO (6 equiv) in dry CH₂Cl₂ (2 mL). After 5 min at -55 °C, a solution of the corresponding dihydroxyester (1 equiv) in dry CH₂Cl₂ was slowly added. The mixture was stirred at the same temperature for 30 min and then triethylamine (10 equiv) was added dropwise. The mixture was warmed to 0 °C over 1 h, quenched with water and extracted with CH₂Cl₂. The organic phase was washed with 2 N HCl and sat aq solutions of NaHCO₃ and NaCl and the solvent was evaporated off. Column chromatography of the reaction products gave the corresponding aldehydes.

4.1.2.1. 6-(*Purin-9-yl*)*hexyl* 9-*deoxy-9-oxo-\alpha-apopicropodophyllate* **18**. From **3** (200 mg, 0.463 mmol), K₂CO₃ (191 mg, 1.39 mmol) and purine **6** (130 mg, 0.64 mmol) in dry DMF (2.0 mL) using the general method during 16 h. The crude hybrid dihydroxyester 12 (185 mg, 0.29 mmol) was transformed to the corresponding aldehyde under Swern conditions and the reaction product was chromatographed on silica gel, eluting with 10% EtOH/CH₂Cl₂ to give compound 18 (62 mg, 35%). ¹H NMR (CDCl₃): δ 1.25 (m, 4H, COO–(CH₂)₂–(CH₂)₂– (CH₂)₂-N₉), 1.50 (m, 2H, COO-CH₂-CH₂-(CH₂)₄-N₉), 1.85 (m, 2H, COO-(CH₂)₄-CH₂-CH₂-N₉), 3.70 (m, 1H, H8'), 3.73 (s, 6H, H10', H12'), 3.78 (s, 3H, H11'), 3.95 (m, 2H, COO-CH₂-(CH₂)₅-N₉), 4.27 $(t, 2H, J = 6.8 \text{ Hz}, COO - (CH_2)_5 - CH_2 - N_9), 4.57 (d, 1H, J = 4.7 \text{ Hz}, H7'),$ 5.97 (d, 1H, I = 1.1 Hz, H10a), 5.99 (d, 1H, I = 1.1 Hz, H10b), 6.22 (s, 2H, I)H2', H6'), 6.62 (s, 1H, H3), 6.87 (s, 1H, H6), 7.33 (s, 1H, H7), 8.18 (s, 1H, H8-purine), 8.99 (s, 1H, H2-purine), 9.15 (s, 1H, H6-purine), 9.59 (s, 1H, H9); ¹³C NMR (CDCl₃): δ 25.1 (COO-(CH₂)₃-CH₂- $(CH_2)_2 - N_9$, 26.1 $(COO - (CH_2)_2 - CH_2 - (CH_2)_3 - N_9)$, 28.2 $(COO - CH_2)_2 - CH_2 - (CH_2)_3 - N_9$ CH₂-CH₂-(CH₂)₄-N₉), 29.7 (COO-(CH₂)₄-CH₂-CH₂-N₉), 43.9 (COO-(CH₂)₅-CH₂-N₉), 45.0 (C8'), 46.6 (C7'), 56.1 (2CH₃, C10', C12'), 60.8 (C11'), 65.0 (COO-CH2-(CH2)5-N9), 101.9 (C10), 104.9 (2CH, C2', C6'), 108.9 (C6), 110.0 (C3), 125.1 (C1), 133.4 (C8), 133.9 (C2), 137.0 (C7'), 137.1 (C1'), 145.7 (C7), 146.0 (C8-purine), 147.4 (C5), 147.7 (C6-purine), 150.5 (C4-purine), 151.9 (C2-purine), 153.3 (2C, C3', C5'), 172.0 (C9'), 191.3 (C9). IR (film, cm⁻¹): 1726 (COOR), 1669 (CHO). $[\alpha]^{20}(\lambda)$: -104° (*c* 0.5%). Anal. (C₃₃H₃₄N₄O₈) C, H, N. HRMS: calcd for C₃₃H₃₄N₄O₈ + H: 615.2449 u; found: 615.2466 *m/z*.

4.1.2.2. 6-(6-Chloropurin-9-yl)hexyl 9-deoxy-9-oxo- α -apopicropodophyllate **19** and 6-(6-chloropurin-7-yl)hexyl 9-deoxy-9-oxo- α apopicropodophyllate **19a**. From **3** (205 mg, 0.475 mmol), K₂CO₃ (197 mg, 1.42 mmol) and a mixture of purines **7/7a** (150 mg, 0.475 mmol) in dry DMF (2.3 mL) using the general method during 15 h to give a mixture of products **13/13a** (279 mg, 88%). The crude hybrid dihydroxyesters **13** (180 mg, 0.269 mmol) was transformed to the corresponding aldehyde under Swern conditions and the reaction product was chromatographed on silica gel, eluting with 10% acetone/CH₂Cl₂ to give compound **19** (48 mg, 28%) and with 30% acetone/CH₂Cl₂ to give compound **19a** (13 mg, 8%).

4.1.2.2.1. Compound **19**. ¹H NMR (CDCl₃): δ 1.25 (m, 4H, COO– (CH₂)₂–(CH₂)₂–(CH₂)₂–N₉), 1.50 (m, 2H, COO–CH₂–CH₂–(CH₂)₄–N₉), 1.85 (m, 2H, COO–(CH₂)₄–CH₂–CH₂–N₉), 4.01 (m, 2H, COO– CH₂–(CH₂)₅–N₉), 4.28 (t, 2H, *J* = 7.2 Hz, COO–(CH₂)₅–CH₂–N₉), 7.33 (s, 1H, H7), 8.21 (s, 1H, H8-purine), 8.75 (s, 1H, H2-purine), 9.59 (s, 1H, H9); ¹³C NMR (CDCl₃): δ 25.1 (COO–(CH₂)₃–CH₂–(CH₂)₂–N₉), 26.0 (COO–(CH₂)₂–CH₂–(CH₂)₃–N₉), 28.2 (COO–CH₂–CH₂– (CH₂)₄–N₉), 29.7 (COO–(CH₂)₄–CH₂–CH₂–N₉), 44.3 (COO–(CH₂)₅– CH₂–N₉), 64.9 (COO–CH₂–(CH₂)₅–N₉), 131.6 (C5-purine), 145.5 (C8-purine), 145.7 (C7), 151.0 (C6-purine), 151.9 (2C, C2-purine, C4-purine), 172.0 (C9'), 191.3 (C9). IR (film, cm⁻¹): 1732 (COOR), 1669 (CHO). [α]²⁰(λ): –103° (*c* 1%). UV λ_{max} (log ε): 206 (4.1), 258 (4.2), 358 (4.4). Anal. (C₃₃H₃₃N₄O₈Cl) C, H, N. HRMS: calcd for C₃₃H₃₃N₄O₈ClNa: 671.1879 u; found: 671.1890 *m*/*z*.

4.1.2.2.2. Compound **19a**. ¹H NMR (CDCl₃): δ 1.25 (m, 4H, COO– (CH₂)₂–(CH₂)₂–(CH₂)₂–N₇), 1.50 (m, 2H, COO–CH₂–CH₂–(CH₂)₄– N₇), 1.85 (m, 2H, COO–(CH₂)₄–CH₂–CH₂–N₇), 4.01 (m, 2H, COO– CH₂–(CH₂)₅–N₇), 4.45 (t, 2H, *J* = 7.5 Hz, COO–(CH₂)₅–CH₂–N₇), 7.34 (s, 1H, H7), 8.32 (s, 1H, H8-purine), 8.88 (s, 1H, H2-purine), 9.59 (s, 1H, H9); ¹³C NMR (CDCl₃): δ 25.1, 25.8 (2CH₂, COO–(CH₂)₂– (CH₂)₂–(CH₂)₂–N₉), 28.2 (COO–CH₂–CH₂–(CH₂)₄–N₉), 31.5 (COO– (CH₂)₄–CH₂–CH₂–N₉), 47.3 (COO–(CH₂)₅–CH₂–N₉), 64.8 (COO– CH₂–(CH₂)₅–N₉), 122.4 (C5-purine), 143.0 (C6-purine), 145.7 (C7), 149.3 (C8-purine), 152.4 (C2-purine), 162.0 (C4-purine), 172.0 (C9'), 191.3 (C9). IR (film, cm⁻¹): 1731 (COOR), 1669 (CHO). [α]²⁰ (λ): –88° (*c* 1%). HRMS: calcd for C₃₃H₃₃N₄O₈ClNa: 671.1879 u; found: 671.1891 *m*/*z*.

4.1.2.3. α'-(6-Chloropurin-9-yl)-p-xylenyl 9-deoxy-9-oxo-α-apopicropodophyllate **20**. From **3** (192 mg, 0.445 mmol), K₂CO₃ (184 mg,

1.33 mmol) and purine 8 (150 mg, 0.445 mmol) in dry DMF (2.8 mL) using the general method during 1.5 h to give dihydroxyester 14 (270 mg, 0.392 mmol, 88%) that was transformed to the corresponding aldehyde under Swern conditions. The reaction product was chromatographed on silica gel, eluting with 60% EtOAc/CH₂Cl₂ to give compound **20** (164 mg, 62%). ¹H NMR (CDCl₃): δ 4.94 (d, 1H, I = 12.8 Hz, COO-CH₂-C₆H₄-CH₂-N₉), 5.10 (d, 1H, I = 12.8 Hz, $COO-CH_2-C_6H_4-CH_2-N_9$, 5.41 (s, 2H, $COO-CH_2-C_6H_4-CH_2-N_9$), 7.08, 7.19 (2d, 4H, I = 8.2 Hz, COO-CH₂-C₆H₄-CH₂-N₉), 7.32 (s, 1H, H7), 8.14 (s, 1H, H8-purine), 8.75 (s, 1H, H8-purine), 9.56 (s, 1H, H9); ¹³C NMR (CDCl₃): δ 47. 5 (COO-CH₂-C₆H₄-CH₂-N₉), 66.1 (COO-CH2-C6H4-CH2-N9), 128.0, 128.3 (4CH, COO-CH2-C6H4-CH2-N₉), 131.5 (C5-purine), 134.5 (1C, COO-CH₂-C₆H₄-CH₂-N₉), 136.6 (1C, COO-CH₂-C₆H₄-CH₂-N₉), 145.3 (C8-purine), 145.9 (C7), 151.1 (C6-purine), 151.9 (C4-purine), 152.2 (C2-purine), 171.8 (C9'), 191.2 (C9). IR (film, cm⁻¹): 1731 (COOR), 1668 (CHO). [α]²⁰ (λ): -151° (c 1%). UV λ_{max} (log ε): 208 (4.2), 259 (4.3), 360 (4.5). Anal. (C₃₅H₂₉N₄O₈Cl) C, H, N. HRMS: calcd for C₃₅H₂₉N₄O₈Cl + H: 669.1747 u; found: 669.1749 m/z.

4.1.2.4. 6-(6-Propylaminopurin-9(7)-yl)hexyl 9-deoxy-9-oxo- α -apopicropodophyllate 21(21a). From 3 (85 mg, 0.19 mmol), K₂CO₃ (40 mg, 1.5 mmol) and purines 9(9a) (100 mg, 1.50 mmol) in dry DMF (1.8 mL) using the general method during 13 h. The crude hybrid dihydroxyester 15 (120 mg), was transformed to the corresponding aldehyde under Swern conditions and the reaction product was chromatographed on silica gel, eluting with 50% acetone/CH₂Cl₂ to give a mixture of compounds **21** and **21a** (24 mg, 20%) which could not be separated. ¹H NMR (CDCl₃): δ 1.00 (t. 3H. I = 7.5 Hz, NH-CH₂-CH₂-CH₃), 1.20 (m, 4H, COO-(CH₂)₂-(CH₂)₂-(CH₂)₂-N_{9/7}), 1.40 (m, 2H, NH-CH₂-CH₂-CH₃), 1.75 (m, 4H, COO- $CH_2-CH_2-(CH_2)_2-CH_2-CH_2-N_{9/7}$), 3.36 (t, 2H, J = 6.5 Hz, NH- $CH_2-CH_2-CH_3$, 21), 3.49 (t, 2H, J = 6.5 Hz, $NH-CH_2-CH_2-CH_3$, 21a), 3.96 (m, 2H, COO-CH2-(CH2)5-N9/7), 4.13 (m, 2H, COO-(CH₂)₅-CH₂-N_{9/7}), 7.32 (s, 1H, H7), 7.71 (s, 1H, H8-purine, 21a), 7.74 (s, 1H, H8-purine, 21), 8.37 (s, 1H, H2-purine), 9.58 (1H, H9); ¹³C NMR (CDCl₃): δ 11.5 (NH–CH₂–CH₂–CH₃), 23.1 (NH–CH₂– CH2-CH3), 25.9, 27.6, 32.5, 33.6 (4CH2, COO-CH2-(CH2)4-CH2-N₇, 21b), 25.2 (COO-(CH₂)₃-CH₂-(CH₂)₂-N₉), 26.1 (COO- $(CH_2)_2 - CH_2 - (CH_2)_3 - N_9),$ 28.3 $(COO-CH_2-CH_2-(CH_2)_4-N_9),$ 30.1 (COO-(CH₂)₄-CH₂-CH₂-N₉), 42.7 (COO-CH₂-(CH₂)₅-N_{9/7}), 43.7 (NH-CH2-CH2-CH3), 65.1 (COO-CH2-(CH2)5-N9/7), 119.7 (C6-purine), 139.5 (C8-purine, 21), 139.6 (C8-purine, 21a), 145.5 (C7), 149.1 (C5-purine), 153.2 (C2-purine), 154.9 (C4-purine), 171.8 (C9'), 191.3 (C9). Anal. (C36H41N5O8) C, H, N. HRMS: calcd for C₃₆H₄₁N₅O₈ + H: 672.3028 u; found: 672.3030 *m*/*z*.

4.1.3. General method for the preparation of hybrid aldehydes **16** and **17** using route B1

A mixture of picropodophyllic acid **3** and K₂CO₃ was dissolved in dry DMF and stirred at room temperature for 30 min. Then the alkylating agent was added and the mixture stirred at room temperature for 1–2 h. The crude product was diluted with EtOAc and filtered, and the organics were washed with sat aq NaCl, dried over Na₂SO₄, filtered and concentrated under vacuum. The obtained dihydroxyesters 22-24 were oxidized to the corresponding aldehydes 25–27 under Swern conditions as described above. After purification by column chromatography, each aldehyde was added to a solution of the corresponding purine and K₂CO₃ in dry DMF and the mixture stirred at room temperature for 3-6 d. The crude product was diluted with EtOAc and filtered, and the organics were washed with sat aq NaCl, dried over Na₂SO₄ and filtered. The solvent was removed under vacuum to give a mixture of the hybrid aldehydes (16, 17, 19, 20) and their derivatives aromatized at the C-ring (28, 29, 30, 31).

4.1.3.1. 3-Bromopropyl picropodophyllate **22**. From **3** (250 mg, 0.58 mmol), K_2CO_3 (240 mg, 1.74 mmol) and 1,3-dibromopropane (0.177 mL, 1.74 mmol) in dry DMF (1.8 mL) using the general method B_1 during 1 h to give compound **22** (263 mg, 82%) [26].

4.1.3.2. 6-Bromohexyl picropodophyllate 23. From 3 (200 mg, 0.463 mmol), K₂CO₃ (192 mg, 1.39 mmol) and 1,6-dibromohexane (0.214 mL, 1.39 mmol) in dry DMF (1.5 mL) using the above method B₁ during 1 h to give compound **23** (175 mg, 63%). ¹H NMR (CDCl₃): δ 1.10–1.60 (m, 6H, COO–(CH₂)₂–(CH₂)₃–CH₂–Br), 1.80 (m, 2H, COO-CH₂-CH₂-(CH₂)₄-Br), 2.47 (m, 1H, H8), 3.35 (m, 1H, H8'), $3.37 (t, 2H, I = 6.5 \text{ Hz}, \text{COO}-(\text{CH}_2)_5-\text{CH}_2-\text{Br}), 3.67 (m, 1H, H9),$ 3.76 (s, 6H, H10', H12'), 3.81 (s, 1H, H11'), 4.00 (t, 2H, J = 6.2 Hz, $COO-CH_2-(CH_2)_5-Br$), 4.27 (d, 1H, J = 7.9 Hz, H7'), 4.83 (d, 1H, *J* = 5.4 Hz, H7), 5.90 (s, 2H, H10), 6.35 (s, 1H, H3), 6.85 (s, 1H, H6); ¹³C NMR (CDCl₃): δ 27.4, 27.7, 28.4 (3CH₂, COO–(CH₂)₂–(CH₂)₃– CH₂-Br), 32.5 (COO-CH₂-CH₂-(CH₂)₄-Br), 33.8 (COO-(CH₂)₅-CH2-Br), 43.5 (C8), 45.7 (C7'), 47.3 (C8'), 56.2 (2CH3, C10', C12'), 60.9 (C11'), 62.7 (C9), 64.8 (COO-CH2-(CH2)5-Br), 69.8 (C7), 101.2 (C10), 106.3 (2CH, C2', C6'), 108.4 (6), 109.3 (C3), 130.2 (C1), 130.7 (C2), 136.7 (C4'), 140.0 (C1'), 146.8 (C4), 147.7 (C5), 153.1 (2C, C3', C5'), 174.4 (C9'). IR (film, cm⁻¹): 3407, 1125, 1037 (OH), 1725 (COOR). $[\alpha]^{20}$ (λ): -56° (*c* 0.93%). HRMS: calcd for C₂₈H₃₅O₉BrNa: 617.1357 u; found: 617.1335 *m*/*z*.

4.1.3.3. 3-Bromopropyl 9-deoxy-9-oxo- α -apopicropodophyllate **25**. From **22** (630 mg, 1.14 mmol) under Swern conditions to yield **25** (520 mg, 86%) [28].

4.1.3.4. 6-Bromohexyl 9-deoxy-9-oxo-α-apopicropodophyllate **26**. From **23** (210 mg, 0.353 mmol). The crude product from the Swern reaction was chromatographed on silica gel, eluting with 5% EtOAc/CH₂Cl₂ to give compound **26** (100 mg, 49%). ¹H NMR (CDCl₃): δ 1.15–1.60 (m, 6H, COO–(CH₂)₂–(CH₂)₃–CH₂–Br), 1.80 (m, 2H, COO–CH₂–CH₂–(CH₂)₄–Br), 3.38 (t, 2H, J = 6.5 Hz, COO–(CH₂)₅–CH₂–Br), 4.0 (m. 2H, COO–CH₂–(CH₂)₅–Br), 7.34 (s, 1H, H7), 9.60 (1H, H9); ¹³C NMR (CDCl₃): δ 24.9, 27.7, 28.3 (3CH₂, COO–(CH₂)₂–(CH₂)₃–CH₂–Br), 32.6 (COO–CH₂–(CH₂)₅–Br), 145.5 (C7), 171.8 (C9'), 191.3 (C9). IR (film, cm⁻¹): 1727 (COOR), 1671 (CHO). [α]²⁰ (λ): –95° (c 0.93%). HRMS: calcd for C₂₈H₃₁O₈BrNa: 597.1094 u; found: 597.1109 *m/z*.

4.1.3.5. α' -Bromo-p-xylenyl 9-deoxy-9-oxo- α -apopicropodophyllate 27. From 3 (150 mg, 0.347 mmol), K₂CO₃ (96 mg, 0.69 mmol) and α,α'-dibromo-p-xylene (183 mg, 0.694 mmol) in dry DMF (1.3 mL) using the general method B_1 during 2 h. The crude ester 24 (300 mg), was transformed to the corresponding aldehyde under Swern conditions and the reaction product was chromatographed on silica gel, eluting with 40% EtOAc/CH₂Cl₂ to give compound 27 (112 mg, 40%). ¹H NMR (CDCl₃): δ 4.45 (s, 2H, COO-CH₂-C₆H₄-CH₂-Br), 5.04 ((AB system, 2H, J = 12.8 Hz, COO-CH₂-C₆H₄-CH₂-Br), 7.10, 7.30 (AB system, 4H, J = 8.2 Hz, COO-CH₂-C₆H₄-CH₂-Br), 7.35 (s, 1H, H7), 9.60 (s, 1H, H9); ¹³C NMR (CDCl₃): δ 33.1 (COO-CH₂-C₆H₄-CH₂-Br), 65.5 (COO-CH₂-C₆H₄-CH₂-N₉), 128.0, 128.7, 129.2 (4CH, COO-CH₂-C₆H₄-CH₂-N₉), 133.8 (1C, COO-CH₂-C₆H₄-CH₂-N₉), 136.0 (1C, COO-CH₂-C₆H₄-CH₂-N₉), 145.8 (C7), 171.7 (C9'), 191.3 (C9). IR (film, cm⁻¹): 1732 (COOR), 1668 (CHO). $[\alpha]^{20}$ (λ): -132° (c 1%). HRMS: calcd for C₃₀H₂₇O₈BrNa: 617.0781 u; found: 617.0804 m/z.

4.1.3.6. 3-(*Purin-9-yl*)*propyl* 9-*deoxy-9-oxodidehydropodophyllate* **28**. From **25** (80 mg, 0.15 mmol), K_2CO_3 (21 mg, 0.15 mmol) and purine (25 mg, 0.21 mmol) in dry DMF (2.0 mL) using the general method B₁ during 6 d. The reaction product was a mixture containing compounds **16** and **28** on a 1:1 ratio. A fraction of this reaction product (20 mg) was redissolved in DMF (2.0 mL) and reacted with K₂CO₃ (10 mg, 0.07 mmol) for 40 h at room temperature. The crude was processed according to the above general methodology to give compound **28** (8 mg, 40%). ¹H NMR (CDCl₃): δ 2.07 (m, 2H, COO–CH₂–CH₂–CH₂–Br), 3.68 (s, 3H, H11'), 3.69 (s, 6H, H10', H12'), 4.03 (t, 2H, *J* = 5.8 Hz, COO–CH₂–CH₂–CH₂–Br), 4.20 (t, 2H, *J* = 6.5 Hz, COO–CH₂–CH₂–CH₂–Br), 6.03 (s, 2H, H10), 6.45 (s, 2H, H2', H6'), 6.74 (s, 1H, H3), 7.36 (s, 1H, H6), 8.24 (s, 1H, H7), 8.36 (s, 1H, H8-purine), 8.78 (s, 1H, H2-purine), 8.82 (s, 1H, H6purine), 9.88 (s, 1H, H9); IR (film, cm⁻¹): 1728 (COOR), 1689 (CHO). HRMS: calcd for C₃₀H₂₆N₄O₈Na: 593.1643 u; found: 593.1641 *m/z*.

4.1.3.7. 3-(6-Chloropurin-9-yl)propyl 9-deoxy-9-oxodidehydropodophyllate 29. From 25 (275 mg, 0.516 mmol), K₂CO₃ (214 mg, 1.55 mmol) and 6-chloropurine (80 mg, 0.52 mmol) in dry DMF (3.8 mL) using the general method B_1 during 3 d. The reaction product was a mixture of compounds 17 and 29 on a 1:1 ratio, from which compound 29 (25 mg, 8%) was purified by column chromatography on silica gel, eluting with 70% EtOAc/CH₂Cl₂. ¹H NMR (CDCl₃): δ 2.07 (m, 2H, COO-CH₂-CH₂-CH₂-Br), 3.68 (s, 6H, H10', H12'), 3.69 (s, 3H, H11'), 4.03 (t, 2H, J = 6.5 Hz, COO-CH₂ Br), 4.26 (t, 2H, J = 6.5 Hz, COO-CH₂-CH₂-CH₂-Br), 6.12 (s, 2H, H10), 6.54 (s, 2H, H2', H6'), 6.96 (s, 1H, H3), 7.32 (s, 1H, H6), 8.18 (s, 1H, H7), 8.28 (s, 1H, H8-purine), 8.68 (s, 1H, H2-purine), 10.06 (s, 1H, H9); ¹³C NMR (CDCl₃): δ 28.8 (COO-CH₂-CH₂-CH₂-Br), 41.5 (COO-CH2-CH2-CH2-Br), 56.3 (2CH3, C10', C12'), 61.1 (C11'), 61.7 (COO-CH₂-CH₂-CH₂-Br), 102.2 (C10), 103.8 (C6), 105.2 (C3), 107.5 (3CH, C7, C2', C6'), 127.3 (C1), 129.0 (C7'), 130.1 (C8'), 133.1 (C8), 131.8 (C5purine), 134.8 (C2), 137.8 (2C, C1', C4'), 146.1 (2C, C5, C8-purine), 149.2 (C4), 150.9 (C6-purine), 151.3 (C4-purine), 151.8 (C2-purine), 153.1 (2C, C3', C5'), 168.6 (C9'), 190.2 (C9). IR (film, cm⁻¹): 1728 (COOR), 1690 (CHO). Anal. (C₃₀H₂₅N₄O₈Cl) C, H, N. HRMS: calcd for C₃₀H₂₅N₄O₈ClNa: 627.1253 u; found: 627.1222 *m*/*z*.

4.1.3.8. 6-(6-Chloropurin-9-yl)hexyl 9-deoxy-9-oxo- α -apopicropodophyllate **19** and 6-(6-chloropurin-9-yl)hexyl 9-deoxy-9oxodidehydropodophyllate **30**. From **26** (70 mg, 0.131 mmol), K₂CO₃ (54 mg, 0.393 mmol) and 6-chloropurine (41 mg, 0.26 mmol) in dry DMF (2.3 mL) using the general method B₁ during 3 d. The reaction product was a mixture of compounds **19** and **30** in a 2:1 ratio that were inseparable.

4.1.3.9. α' -(6-Chloropurin-9-yl)-p-xylenyl 9-deoxy-9-oxo- α -apopicropodophyllate **20** and α' -(6-chloropurin-9-yl)-p-xylenyl 9-deoxy-9oxodidehydropodophyllate **31**. From **27** (78 mg, 0.131 mmol), K₂CO₃ (54 mg, 0.393 mmol) and 6-chloropurine (41 mg, 0.26 mmol) in dry DMF (2.3 mL) using the general method B₁ during 3 d. The reaction product was a complex mixture containing compounds **20** and **31** in a 1:1 ratio that were inseparable.

4.1.4. Preparation of hybrid aldehydes 16 and 17 using route B3

A solution of acetonide **32** (obtained from **22**) in dry DMF was added to a solution of the corresponding purine and K_2CO_3 in dry DMF and the mixture stirred at 100 °C for 24 h. Water was added to the reaction mixture and the crude was extracted with EtOAc. The organics were washed with sat aq NaCl, dried over Na₂SO₄ and filtered. The solvent was removed under vacuum and the crude was purified by column chromatography to give the corresponding protected conjugates (**33** and **34**). The hybrids were dissolved in a water/acetone (2:8) solution (10 mL) and deprotected by reaction with *p*-toluenesulfonic acid at room temperature during 40–48 h. After this time, the acetone was concentrated under vacuum and the crude mixture was diluted with sat aq NaHCO₃ and extracted with EtOAc. The organics were washed with sat aq NaCl, dried over Na₂SO₄ and filtered. The obtained hybrid dihydroxyesters (**10**, **11**) were transformed into the corresponding aldehydes **16** and **17** without further purification by oxidation under Swern conditions.

4.1.4.1. 7,9-Acetonide of 3-bromopropyl picropodophyllate **32**. A catalytic amount (8 mg) of *p*-toluenesulfonic acid was added to a solution of 22 (240 mg, 0.43 mmol) in 2,2-dimethoxypropane (10 mL 82 mmol). The mixture was stirred at room temperature for 6.5 h, K₂CO₃ (500 mg, 3.6 mmol) was then added and the reaction mixture stirred for 1 h. After addition of water and evaporation of the 2,2-dimethoxypropane, the crude was extracted with EtOAc. The organics were washed with a sat aq solution of NaCl, dried over Na₂SO₄ and filtered. The solvent was removed under vacuum to give 220 mg (86%) of compound **32**. ¹H NMR (CDCl₃): δ 1.49, 1.58 (2s, 6H, H12, H13), 2.12 (m, 2H, COO-CH₂-CH Br), 2.27 (m, 1H, H8), 2.80 (dd, 1H, J = 4.8 and 2.2 Hz, H8'), 3.30 (m, 2H, COO-CH2-CH2-CH2-Br), 3.77 (s, 6H, H10', H12'), 3.82 (s, 3H, H11'), 4.09 (d, 1H, J = 11.3 Hz, H9a), 4.14 (d, 1H, J = 11.3 Hz, H9b), 4.30 (m, 2H, COO-CH₂-CH₂-CH₂-Br), 4.37 (d, 1H, J = 2.2 Hz, H7'), 4.90 (d, 1H, J = 11.2 Hz, H7), 5.90 (s, 2H, H10), 6.26 (s, 2H, H2', H6'), 6.38 (s, 1H, H3), 6.99 (s, 1H, H6); ¹³C NMR (CDCl₃): δ 19.6, 29.9 (2CH₃, C12, C13), 29.2 (COO-CH₂-CH₂-CH₂-Br), 31.2 (COO-CH₂-CH2-CH2-Br), 34.9 (C8), 46.8 (C7'), 48.0 (C8'), 56.3 (2CH3, C10', C12'), 60.9 (C11'), 62.6, 63.1 (2 CH2, C9, COO-CH2-CH2-CH2-Br), 68.0 (C7), 99.8 (C11), 101.0 (C10), 104.8 (C6), 106.1 (2CH, C2', C6'), 109.5 (C3), 128.7 (C1), 130.9 (C2), 136.9 (C4'), 140.5 (C1'), 146.9 (C5), 147.1 (C4), 153.2 (2C, C3', C5'), 172.8 (C9'). IR (film, cm⁻¹): 1729 (COOR). HRMS: calcd for C₂₈H₃₃O₉BrNa: 615.1200 u; found: 615.1221 *m/z*.

4.1.4.2. 7,9-Acetonide of 3-(purin-9-yl)propyl picropodophyllate 33. From acetonide **32** (220 mg, 0.370 mmol), K₂CO₃ (51 mg, 0.37 mmol) and purine (62 mg, 0.52 mmol) in dry DMF (5.0 mL) using the general method B₃ during 24 h. The reaction product was chromatographed silica gel, eluting with 10% EtOH/CH₂Cl₂ to give compound **33** (140 mg, 60%). ¹H NMR (CDCl₃): δ 1.19, 1.24 (2s, 2H, H12, H13), 2.25 (m, 3H, H8, COO-CH₂-CH₂-CH₂-N₉), 4.07 (m, 1H, H9), 4.11 (m, 2H, COO $-CH_2-CH_2-CH_2-N_9$), 4.21 (t, 2H, J = 6.9 Hz, $COO-CH_2-CH_2-CH_2-N_9$, 4.91 (d, 1H, J = 11.0 Hz, H7), 7.88 (s, 1H, H8-purine), 8.94 (s, 1H, H2-purine), 9.12 (s, 1H, H6-purine); ¹³C NMR (CDCl₃): δ 19.6, 29.9 (2CH₃, C12, C13), 29.1 (COO-CH₂-CH₂-CH2-N9), 35.0 (C8), 40.6 (COO-CH2-CH2-CH2-N9), 61.3 (COO-CH₂-CH₂-CH₂-N₉), 63.0 (C9), 67.9 (C7), 99.8 (C11), 134.1 (C5-purine), 145.1 (C8-purine), 148.8 (C6-purine), 151.4 (C4-purine), 152.7 (C2-purine), 174.6 (C9'). IR (film, cm⁻¹): 1729 (COOR), HRMS: calcd for C₃₃H₃₆N₄O₉Na: 655.2374 u; found: 655.2377 m/z.

4.1.4.3. 3-(Purin-9-yl)propyl 9-deoxy-9-oxo- α -apopicropodophyllate 16. From 33 (80 mg, 0.13 mmol) and p-toluenesulfonic acid (10 mg, 0.05 mmol) in a water/acetone (2:8) solution (10 mL) using the general deprotection described in method B₃ during 40 h, to yield the hybrid ester 10 (55 mg, 74%) that was transformed to the corresponding aldehyde **16** under Swern conditions (40 mg, 75%). ¹H NMR (CDCl₃): δ 2.18 (m, 2H, COO-CH₂-CH₂-CH₂-N₉), 4.00 (t, 2H, J = 6.2 Hz, COO $-CH_2-CH_2-CH_2-N_9$), 4.25 (t, 2H, J = 6.6 Hz, COO-CH₂-CH₂-CH₂-N₉), 7.38 (s, 1H, H7), 8.09 (s, 1H, H8-purine), 8.94 (s, 1H, H2-purine), 9.11 (s, 1H, H6-purine), 9.64 (s, 1H, H9); ¹³C NMR (CDCl₃): δ 28.5 (COO-CH₂-CH₂-CH₂-N₉), 40.5 (COO-CH₂-CH₂-CH₂-N₉), 61.4 (COO-CH₂-CH₂-CH₂-N₉), 133.1 (C8), 134.2 (C5purine), 146.0 (C7), 146.2 (C8-purine), 148.5 (C6-purine), 151.4 (C4-purine), 152.5 (C2-purine), 171.9 (C9'), 191.5 (C9). IR (film, ¹): 1729 (COOR), 1667 (CHO). $[\alpha]^{20}(\lambda)$: -134° (*c* 0.83%). HRMS: cm^{-1} calcd for C₃₀H₂₈N₄O₈ + H: 573.1980 u; found: 573.1979 m/z.

4.1.4.4. 3-(6-Chloropurin-9-yl)propyl 9-deoxy-9-oxo- α -apopicropodophyllate **17**. From acetonide **32** (345 mg, 0.582 mmol), K₂CO₃ (75 mg, 0.58 mmol) and 6-chloropurine (98 mg, 0.81 mmol) in dry DMF (5.0 mL) using the general method B₃ during 24 h. The reaction product, containing acetonide **34**, (340 mg) was treated with *p*-toluenesulfonic acid (10 mg, 0.05 mmol) in a water/acetone (2:8) solution (10 mL) during 48 h and the obtained hybrid dihydroxvester 11 (225 mg, 0.359 mmol) was transformed to the corresponding aldehyde under Swern conditions. The reaction product was chromatographed on silica gel, eluting with EtOAc to give compound **17** (105 mg, 54%). ¹H NMR (CDCl₃): δ 2.16 (m, 2H, COO– CH₂-CH₂-CH₂-N₉), 4.07 (m, 2H, COO-CH₂-CH₂-CH₂-N₉), 4.23 (m, 2H, COO-CH₂-CH₂-CH₂-N₉), 7.39 (s, 1H, H7), 8.16 (s, 1H, H8purine), 8.71 (s, 1H, H2-purine), 9.64 (s, 1H, H9); ¹³C NMR (CDCl₃): δ 28.5 (COO-CH₂-CH₂-CH₂-N₉), 41.2 (COO-CH₂-CH₂-CH₂-N₉), 61.3 (COO-CH₂-CH₂-CH₂-N₉), 131.8 (C5-purine), 133.1 (C8), 145.9 (C8-purine), 146.2 (C7), 150.8 (C6-purine), 151.1 (C4-purine), 151.9 (C2-purine), 172.0 (C9'), 191.5 (C9), IR (film, cm⁻¹): 1729 (COOR), 1667 (CHO). $[\alpha]^{20}$ (λ): -102° (c 0.91%). HRMS: calcd for $C_{30}H_{27}N_4O_8Cl + H$: 607.1590 u; found: 607.1644 m/z.

4.1.5. General methods for the synthesis of imines 35 and 36

A mixture of aldehyde (1 equiv), anhydrous MgSO₄ (4 equiv) and propylamine (2 equiv) was dissolved in CH_2Cl_2 (3 mL) and stirred at room temperature during 3 d. The reaction mixture was diluted with CH_2Cl_2 , filtered and concentrated under vacuum to give the imine in quantitative yield. Imines were obtained pure enough to carry on complete characterization without being necessary any purification step.

4.1.5.1. 6-(6-Chloropurin-9-vl)hexvl 9-deoxv-9-propvlimino- α -apopicropodophyllate 35. From aldehyde 19 (160 mg, 0.247 mmol) and propylamine (0.041 mL, 0.49 mmol) following the above procedure during 3 d to give compound **35** (170 mg, 99%). ¹H NMR (CDCl₃): δ 0.75 (t, 3H, J = 7.5 Hz, N-CH₂-CH₂-CH₃), 1.05-1.90 (m, 8H, COO-CH₂-(CH₂)₄-CH₂-N₉), 1.46 (m, 2H, N-CH₂-CH₂-CH₃), 3.35 (m, 2H, N-CH₂-CH₂-CH₃), 3.88 (m, 2H, COO-CH₂-(CH₂)₄-CH₂-N₉), 4.20 (t, 2H, J = 7.2 Hz, COO-CH₂-(CH₂)₄-CH₂-N₉), 6.74 (s, 1H, H7), 7.89 (s, 1H, H9), 8.15 (s, 1H, H8-purine), 8.68 (s, 1H, H2purine); ¹³C NMR (CDCl₃): δ 11.7 (N-CH₂-CH₂-CH₃), 24.1 (N-CH₂-CH₂-CH₃), 25.1 (COO-(CH₂)₃-CH₂-(CH₂)₂-N₉), 26.1 (COO-(CH₂)₂-CH₂-(CH₂)₃-N₉), 28.3 (COO-CH₂-CH₂-(CH₂)₄-N₉), 29.8 (COO-(CH₂)₄-CH₂-CH₂-N₉), 44.4 (COO-(CH₂)₅-CH₂-N₉), 63.0 (N-CH₂-CH₂-CH₃), 64.4 (COO-CH₂-(CH₂)₅-N₉), 131.7 (2C, C8, C5-purine), 135.2 (C7), 145.3 (C8-purine), 151.0 (C6-purine), 151.9 (2C, C2-purine, C4-purine), 161.1 (C9), 172.9 (C9'). IR (film, cm⁻¹): 1724 (COOR), HRMS: calcd for $C_{36}H_{40}N_5O_7Cl + H$: 690.2689 u; found: 690.2667 m/z.

4.1.5.2. α' -(6-Chloropurin-9-yl)-p-xylenyl 9-deoxy-9-propylimino- α *apopicropodophyllate* **36**. From aldehvde **20**(120 mg, 0.18 mmol) and propylamine (0.029 mL, 0.36 mmol) following the general procedure during 3 d to give compound **36** (125 mg, 94%). ¹H NMR (CDCl₃): $\delta 0.75(t, 3H, I = 7.5 \text{ Hz}, \text{N}-\text{CH}_2-\text{CH}_3), 1.47(m, 2H, \text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_3)$ CH₃), 3.35 (m, 2H, N–CH₂–CH₂–CH₃), 4.93 (d, 1H, J = 12.9 Hz, COO– $CH_2-C_6H_4-CH_2-N_9$), 5.07 (d, 1H, J = 12.9 Hz, $COO-CH_2-C_6H_4-CH_2-C_6H_4$ N₉), 5.41 (s, 2H, COO-CH₂-C₆H₄-CH₂-N₉), 6.78 (s, 1H, H7), 7.07, 7.18 (AB system, 4H, J = 7.9 Hz, COO-CH₂-C₆H₄-CH₂-N₉), 7.93 (s, 1H, H9), 8.13 (s, 1H, H8-purine), 8.76 (s, 1H, H8-purine); ¹³C NMR (CDCl₃): δ 11.7 (N-CH₂-CH₂-CH₃), 24.1 (N-CH₂-CH₂-CH₃), 47. 5 (COO-CH₂-C₆H₄-CH₂-N₉), 62.9 (N-CH₂-CH₂-CH₃), 65.9 (COO-CH₂-C₆H₄-CH₂-N₉), 127.8, 128.3 (4CH, COO-CH₂-C₆H₄-CH₂-N₉), 131.7 (2C, C8, C5-purine), 134.3 (1C, COO-CH₂-C₆H₄-CH₂-N₉), 135.3 (C7), 136.8 (1C, COO-CH₂-C₆H₄-CH₂-N₉), 145.2 (C8-purine), 151.0 (C6purine), 151.8 (C4-purine), 152.1 (C2-purine), 161.0 (C9), 172.6 (C9'). IR (film, cm^{-1}): 1728 (COOR). HRMS: calcd for $C_{38}H_{36}N_5O_7Cl + H$: 710.2376 u; found: 710.2372 m/z.

4.1.6. General methods for the synthesis of amines 37 and 38

The solution of the imine, obtained as described above, in MeOH (20 mL) was treated with excess of NaBH₄ at 0 °C for 1 h. Water was added to the reaction mixture and MeOH was partially removed in vacuum to give an aqueous solution that was adjusted with sat Na₂CO₃ to pH > 7 and then extracted with EtOAc. The organic phase was washed with a sat aq solution of NaCl, dried over Na₂SO₄ and filtered and the solvent removed under vacuum. Purification by column chromatography under the presence of triethylamine (1% v/v) yielded the corresponding amine.

4.1.6.1. 6-(6-Chloropurin-9-yl)hexyl 9-deoxy-9-propylamino- α -apopicropodophyllate 37. From imine 35 (130 mg, 0.19 mmol) and NaBH₄ (130 mg, 3.44 mmol) following the general procedure during 1 h. The reaction product was chromatographed on silica gel, eluting with 10% EtOAc/CH₂Cl₂ to give compound **37** (16 mg, 9%). ¹H NMR (CDCl₃): δ 0.78 (t, 3H, J = 7.5 Hz, NH–CH₂–CH₂–CH₃), 1.10–1.60 (m, 6H, COO-CH₂-(CH₂)₃-(CH₂)₂-N₉), 1.22 (m, 2H, NH-CH₂-CH2-CH3), 1.85 (m, 2H, COO-CH2-(CH2)3-CH2-CH2-N9), 2.27 (m, 2H, NH $-CH_2-CH_2-CH_3$), 3.27 (d, 1H, J = 14.0 Hz, H9a), 3.41 (d, 1H, J = 14.0 Hz, H9b), 3.99 (m, 2H, COO-CH₂-(CH₂)₄-CH₂-N₉), 4.24 $(t, 2H, J = 7.2 \text{ Hz}, \text{COO}-\text{CH}_2-(\text{CH}_2)_4-\text{CH}_2-\text{N}_9), 6.42 (s, 1H, H7), 8.18$ (s, 1H, H8-purine), 8.74 (s, 1H, H2-purine); 13 C NMR (CDCl₃): δ 11.6 (NH-CH2-CH2-CH3), 22.8 (NH-CH2-CH2-CH3), 25.2 (COO-(CH₂)₃-CH₂-(CH₂)₂-N₉), 26.2 (COO-(CH₂)₂-CH₂-(CH₂)₃-N₉), 28.4 (COO-CH2-CH2-(CH2)4-N9), 29.8 (COO-(CH2)4-CH2-CH2-N₉), 44.3 (COO-(CH₂)₅-CH₂-N₉), 50.4 (NH-CH₂-CH₂-CH₃), 53.5 (C9), 64.6 (COO-CH₂-(CH₂)₅-N₉), 126.0 (C7), 131.5 (C5-purine), 145.5 (C8-purine), 150.9 (C6-purine), 151.9 (2C, C2purine, C4-purine), 172.9 (C9'). IR (film, cm⁻¹): 3366 (NH), 1724 (COOR), HRMS: calcd for $C_{36}H_{42}N_5O_7Cl + H$: 692.2845 u; found: 692.2822 m/z.

4.1.6.2. α' -(6-Chloropurin-9-yl)-p-xylenyl 9-deoxy-9-propylamino- α -apopicropodophyllate **38**. From imine **36** (120 mg, 0.17 mmol) and NaBH₄ (120 mg, 3.17 mmol) following the general procedure during 1 h. The reaction product was chromatographed on silica gel, eluting with 80% EtOAc/CH₂Cl₂ to give compound **38** (9 mg, 7%). ¹H NMR (CDCl₃): δ 0.77 (t, 3H, J = 7.2 Hz, NH–CH₂–CH₂–CH₃), 1.30 (m, 2H, NH-CH₂-CH₂-CH₃), 2.30 (m, 2H, NH-CH₂-CH₂-CH₃), 3.26 (d, 1H, J = 14.4 Hz, H9a), 3.41 (d, 1H, J = 14.4 Hz, H9b), 4.96 (d, 1H, J = 12.6 Hz, COO-CH₂-C₆H₄-CH₂-N₉), 5.09 (d, 1H, J = 12.6 Hz, COO-CH₂-C₆H₄-CH₂-N₉), 5.44 (s, 2H, COO-CH₂-C₆H₄-CH₂-N₉), 6.43 (s, 1H, H7), 7.08, 7.20 (AB system, 4H, J = 7.9 Hz, COO-CH₂-C₆H₄-CH₂-N₉), 8.16 (s, 1H, H8-purine), 8.78 (s, 1H, H8-purine); ¹³C NMR (CDCl₃): δ 11.5 (NH–CH₂–CH₂–CH₃), 23.1 (NH-CH2-CH2-CH3), 47. 5 (COO-CH2-C6H4-CH2-N9), 50.0 (NH-CH₂-CH₂-CH₃), 53.5 (C9), 65.9 (COO-CH₂-C₆H₄-CH₂-N₉), 127.4 (C7), 127.9, 128.5 (4CH, COO-CH₂-C₆H₄-CH₂-N₉), 130.9 (C8), 145.2 (C8-purine), 152.3 (C4-purine), 152.5 (C2-purine), 172.3 (C9'). IR (film, cm⁻¹): 3395 (NH), 1728 (COOR). HRMS: calcd for $C_{38}H_{38}N_5O_7Cl + H$: 712.2532 u; found: 712.2501 *m*/*z*.

4.2. Cell growth inhibition assays

A colourimetric assay using sulforhodamine B (SRB) was adapted for a quantitative measurement of cell growth and viability, following a previously described method [36]. Cells were seeded in 96-well microtitre plates, at 5×10^3 cells per well in aliquots of 195 µL of RPMI medium, and they were allowed to attach to the plate surface by growing in a drug-free medium for 18 h. Afterwards, samples were added in aliquots of 5 µL (dissolved in DMSO/H₂O, 3:7). After 72 h exposure, the antitumour effect was measured by the SRB methodology: cells were fixed by adding 50 µL of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubating

for 60 min at 4 °C. Plates were washed with deionised water and dried; 100 μ L of SBR solution (0.4% wt/vol in 1% acetic acid) was added to each microtitre well and incubated for 10 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air-dried and bound stain was solubilized with Tris buffer. Optical densities were read on an automated spectrophotometer plate reader at single wavelength of 490 nm. Data analyses were generated automatically by the LIMS implementation. Using control OD values (C), test OD values (T) and time zero OD values (T_0), the drug concentration that caused a 50% growth inhibition (GI₅₀ value) was calculated from the equation: $100 \times [(T - T_0)/(C - T_0)] = 50$. Each value represents the mean from triplicate determinations.

4.3. Cell cycle analysis

For cell cycle analysis, we used the human cervical cancer HeLa cell line grown in DMEM supplemented with 10% (v/v) heat-inactivated foetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air. Cells were periodically tested for *Mycoplasma* infection and found to be negative. Untreated and drug-treated cells ($3-5 \times 10^5$) were centrifuged and fixed overnight in 70% ethanol at 4 °C. Then, cells were washed three times with PBS, incubated for 1 h with 1 mg/mL RNAse A and 20 µg/mL propidium iodide at room temperature, and analyzed with a Becton Dickinson FACSCalibur flow cytometer (San Jose, CA) as described previously [38,39] to determine the percentage of cells in each cell cycle stage.

4.4. Confocal microscopy

HeLa cells were grown on poly-L-lysine coated coverslips, and after drug treatment coverslips were washed three times with HPEM (25 mM HEPES, 60 mM PIPES, 10 mM EGTA, 3 mM MgCl₂, pH6.6), fixed with 4% paraformaldehyde in HPEM buffer for 15 min, and permeabilized with 0.5% Triton X-100 as previously described [40]. Coverslips were incubated with a specific Ab-1 anti-alphatubulin mouse monoclonal antibody (diluted 1:150 in PBS) (Calbiochem) for 1 h, washed 4 times with PBS, and then incubated with CY2-conjugated anti-mouse IgG (Jackson ImmunoResearch) for 1 h at 4 °C. After washing 4 times with PBS, cell nuclei were stained with DAPI (Sigma) for 5-10 min, washed with PBS, and then samples were analyzed by confocal microscopy using a ZeissLSM310 laser scan confocal microscope (Oberkochen, Germany). A drop of SlowFade Light Antifading reagent (Molecular Probes) was added to preserve fluorescence. Negative controls, lacking the primary antibody or using an irrelevant antibody, showed no staining.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.10.

026. These data included complete $^1\mathrm{H}$ and $^{13}\mathrm{C}$ data assignment and MOL files.

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