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Synthesis and cytotoxic evaluation of new terpenylpurines†

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Several new terpenylpurine derivatives were prepared through alkylation of different purines with halogenated reagents derived from natural terpenoids, commercially available or isolated from cones of *C. sempervirens* L. and further transformed into appropriate alkylated agents. Alkylation of the purines gave mixtures of 9- and 7-alkylpurines, being the 9-alkylpurines the major regioisomers. The presence of the terpenyl residue induced cytotoxicity on simple purines and, in general, that activity improved as the substituent was larger. The 7-diterpenyl-6-chloropurine *E*-**21b** was the most cytotoxic in the series and it can be considered an analogue of the marine natural compounds agelasines and agelasimines, which were taken as models for this work.

1. Introduction

The purine heterocycle is one of the most widely distributed in Nature since it can be found in the nucleic acids and in other important primary metabolites as NADP or ATP.[1] Although unsubstituted purine does not exist in Nature, there are a great number of purine derivatives, particularly adenine derivatives, that are involved in numerous metabolic processes. Many structurally modified purine nucleosides and nucleotides are biologically significant with activities ranging from antineoplastic and antiviral to antihypertensive, antiasthmatic or antituberculosis among others[2] and the scope of therapeutic applications seems to be far from being completed.

Among the purine derivatives, nucleosides and nucleotides, either from natural or synthetic origin, are the best known, but we have put our attention on other natural *N*-alkylpurines,[3] in which the alkyl chains can be found on any nitrogen atom at the purine moiety. Those alkyl chains can go from a simple methyl group in xanthines to large diterpenoids in agelasines or asmarines.[4]

Some representative examples of natural *N*-alkylpurines are shown in Fig. 1 and usually those natural products have served as models for the design and synthesis of a great number of derivatives and analogues for which a variety of biological activities have been described.[2,3]

In the course of our research towards new antitumour cytotoxics based on natural products, we were particularly interested on those secondary metabolites of marine origin formed by a diterpenoid attached to the 7-nitrogen atom of an adenine derivative as asmarines or agelasimines, some examples are shown in Fig. 1.[3] Those derivatives can be called terpene–adenine hybrids or terpenylpurines. These compounds have attracted the scientific interest because of their biological properties,[3] such as cytotoxic, antimicrobial, antiprotozoal, antifungal, antifouling, *etc.*

Our interest in this type of compounds is related mainly with the potent cytotoxicity described for some of them. Our research group has been involved for years in the design, synthesis and biological evaluation of cytotoxic compounds related to natural products. We have obtained very interesting results on the chemomodulation of cytotoxicity in podophyllotoxin related lignans,[5] and also in the chemoinduction of bioactivity on inactive terpenoids such as communic acids.[6] In this sense, we have synthesized a large number of derivatives, named terpenylquinones that showed very interesting cytotoxicity.[6] These terpenylquinones can be considered hybrids of a terpenoid rest and a quinone moiety and can also be considered analogues of other cytotoxic marine natural products such as avarone.[7] Recently, we have also described the synthesis and cytotoxicity of a new family of hybrids, lignopurines, between lignans and purines[8] that revealed the importance of the purine core on their cytotoxicity.

This background, and the fact that the natural alkylpurines are usually isolated in very small quantities which limited their

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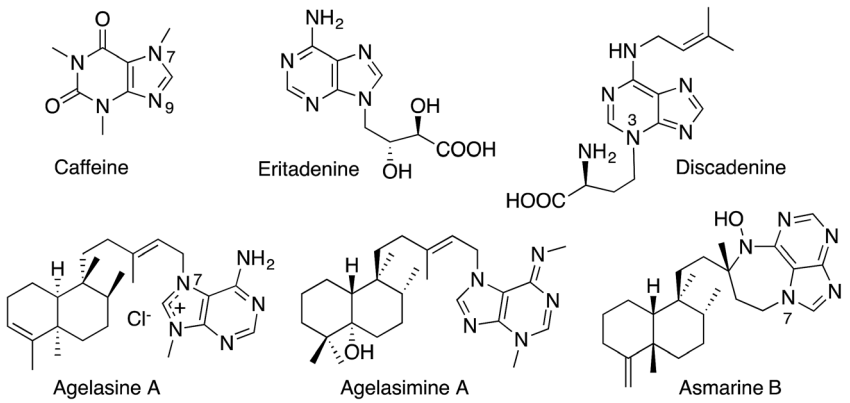


Fig. 1 Chemical structure of several natural alkylpurines.

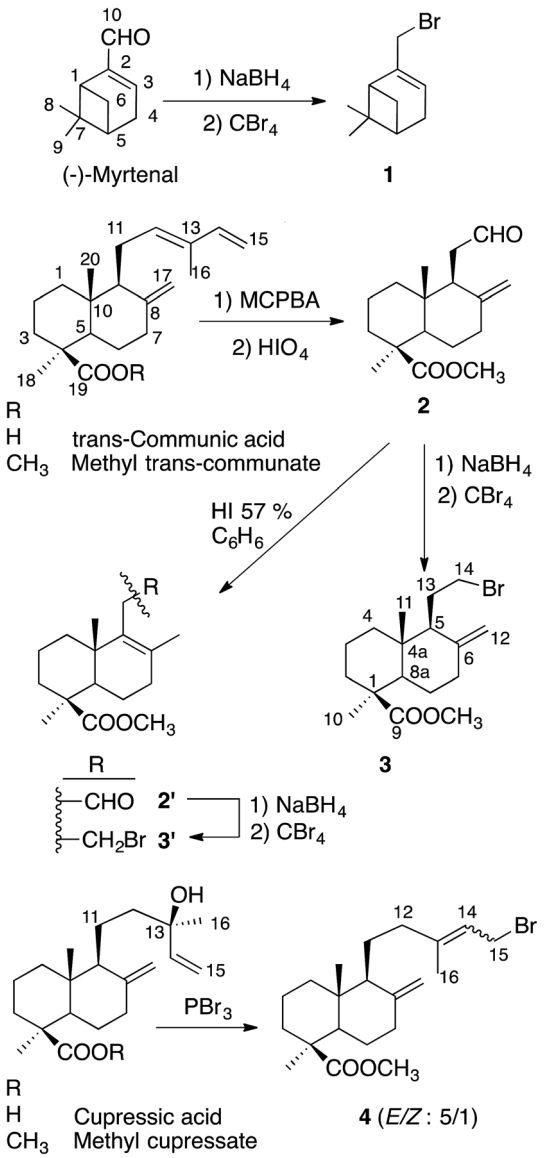
structure–activity relationship studies,[3] prompted us to design and prepare new terpenylpurine derivatives starting from natural monoterpenoids and diterpenoids, either commercially available or isolated by us from their natural sources, and to evaluate the influence of the terpenoid size on their cytotoxic properties.

2. Results and discussion

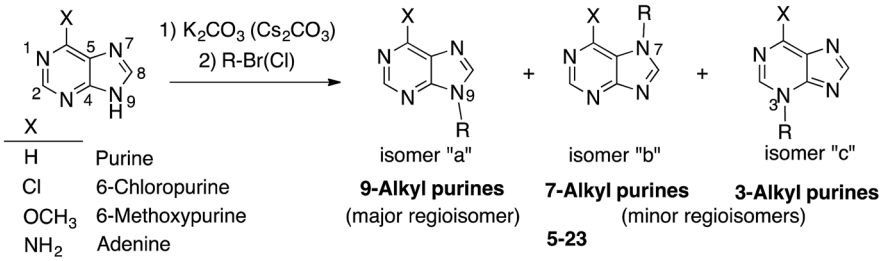
2.1. Chemistry

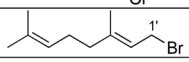
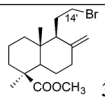
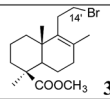
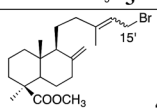
The *N*-alkylpurines described in this work have been prepared by the classical procedure of alkylation of purines with alkyl halides.[9] As starting materials for the synthesis of terpenyl bromides we used the commercial monoterpenoid myrtenal and the diterpenoids *trans*-communic and cupressic acids isolated from their natural sources. We also used other commercially available alkyl halides such as 1-bromopentane, cynnamyl bromide, isoprenyl chloride and geranyl bromide.

Myrtenal was easily transformed into the myrtenyl bromide **1** through $NaBH_4$ reduction followed by substitution of the hydroxyl group with CBr_4,[10] in this way, compound **1** was obtained in 87% overall yield from myrtenal (Scheme 1). *trans*-Communic and cupressic acids were isolated from the acid fraction of the *n*-hexane extract of *Cupressus sempervirens* L. cones (Cupressaceae). Both acids were quantitatively transformed into their corresponding methyl esters by treatment with trimethylsilyldiazomethane[11] and then transformed into the terpenyl bromides **3**, **3′** and **4** as shown in Scheme 1. Epoxidation of the trisubstituted double bond in methyl *trans*-communate, followed by oxidation with periodic acid[12] yielded the tetranorditerpenic aldehyde **2**, which was transformed into the bromide **3** by reduction and substitution as described for myrtenal. Bromide **3′** was prepared following the same procedure, previous isomerization of the exocyclic double bond.[6b] Diterpenyl bromide **4** was obtained in 77% yield by treatment of methyl cupressate with PBr_3 at −35 °C.[13] Nucleophilic substitution and allylic isomerization took place at once and compound **4** was obtained as an unresoluble mixture of the *E* and *Z* isomers in a 5 : 1 ratio that was used for the alkylation step.



Scheme 1 Preparation of the bromo-derivatives, used as electrophiles, from natural terpenoids.



Entry	R-Br(Cl)	Starting Purine	Products
1		Purine	**5a** + **5b**
2		6-Chloropurine	**6a** + **6b**
3		6-Methoxypurine	**7a** + **7b**
4		6-Chloropurine	**8a** + **8b**
5		6-Chloropurine	**9a** + **9b**
6		6-Chloropurine	**10a** + **10b**
7	 **1**	Purine	**11a**
8		6-Chloropurine	**12a** + **12b**
9		6-Methoxypurine	**13a** + **13b**
10		Adenine	**14a**
11	 **3**	Purine	**15a**
12		6-Chloropurine	**16a**
13		6-Methoxypurine	**17a**
14		Adenine	**18a**
15	 **3'**	6-Methoxypurine	**19a**
16	 **4**	Purine	(*Z*)-**20a**+(*E*)-**20a**+(*Z*)-**20b**+(*E*)-**20b**+(*E*)-**20c**
17		6-Chloropurine	(*E*)-**21a**+(*Z*)-**21b**+(*E*)-**21b**+(*E*)-**21c**
18		6-Methoxypurine	(*Z*)-**22a**+(*E*)-**22a**+(*Z*)-**22b**+(*E*)-**22b**+(*Z*)-**22c**+(*E*)-**22c**
19		Adenine	(*Z*)-**23a**+(*E*)-**23a**+(*Z*)-**23c**+(*E*)-**23c**

Scheme 2 Preparation of the alkylpurines **5**–**23** by treatment with alkyl halides.

The alkylation of purines was performed in DMF, using potassium carbonate or cesium carbonate as a base[14] (Scheme 2). In general, the reaction products were mixtures of alkylpurines, in which the major regioisomer was the corresponding 9-alkylated product (isomer "a"). In most of the cases, the minor regioisomer, 7-alkylated purine (isomer "b"), was also separated by chromatographic procedures (Scheme 2, entries 1–9). When the diterpenyl bromide **4** was used (entries 16–19), not only isomers "a" and "b" were detected but also the 3-alkylated products (isomers "c") were isolated and even the *Z* and *E* stereoisomers were also separated and characterized on the bases of the chemical shift (δ) observed in ^{13}C NMR spectra for the C-16′ methyl group on the terpenyl moiety. In the *Z* isomers, this methyl signal appeared at ≈23 ppm whereas in the *E* isomers, δ was at ≈16 ppm.[15] For the assignments of the NMR data in the synthesized alkylpurines, the purine positions were numbered from 1 to 9, whereas prime numbers were used for the alkyl side chain, keeping the terpenoid numbering system stated in Scheme 1: from 1′ to 10′ in those alkylpurines derived from monoterpenes and from 1′ to 20′ in those derived from the diterpenes. In the case of the terpenylpurines obtained from the tetranor-derivatives **3** and **3′**, the numbering shown in Scheme 1 with primes was used.

All the alkylpurines isolated are shown in Scheme 2. Some of the regioisomeric 7- and 9-pairs of alkylated purines showed characteristic chemical shift differences in their ^{1}H and ^{13}C spectra as those described for similar alkylpurines,[16] however we had several compounds in which the differences were not so evident and so that, the position of the radical was unequivocal assigned by two-dimensional HMBC correlations obtained for purines **6a**, **7a**, **7b**, **13a**, **13b**, **17a**, *Z*-**20b**, *E*-**20b**, *E*-**20c**, *E*-**21a**, *E*-

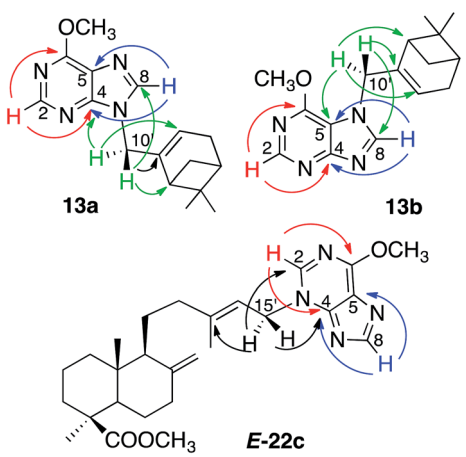


Fig. 2 Selected long-range H/C connectivities found for some regioisomers.

21b, *E*-**21c**, *E*-**22a**, *E*-**22b**, *E*-**22c**, *Z*-**23a**, *E*-**23a**, *Z*-**23c** and *E*-**23c**. Correlations observed between 2-H, 8-H and the CH_2 group of the side chain attached to N-7 (N-9), with the quaternary carbons C-4 (C-5) in the purine ring were the most determinant. NMR spectra and complete ^1H and ^{13}C NMR data assignments are included in the ESI.† As an example of the three isolated regioisomers, representative correlations experimentally observed for **13a**, **13b** and *E*-**22c** are shown in Fig. 2.

2.2. Cytotoxicity on tumour and normal cell lines

Most of the compounds prepared were evaluated *in vitro*, using the sulforhodamine B colorimetric assay,[17] to establish their cytotoxicity against the following human tumoural cell lines: NCI-H460 (non-small cell lung carcinoma), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma). The toxicity on non-tumour cell lines was also evaluated using a porcine liver primary cell culture designated as PLP2. The results were expressed as GI_{50}, compound concentration in μM that inhibited 50% of the net cell growth and they are shown in Table 1. Only those derivatives that showed GI_{50} values lower that 125 μM against one or more cell lines were considered active. In general, those compounds with a GI_{50} value under 20 μM were considered good cytotoxic, while values over 70 μM were considered slightly cytotoxic. The starting purines (purine, 6-chloropurine, 6-methoxypurine and adenine) together with the anticancer drugs 6-mercaptopurine and ellipticine were included in the assays as references.

As it can be seen in Table 1, it is possible to establish some general considerations of structure–activity relationship. First,

Table 1 Cytotoxicity data (GI_{50} in μM)[a] for the synthesized alkylpurines

Compound	NCI-H460	HeLa	HepG2	MCF-7	PLP2
5a	>125	>125	>125	>125	>125
5b	>125	>125	>125	>125	>125
6a	84.9 ± 8.1	65.8 ± 6.8	19.1 ± 0.8	90.3 ± 0.9	120.2 ± 2.6
6b	>125	>125	>125	>125	>125
8a	33.4 ± 0.3	32.0 ± 0.3	37.7 ± 2.8	15.3 ± 1.4	>125
8b	38.4 ± 2.9	22.5 ± 1.9	64.1 ± 5.8	60.3 ± 0.8	44.5 ± 3.2
9a	119 ± 3	90.6 ± 9.2	>125	>125	>125
9b	96 ± 10	40.0 ± 3.9	>125	13.6 ± 0.8	>125
10a	32.1 ± 1.1	28.9 ± 1.0	32.0 ± 1.0	40.2 ± 2.7	70.8 ± 3.7
10b	27.2 ± 0.5	29.1 ± 0.7	39.3 ± 0.6	36.5 ± 3.1	75.2 ± 5.1
11a	102 ± 5	90.8 ± 6.2	>125	109 ± 7	>125
12b	>125	>125	119 ± 3	>125	>125
13b	>125	97.3 ± 9.1	>125	>125	>125
15a	>125	>125	>125	>125	>125
17a	61.1 ± 2.9	86.9 ± 3.6	21.7 ± 0.9	82.4 ± 6.1	>125
18a	>125	103 ± 10	>125	76.2 ± 5.0	>125
(*E*)-**20a**	26.6 ± 0.1	32.3 ± 3.3	37.6 ± 2.1	34.7 ± 2.7	60.0 ± 5.0
(*Z*)-**20a**	97.0 ± 3.1	52.6 ± 2.7	48.9 ± 2.6	32.7 ± 0.8	72.8 ± 1.8
(*E*)-**20b**	28.0 ± 2.0	40.1 ± 2.5	35.3 ± 1.5	37.4 ± 0.5	63.9 ± 3.9
(*Z*)-**20b**	>125	>125	>125	>125	>125
(*E*)-**20c**	38.9 ± 3.8	61.4 ± 0.1	33.8 ± 0.9	43.4 ± 0.1	106 ± 6
(*E*)-**21a**	3.98 ± 0.41	12.8 ± 1.3	11.2 ± 0.3	13.7 ± 0.2	30.2 ± 2.0
(*E*)-**21b**	7.58 ± 0.89	3.30 ± 0.19	11.0 ± 0.4	10.7 ± 0.9	55.9 ± 1.0
(*Z*)-**21b**	24.4 ± 2.0	118 ± 3	>125	39.7 ± 0.6	>125
(*E*)-**21c**	3.06 ± 0.42	16.0 ± 0.3	15.2 ± 0.4	27.6 ± 2.0	86.9 ± 2.3
(*Z*)-**22a**	>125	>125	>125	>125	>125
(*E*)-**22a**	41.4 ± 1.7	31.8 ± 2.0	43.4 ± 2.5	35.5 ± 1.8	51.5 ± 2.7
(*E*)-**22b**	>125	>125	>125	>125	>125
(*Z*)-**22b**	>125	>125	>125	>125	>125
(*E*)-**22c**	>125	100 ± 8	20.2 ± 1.5	72.0 ± 7.4	>125
(*E*/*Z*)-**23a**	80.8 ± 6.4	64.1 ± 6.1	105 ± 4	>125	>125
(*Z*)-**23a**	>125	>125	>125	>125	>125
(*E*/*Z*)-**23b**	23.3 ± 0.5	13.5 ± 1.1	34.5 ± 1.5	34.9 ± 2.5	79.1 ± 1.6
(*E*)-**23c**	35.9 ± 1.4	20.4 ± 1.6	41.1 ± 3.5	34.1 ± 1.8	70.9 ± 6.6
(*Z*)-**23c**	30.1 ± 3.1	29.6 ± 1.7	26.4 ± 2.6	35.4 ± 0.4	68.2 ± 2.9
Purine	70.8 ± 6.0	>125	29.3 ± 0.6	58.2 ± 1.1	>125
Chloropurine	>125	>125	>125	>125	>125
Methoxypurine	>125	>125	>125	>125	>125
Adenine	>125	>125	>125	>125	>125
Mercaptopurine	>125	>125	28.2 ± 1.9	>125	>125
Ellipticine	7.96 ± 0.25	4.75 ± 0.55	13.1 ± 0.5	3.69 ± 0.16	8.57 ± 0.13

[a] GI_{50} values are expressed as mean ± standard deviation of two independent experiments, each carried out in duplicate.

it could be said that the presence of alkyl groups in a purine system promotes cytotoxicity compared to simple analogues as 6-chloropurine and adenine, which were inactive in the tests, and even better than 6-mercaptopurine which was only active in the HepG2 cell line.

The increasing of chain size led to a better cytotoxic activity. Thus, purines with linear and shorter side chains were inactive (**5a**, **5b** and **6b**) or slightly active (**6a**, **9a**, and **9b**) against any of the four tumour cell lines tested, only purines **6a** and **9b** showed an interesting cytotoxicity against HepG2 (19.1 μM) and MCF-7 cells (13.6 μM) respectively. The presence of a phenyl group improved the cytotoxicity as happened with **8a** and **8b**, which had a cinnamyl residue and showed moderate activity.

Among the monoterpenyl substituents, a linear geranyl substituent was better than the bulky pinene moiety (**10a**, **10b** *vs.* **11a**, **12b**, **13b**) with similar GI_{50} values for all the tumour cell lines and the same applied to the evaluated tetranorditerpenyl derivatives **15a**, **17a** and **18a**. It is interesting to note the activity of the purine **8a** against MCF-7 line (15.3 μM) and the purine **17a** against HepG2 (21.7 μM) without showing toxicity in the primary line PLP2.

Purines with a diterpenoid moiety included the most cytotoxic derivatives obtained, although the terpenoid is not as determinant as the substitution on the C-6 position of the purine ring: 6-chloropurine derivatives (**21**) were most potent than purine (**20**), 6-methoxypurine (**22**) and adenine (**23**) derivatives, independently if the terpenoid was at N-9, N-7 or N-3. Exceptionally some differences were observed between several *Z*/*E* isomers at the diterpenoid moiety, being more potent the *E*-isomers (*E*-**20a**, *E*-**20b**, *E*-**21b** and *E*-**22a** *vs.* *Z*-**20a**, *Z*-**20b**, *Z*-**21b** and *Z*-**22a**).

The most cytotoxic of all compounds tested on the different tumour cell lines were diterpenylpurines *E*-**21a** and *E*-**21b** (3.30–13.7 μM), being several times more potent against tumour lines than against non-tumour line PLP2. Particularly, compound *E*-**21a** was the most potent against NCI-H460 line (3.98 μM) and *E*-**21b** presented the best value cytotoxicity against HeLa cell line (3.30 μM). Both compounds improved cytotoxicity values of ellipticine in NCI-H460 and HepG2 tumour lines and besides showed less toxicity to non-tumour cells.

3. Conclusions

As a conclusion, several new terpenylpurine derivatives were efficiently prepared through alkylation of different purines with halogenated reagents derived from natural terpenoids, commercially available or isolated from their natural sources. Thus, cupressic acid was easily isolated in a good yield from cones of *C. sempervirens* L. and further transformed into appropriate alkylated agents. Alkylation of the purines gave mixtures of 9- and 7-alkylpurines, being the 9-alkylpurines the major regioisomers. Sometimes the 3-alkylpurine derivatives were also isolated in low yield from the reaction product. The presence of the terpenyl residue induced cytotoxicity on simple purines and, in general, that activity improved as the substituent was larger, like those present in the marine terpenylpurines. Although more derivatives are necessary to obtain more significant conclusions on structure–activity relationship, the fact that derivative *E*-**21b** was the most cytotoxic in the series, encourage us to continuous with our research towards the selective preparation of 7-alkylated purines, which could be considered analogues of agelasines and agelasimines, which were the marine natural terpenylpurines taken as models for this work.

4. Experimental section

4.1. Chemistry

^1H and ^{13}C NMR experiments were recorded on a Bruker AC 200 (200 or 50.3 MHz, respectively) or Bruker Avance 400DRX (400 or 100 MHz) spectrometers in $CDCl_3$ or CD_3OD using the residual solvent signal as reference. Chemical shift (δ) values are expressed in ppm followed by multiplicity and coupling constants (J) in Hz. Only representative chemical shift values of ^1H NMR data are described and assigned in this section for the new compounds and complete ^1H and ^{13}C NMR assignments are included in the ESI.†

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter in $CHCl_3$ solution and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV spectra were obtained on a Hitachi 100-60 spectrophotometer. IR spectra were obtained on a Nicolet Impact 410 spectrophotometer in NaCl film. HRMS were run in a QSTAR XL Q-TOF (Applied Biosystems) using electrospray ionization (ES) at 5500 V with an HPLC Agilent 1100 chromatograph. Solvents and reagents were purified by standard procedures as necessary and the chlorinated solvents, including $CDCl_3$, were filtered through $NaHCO_3$ prior its use, in order to eliminate acid traces. DMF was dried over molecular sieves and treated with K_2CO_3 for the same reason. Column chromatography (CC) purifications were performed using silica gel 60 (40–63 μm, 230–400 mesh, Merck).

4.1.1. Starting materials. Myrtenal, 1-bromopentane, cynnamyl bromide, isoprenyl chloride and geranyl bromide were commercially available and *trans*-communic and cupressic acids were isolated from cones of *Cupressus sempervirens* L. (Cupressaceae) as follows. Dry cones (2.3 kg) were extracted with *n*-hexane in a Soxhlet over 10 h. After cooling overnight, the insoluble part was separated, the solvent was evaporated and the residue was redissolved in Et_2O and extracted with 4% NaOH yielding an acid part (7.4 g). CC of this fraction, using hexane/EtOAc 8 : 2 as eluent, afforded *trans*-communic acid[18] (4.8 g, 64%) and cupressic acid[19] (1.5 g, 20%). Both acids were quantitatively transformed into their methyl esters by treatment with trimethylsilyldiazomethane[11] in C_6H_6/MeOH (1 : 1).

4.1.2. 10-Bromo-2-pinene 1. To a solution of myrtenal (500 mg, 3.3 mmol) in THF (8 mL) $NaBH_4$ (400 mg, 10.5 mmol) was added and kept stirring at room temperature for 8 h. Then, the reaction mixture was quenched with a saturated aqueous solution of NH_4Cl and extracted with EtOAc. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent gave a reaction product that was dissolved in CH_2Cl_2 (10 mL); then, CBr_4 (1.58 g, 4.78 mmol) and PPh_3 (1.25 g, 4.78 mmol) were added at 0 °C and stirred at this temperature for 30 min. The solvent was evaporated and filtered

over celite and chromatographed on silica gel to give **1** (600 mg, 84%).

4.1.3. Bromoderivative 3. Methyl *trans*-communate was transformed into the aldehyde **2** (55%) by a described procedure.[12] Treatment of **2** with $NaBH_4$ first, and then with CBr_4 and PPh_3 following the same procedure described above for **1** afforded **3** (60%).

4.1.4. Bromoderivative 3′. To a solution of **2** (50 mg, 0.18 mmol) in C_6H_6 (18 mL) was added HI 57% (0.40 mL, 0.18 mmol). The mixture was stirred at 80 °C for 10 min. Then, EtOAc was added and the organic layer was washed with aq satd $NaHCO_3$, brine, dried over Na_2SO_4, filtered and evaporated off yielding the isomerised product **2′** (35 mg, 70%). Treatment of **2′** with $NaBH_4$ first, and then with CBr_4 and PPh_3 following the same procedure described above for **1** afforded **3′** (50%).

4.1.5. Bromoderivative 4. To a solution of methyl cupressate (687 mg, 2.06 mmol) and pyridine (166 μL, 2.06 mmol) in dry Et_2O (10 mL) at −35 °C, phosphorous tribromide in dry Et_2O (5 mL) was added dropwise and stirred under inert atmosphere at the same temperature for 1 h. The reaction mixture was diluted with EtOAc and the organic layer was washed with 2 N HCl, aq satd $NaHCO_3$, brine, dried over Na_2SO_4, filtered and evaporated off to give **4** (634 mg, 77%). ^{1}H NMR ($CDCl_3$) δ: 1.65 (3H, s, 16′-H), 3.55 (3H, s, 19′-OCH_3), 3.95 (2H, d, 8.4, 15′-H), 4.43 (1H, s, 17′-Ha), 4.78 (1H, s, 17′-Hb), 5.42 (1H, t, 8.4, 14′-H).

4.1.6. General method for the synthesis of alkylated purines 5–23. A solution of purine and K_2CO_3 or Cs_2CO_3 in dry DMF was stirred at room temperature for 15 min. The alkylating agent was then added and the mixture stirred for a specified time and temperature under inert atmosphere. The crude product was diluted with water, extracted with EtOAc, and washed with sat aq NaCl, dried over Na_2SO_4 and filtered. The solvent was removed under vacuum to give the alkylated purines **5–23**.

*9(7)-Pentylpurine **5**.* From purine (150 mg, 1.25 mmol), K_2CO_3 (173 mg, 1.25 mmol) and 1-bromopentane (160 μL, 1.25 mmol) in dry DMF (3 mL), at 80 °C for 24 h. The reaction product was chromatographed on silica gel, eluting with CH_2Cl_2/EtOH 97 : 3 to give: (a) **5a** (114 mg, 48%). ^{1}H NMR ($CDCl_3$) δ: 4.26 (2H, t, 7.4, 1′-H), 8.07 (1H, s, 8-H), 8.95 (1H, s, 2-H), 9.11 (1H, s, 6-H). HRMS (ES, M + Na): *m*/*z* 213.1110 (calc. for $C_{10}H_{14}N_4Na$: 213.1111). (b) **5b** (16 mg, 7%). ^{1}H NMR ($CDCl_3$) δ: 4.22 (2H, t, 7.0, 1′-H), 8.16 (1H, s, 8-H), 8.91 (1H, s, 6-H), 9.09 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 191.1287 (calc. for $C_{10}H_{15}N_4$: 191.1291).

*6-Chloro-9(7)-pentylpurine **6**.* From 6-chloropurine (150 mg, 0.97 mmol), Cs_2CO_3 (125 mg) and 1-bromopentane (100 μL, 0.81 mmol) in dry DMF (5 mL) at 100 °C for 24 h. The reaction product was chromatographed on silica gel (Et_2O/acetone 95 : 5) to give: (a) **6a** (80 mg, 44%). ^{1}H NMR ($CDCl_3$) δ: 4.28 (2H, t, 7.2, 1′-H), 8.11 (1H, s, 8-H), 8.74 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 225.0897 (calc. for $C_{10}H_{14}N_4Cl$: 225.0901). (b) **6b** (17 mg, 9%). ^{1}H NMR ($CDCl_3$) δ: 4.46 (2H, t, 7.2, 1′-H), 8.21 (1H, s, 8-H), 8.88 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 225.0905 (calc. for $C_{10}H_{14}N_4Cl$: 225.0901).

*6-Methoxy-9(7)-pentylpurine **7**.* From 6-methoxypurine (100 mg, 0.66 mmol), Cs_2CO_3 (125 mg) and 1-bromopentane (100 μL, 0.80 mmol) in dry DMF (5 mL) at 100 °C for 24 h. The reaction product was chromatographed on silica gel to give: (a) **7a** (Et_2O/EtOAc 1 : 1) (75 mg, 52%). ^{1}H NMR ($CDCl_3$) δ: 4.20 (2H, t, 7.2, 1′-H), 4.15 (3H, s, 6-OCH_3), 7.87 (1H, s, 8-H), 8.50 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 243.1217 (calc. for $C_{11}H_{16}N_4ONa$: 243.1216). (b) **7b** (Et_2O/EtOAc 3 : 7) (15 mg, 10%). ^{1}H NMR ($CDCl_3$) δ: 4.30 (2H, t, 7.6, 1′-H), 4.15 (3H, s, 6-OCH_3), 7.97 (1H, s, 8-H), 8.62 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 243.1215 (calc. for $C_{11}H_{16}N_4ONa$: 243.1216).

*6-Chloro-9(7)-(3-phenyl-2-propenyl)purine **8**.* From 6-chloropurine (300 mg, 1.94 mmol), K_2CO_3 (268 mg, 1.94 mmol) and cynnamyl bromide (395 mg, 1.94 mmol) in dry DMF (32 mL) at 80 °C for 20 h. The reaction product was chromatographed on silica gel (CH_2Cl_2/EtOH 97 : 3) to give: (a) **8a** (100 mg, 20%). ^{1}H NMR (CD_3OD) δ: 4.93 (2H, d, 6.1, 1′-H), 8.43 (1H, s, 2-H), 8.54 (1H, s, 8-H). HRMS (ES, M + H): *m*/*z* 271.0750 (calc. for $C_{14}H_{12}N_4Cl$: 271.0745). (b) **8b** (20 mg, 4%). ^{1}H NMR (CD_3OD) δ: 5.22 (2H, d, 4.6, 1′-H), 8.62 (1H, s, 2-H), 8.67 (1H, s, 8-H). HRMS (ES, M + H): *m*/*z* 271.0741 (calc. for $C_{14}H_{12}N_4Cl$: 271.0745).

*6-Chloro-9(7)-(3-methyl-2-butenyl)purine **9**.* From 6-chloropurine (300 mg, 1.94 mmol), K_2CO_3 (268 mg, 1.94 mmol) and isoprenyl chloride (0.22 mL, 1.9 mmol) in dry DMF (32 mL) at 80 °C for 20 h. The reaction product was chromatographed on silica gel (CH_2Cl_2/EtOH 95 : 5) to give: (a) **9a** (56 mg, 13%). ^{1}H NMR (CD_3OD) δ: 4.84 (2H, d, 7.2, 1′-H), 8.42 (1H, s, 2-H), 8.63 (1H, s, 8-H). HRMS (ES, M + H): *m*/*z* 223.0740 (calc. for $C_{10}H_{12}N_4Cl$: 223.0745). (b) **9b** (14 mg, 3%). ^{1}H NMR (CD_3OD) δ: 5.13 (2H, d, 6.7, 1′-H), 8.60 (1H, s, 2-H), 8.70 (1H, s, 8-H). HRMS (ES, M + H): *m*/*z* 223.0743 (calc. for $C_{10}H_{12}N_4Cl$: 223.0745).

*6-Chloro-9(7)-geranylpurine **10**.* From 6-chloropurine (200 mg, 1.29 mmol), K_2CO_3 (178 mg, 1.29 mmol) and geranyl bromide (0.26 mL, 1.3 mmol) in dry DMF (7 mL) at 80 °C for 20 h. The reaction product was chromatographed on silica gel (CH_2Cl_2/EtOH 98 : 2) to give: (a) **10a** (157 mg, 42%). IR ν_{max}/cm^{-1}: 3050, 2973, 1592, 1563, 1490, 1440, 933, 856. ^{1}H NMR (CD_3OD) δ: 4.92 (2H, d, 7.2, 1′-H), 8.48 (1H, s, 8-H), 8.70 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 291.1378 (calc. for $C_{15}H_{20}N_4Cl$: 291.1371). (b) **10b** (50 mg, 13%). IR ν_{max}/cm^{-1}: 3050, 2980, 1599, 1536, 1476, 1446, 973, 840. ^{1}H NMR (CD_3OD) δ: 5.18 (2H, d, 6.8, 1′-H), 8.62 (1H, s, 8-H), 8.77 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 291.1369 (calc. for $C_{15}H_{20}N_4Cl$: 291.1371).

*9-(2-Pinen-10-yl)purine **11**.* From purine (61 mg, 0.51 mmol), K_2CO_3 (70 mg, 0.51 mmol) and 10-bromo-2-pinene **1** (110 mg, 0.51 mmol) in dry DMF (15 mL) at 50 °C for 5 h. The reaction product was chromatographed on silica gel (hexane/acetone 1 : 1) to give **11a** (45 mg, 36%). IR ν_{max}/cm^{-1}: 2985, 1595, 1578, 1501, 1407, 1347. ^{1}H NMR ($CDCl_3$) δ: 4.76 (2H, ABq, 15.4, 10′-H), 8.09 (1H, s, 8-H), 9.00 (1H, s, 2-H), 9.15 (1H, s, 6-H). HRMS (ES, M + H): *m*/*z* 255.1607 (calc. for $C_{15}H_{19}N_4$: 255.1604).

*6-Chloro-9(7)-(2-pinen-10-yl)purine **12**.* From 6-chloropurine (65 mg, 0.42 mmol), K_2CO_3 (58 mg, 0.42 mmol) and 10-bromo-2-pinene **1** (90 mg, 0.42 mmol) in dry DMF (15 mL) at 50 °C for 5 h. The reaction product was chromatographed on silica gel (hexane/acetone 7 : 3) to give: (a) **12a** (40 mg, 33%). IR ν_{max}/cm^{-1}: 3060, 2983, 1591, 1557, 1495, 1333, 1185. ^{1}H NMR ($CDCl_3$) δ: 4.80 (2H, ABq, 15.0, 10′-H), 8.11 (1H, s, 8-H), 8.76 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 289.1221 (calc. for $C_{15}H_{18}N_4Cl$: 289.1214). (b) **12b** (20 mg, 17%). IR ν_{max}/cm^{-1}:

3080, 2987, 1599, 1537, 1472, 1365. ^{1}H NMR ($CDCl_3$) δ: 4.98 (2H, ABq, 15.4, 10′-H), 8.21 (1H, s, 8-H), 8.89 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 289.1215 (calc. for $C_{15}H_{18}N_4Cl$: 289.1214).

6-Methoxy-9(7)-(2-pinen-10-yl)purine ***13***. From 6-methoxypurine (49 mg, 0.32 mmol), K_2CO_3 (44 mg, 0.32 mmol) and 10-bromo-2-pinene **1** (70 mg, 0.32 mmol) in dry DMF (15 mL) at 50 °C for 5 h. The reaction product was chromatographed on silica gel to give: (a) **13a** (hexane/acetone 8 : 2) (50 mg, 71%). IR ν_{max}/cm^{-1}: 3033, 2985, 1598, 1574, 1477, 1404, 1312. ^{1}H NMR ($CDCl_3$) δ: 4.19 (3H, s, 6-OCH_3), 4.73 (2H, ABq, 15.2, 10′-H), 7.88 (1H, s, 8-H), 8.54 (1H, s, 2-H). (b) **13b** (hexane/acetone 6 : 4) (15 mg, 21%). IR ν_{max}/cm^{-1}: 3071, 2984, 1616, 1558, 1483, 1402, 1354. ^{1}H NMR ($CDCl_3$) δ: 4.13 (3H, s, 6-OCH_3), 4.82 (2H, ABq, 15.2, 10′-H), 7.98 (1H, s, 8-H), 8.63 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 285.1718 (calc. for $C_{16}H_{21}N_4O$: 285.1710).

9-(2-Pinen-10-yl)adenine ***14***. From adenine (57 mg, 0.42 mmol), K_2CO_3 (58 mg, 0.42 mmol) and 10-bromo-2-pinene **1** (90 mg, 0.42 mmol) in dry DMF (15 mL) at 50 °C for 5 h. The reaction product was chromatographed on reverse phase-silica gel (LiChroprep RP-18, 40-63 μm, Merck), eluting with MeOH/H_2O 8 : 2 to give **14a** (25 mg, 22%). IR ν_{max}/cm^{-1}: 3308, 3140, 2985, 1676, 1654, 1623, 1560, 1480, 1418. ^{1}H NMR (CD_3OD) δ: 4.63 (2H, s, 10′-H), 7.97 (1H, s, 8-H), 8.10 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 270.1708 (calc. for $C_{15}H_{20}N_5$: 270.1713).

Tetranorterpenylpurine ***15***. From purine (46 mg, 0.39 mmol), K_2CO_3 (43 mg, 0.39 mmol) and terpenylbromide **3** (133 mg, 0.39 mmol) in dry DMF (15 mL) at reflux for 22 h. The reaction product was chromatographed on silica gel, eluting with Et_2O/acetone 8 : 2 to give **15a** (53 mg, 35%). IR ν_{max}/cm^{-1}: 3077, 2873, 1723, 1683, 1595, 1503, 1407, 1095, 898. ^{1}H NMR ($CDCl_3$) δ: 4.21 (1H, m, 14′-Ha), 4.41 (1H, m, 14′-Hb), 8.03 (1H, s, 8-H), 9.00 (1H, s, 2-H), 9.15 (1H, s, 6-H). HRMS (ES, M + H): *m*/*z* 383.1448 (calc. for $C_{22}H_{31}N_4O_2$: 383.2441).

Tetranorterpenyl-6-chloropurine ***16***. From 6-chloropurine (70 mg, 0.46 mmol), K_2CO_3 (63 mg, 0.57 mmol) and terpenylbromide **3** (157 mg, 0.46 mmol) in dry DMF (15 mL) at reflux for 7 h. The reaction product was chromatographed on silica gel, eluting with CH_2Cl_2/acetone 9 : 1 to give **16a** (18 mg, 10%). IR ν_{max}/cm^{-1}: 2850, 1724, 1698, 1592, 1508, 1409, 1225, 1153, 887. ^{1}H NMR ($CDCl_3$) δ: 4.26 (1H, m, 14′-Ha), 4.41 (1H, m, 14′-Hb), 8.06 (1H, s, 8-H), 8.77 (1H, s, 2-H).

Tetranorterpenyl-6-methoxypurine ***17***. From 6-methoxypurine (106 mg, 0.67 mmol), K_2CO_3 (93 mg, 0.67 mmol) and terpenylbromide **3** (230 mg, 0.67 mmol) in dry DMF (15 mL) at reflux for 7 h. The reaction product was chromatographed on silica gel, eluting with CH_2Cl_2/acetone 9 : 1 to give **17a** (78 mg, 30%). IR ν_{max}/cm^{-1}: 2872, 1722, 1598, 1574, 1477, 1405, 1153, 887. ^{1}H NMR ($CDCl_3$) δ: 4.18 (3H, s, 6-OCH_3), 4.12 (1H, m, 14′-Ha), 4.36 (1H, m, 14′-Hb), 7.82 (1H, s, 8-H), 8.53 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 413.2540 (calc. for $C_{23}H_{33}N_4O_3$: 413.2547).

Tetranorterpenyladenine ***18***. From adenine (84 mg, 0.63 mmol), K_2CO_3 (86 mg, 0.63 mmol) and terpenylbromide **3** (215 mg, 0.63 mmol) in dry DMF (15 mL) at reflux for 23 h. The reaction product was chromatographed on silica gel, eluting with Et_2O/EtOH 9 : 1 to give **18a** (76 mg, 31%). IR ν_{max}/cm^{-1}: 3317, 1722, 1650, 1597, 1416, 1151, 889. ^{1}H NMR ($CDCl_3$) δ: 4.13 (1H, m, 14′-Ha), 4.32 (1H, m, 14′-Hb), 7.74 (1H, s, 8-H), 8.37 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 398.2547 (calc. for $C_{22}H_{32}N_5O_2$: 398.2550).

Tetranorterpenyl-6-methoxypurine ***19***. From 6-methoxypurine (30 mg, 0.22 mmol), K_2CO_3 (26 mg, 0.20 mmol) and terpenylbromide **3′** (67 mg, 0.20 mmol) in dry DMF (15 mL) at reflux for 18 h. The reaction product was chromatographed on silica gel, eluting with CH_2Cl_2/acetone 9 : 1 to give **19a** (18 mg, 23%). IR ν_{max}/cm^{-1}: 2872, 1723, 1598, 1574, 1477, 1160. ^{1}H NMR ($CDCl_3$) δ: 4.18 (3H, s, 6-OCH_3), 4.18 (2H, m, 14′-H), 7.88 (1H, s, 8-H), 8.55 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 413.2544 (calc. for $C_{23}H_{33}N_4O_3$: 413.2547).

Diterpenylpurines ***20***. From purine (41 mg, 0.34 mmol), K_2CO_3 (47 mg, 0.34 mmol) and terpenylbromide **4** (136 mg, 0.34 mmol) in dry DMF (10 mL) at 80 °C for 20 h. The reaction product was chromatographed on silica gel to give the following compounds:

(a) (*Z*)-**20a** (12 mg, 9%), (CH_2Cl_2/EtOH 97 : 3). $[\alpha]_D^{22}$ +24.9 (*c* 0.43 in $CHCl_3$). UV: λ_{max}(EtOH)/nm 266 (ε/dm^3 mol^{-1} cm^{-1} 970). IR ν_{max}/cm^{-1}: 2928, 1723, 1643, 1597, 1451, 1127. ^{1}H NMR ($CDCl_3$) δ: 4.48 (2H, d, 7.0, 15′-H), 8.09 (1H, s, 8-H), 8.99 (1H, s, 6-H), 9.02 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 459.2734 (calc. for $C_{26}H_{36}N_4O_2Na$: 459.2730).

(b) (*E*)-**20a** (16 mg, 12%), (CH_2Cl_2/EtOH 97 : 3). IR ν_{max}/cm^{-1}: 2925, 1723, 1595, 1453, 1123. ^{1}H NMR ($CDCl_3$) δ: 4.85 (2H, d, 7.0, 15′-H), 8.10 (1H, s, 8-H), 9.00 (1H, s, 6-H), 9.14 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 459.2734 (calc. for $C_{26}H_{36}N_4O_2Na$: 459.2730).

(c) (*Z*)-**20b** (7 mg, 6%), (CH_2Cl_2/EtOH 95 : 5). IR ν_{max}/cm^{-1}: 2924, 1723, 1601, 1451, 1418, 1045. ^{1}H NMR ($CDCl_3$) δ: 4.75 (2H, m, 15′-H), 8.18 (1H, s, 8-H), 8.88 (1H, s, 6-H), 9.10 (1H, s, 2-H).

(d) (*E*)-**20b** (6 mg, 4%), (CH_2Cl_2/EtOH 95 : 5). IR ν_{max}/cm^{-1}: 3080, 2923, 1724, 1609, 1449, 1154, 880. ^{1}H NMR ($CDCl_3$) δ: 4.85 (2H, d, 7.0, 15′-H), 8.22 (1H, s, 8-H), 8.92 (1H, s, 6-H), 9.13 (1H, s, 2-H).

(e) (*E*)-**20c** (6 mg, 4%), (CH_2Cl_2/EtOH 95 : 5). IR ν_{max}/cm^{-1}: 3080, 2939, 1721, 1650, 1620, 1444, 1161, 880. ^{1}H NMR ($CDCl_3$) δ: 4.99 (2H, d, 7.0, 15′-H), 8.64 (1H, s, 2-H), 8.68 (1H, s, 6-H), 8.77 (1H, s, 8-H). HRMS (ES, M + Na): *m*/*z* 459.2752 (calc. for $C_{26}H_{36}N_4O_2Na$: 459.2730).

Diterpenyl-6-chloropurines ***21***. From 6-chloropurine (188 mg, 1.21 mmol), K_2CO_3 (168 mg, 1.21 mmol) and terpenylbromide **4** (481 mg, 1.21 mmol) in dry DMF (10 mL) at 80 °C for 20 h. The reaction product was chromatographed on silica gel (CH_2Cl_2/EtOH) to give the following compounds:

(a) (*E*)-**21a** (65 mg, 11%), (CH_2Cl_2/EtOH 99 : 1). $[\alpha]_D^{22}$ + 25.1 (*c* 1.3 in $CHCl_3$). UV λ_{max}(EtOH)/nm: 266 (ε/dm^3 mol^{-1} cm^{-1} 2380). IR ν_{max}/cm^{-1}: 2924, 1724, 1633, 1420, 1066, 878. ^{1}H NMR ($CDCl_3$) δ: 4.87 (2H, m, 15′-H), 8.10 (1H, s, 8-H), 8.75 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 493.2644 (calc. for $C_{26}H_{35}N_4O_2ClNa$: 493.2341).

(b) (*Z*)-**21b** (7 mg, 1%), (CH_2Cl_2/EtOH 99 : 1). IR ν_{max}/cm^{-1}: 2924, 1725, 1640, 1153, 895. ^{1}H NMR ($CDCl_3$) δ: 5.00 (2H, d, 7.0, 15′-H), 8.22 (1H, s, 8-H), 8.87 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 493.2339 (calc. for $C_{26}H_{35}N_4O_2ClNa$: 493.2341).

(c) (*E*)-**21b** (10 mg, 2%), (CH_2Cl_2/EtOH 99 : 1). IR ν_{max}/cm^{-1}: 3081, 2935, 1722, 1642, 1597, 1448, 1155, 975, 889. ^{1}H NMR ($CDCl_3$) δ: 5.07 (2H, d, 7.0, 15′-H), 8.24 (1H, s, 8-H), 8.87 (1H, s, 2-

H). HRMS (ES, M + Na): *m*/*z* 493.2334 (calc. for $C_{26}H_{36}N_4O_2ClNa$: 493.2341).

(d) (*E*)-**21c** (14 mg, 3%), (CH_2Cl_2/EtOH 97 : 3). $[\alpha]_D^{22}$ + 19.3 (*c* 0.51 in $CHCl_3$). IR ν_{max}/cm^{-1}: 2925, 1722, 1597, 1420, 1114, 1049. ^1H NMR ($CDCl_3$) δ: 4.75 (2H, d, 7.0, 15′-H), 7.71 (1H, s, 8-H), 8.36 (1H, s, 2-H).

Diterpenyl-6-methoxypurines ***22***. From 6-methoxypurine (101 mg, 0.68 mmol), K_2CO_3 (93 mg, 0.68 mmol) and terpenylbromide **4** (268 mg, 0.68 mmol) in dry DMF (10 mL) at 80 °C for 20 h. The reaction product was chromatographed on silica gel (CH_2Cl_2/EtOH) to give the following compounds:

(a) (*Z*)-**22a** (6 mg, 2%), (CH_2Cl_2/EtOH 99 : 1). IR ν_{max}/cm^{-1}: 3080, 2933, 2851, 1722, 1620, 1453, 1155, 889. ^1H NMR ($CDCl_3$) δ: 4.18 (3H, s, 6-OCH_3), 4.75 (2H, d, 7.0, 15′-H), 7.87 (1H, s, 8-H), 8.54 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 489.2850 (calc. for $C_{27}H_{38}N_4O_3Na$: 489.2836).

(b) (*E*)-**22a** (23 mg, 7%), (CH_2Cl_2/EtOH 99 : 1). $[\alpha]_D^{22}$ + 33.7 (*c* 0.54 in $CHCl_3$). IR ν_{max}/cm^{-1}: 3078, 2928, 2851, 1722, 1620, 1454, 1155, 889. ^1H NMR ($CDCl_3$) δ: 4.18 (3H, s, 6-OCH_3), 4.82 (2H, m, 15′-H), 7.90 (1H, s, 8-H), 8.55 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 489.2860 (calc. for $C_{27}H_{38}N_4O_3Na$: 489.2836).

(c) (*Z*)-**22b** (3 mg, 1%), (CH_2Cl_2/EtOH 98 : 2). IR ν_{max}/cm^{-1}: 3080, 2939, 2850, 1723, 1643, 1596, 1471, 1156, 888. ^1H NMR ($CDCl_3$) δ: 4.16 (3H, s, 6-OCH_3), 4.93 (2H, d, 7.0, 15′-H), 7.98 (1H, s, 8-H), 8.63 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 489.2855 (calc. for $C_{27}H_{38}N_4O_3Na$: 489.2836).

(d) (*E*)-**22b** (14 mg, 2%), (CH_2Cl_2/EtOH 97 : 3). $[\alpha]_D^{22}$ + 26.1 (*c* 0.39 in $CHCl_3$). IR ν_{max}/cm^{-1}: 3081, 2932, 2850, 1722, 1641, 1597, 1473, 1156, 889. ^1H NMR ($CDCl_3$) δ: 4.16 (3H, s, 6-OCH_3), 4.93 (2H, d, 7.0, 15′-H), 8.02 (1H, s, 8-H), 8.64 (1H, s, 2-H). HRMS (ES, M + Na): *m*/*z* 489.2844 (calc. for $C_{27}H_{38}N_4O_3Na$: 489.2836).

(e) (*Z*)-**22c** (2 mg, 1%), (CH_2Cl_2/EtOH 95 : 5). ^1H NMR ($CDCl_3$) δ: 4.30 (3H, s, 6-OCH_3), 5.09 (2H, d, 7.0, 15′-H), 8.18 (1H, s, 2-H), 8.25 (1H, s, 8-H). HRMS (ES, M + Na): *m*/*z* 489.2834 (calc. for $C_{27}H_{38}N_4O_3Na$: 489.2836).

(f) (*E*)-**22c** (7 mg, 2%), (CH_2Cl_2/EtOH 95 : 5). ^1H NMR ($CDCl_3$) δ: 4.30 (3H, s, 6-OCH_3), 5.15 (2H, m, 15′-H), 8.23 (1H, s, 2-H), 8.26 (1H, s, 8-H). HRMS (ES, M + Na): *m*/*z* 489.2840 (calc. for $C_{27}H_{38}N_4O_3Na$: 489.2836).

Diterpenyladenines ***23***. From adenine (124 mg, 0.90 mmol), K_2CO_3 (122 mg, 0.90 mmol) and terpenylbromide **4** (357 mg, 0.90 mmol) in dry DMF (15 mL) at 110 °C for 22 h. The reaction product was chromatographed on silica gel (CH_2Cl_2/EtOH) to give the following compounds:

(a) (*Z*)-**23a** (6 mg, 1%), (CH_2Cl_2/EtOH 95 : 5). IR ν_{max}/cm^{-1}: 3334, 3083, 2927, 1722, 1649, 1617, 1446, 1160, 848. ^1H NMR ($CDCl_3$) δ: 4.41 (2H, d, 7.0, 15′-H), 7.77 (1H, s, 8-H), 8.36 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 452.3040 (calc. for $C_{26}H_{38}N_5O_2$: 452.3020).

(b) (*E*)-**23a** (17 mg, 4%), (CH_2Cl_2/EtOH 9 : 1). IR ν_{max}/cm^{-1}: 3357, 2923, 1724, 1595, 1451, 1119, 889. ^1H NMR ($CDCl_3$) δ: 4.77 (2H, t, 7.0, 15′-H), 7.80 (1H, s, 8-H), 8.36 (1H, s, 2-H). HRMS (ES, M + H): *m*/*z* 452.3032 (calc. for $C_{26}H_{38}N_5O_2$: 452.3020).

(c) (*Z*)-**23c** (11 mg, 2%), (CH_2Cl_2/EtOH 9 : 1). IR ν_{max}/cm^{-1}: 3372, 3086, 2927, 1723, 1642, 1613, 1453, 1116, 889. ^1H NMR ($CDCl_3$) δ: 4.94 (2H, m, 15′-H), 8.00 (1H, s, 2-H), 8.08 (1H, s, 8-H). HRMS (ES, M + H): *m*/*z* 452.3034 (calc. for $C_{26}H_{38}N_5O_2$: 452.3020).

(d) (*E*)-**23c** (3 mg, 0.5%), (CH_2Cl_2/EtOH 8 : 2). $[\alpha]_D^{22}$ + 24.9 (*c* 0.62 in $CHCl_3$). IR ν_{max}/cm^{-1}: 3358, 3083, 2930, 1723, 1640, 1613, 1478, 1116, 889. ^1H NMR ($CDCl_3$) δ: 5.01 (2H, t, 7.0, 15′-H), 8.03 (1H, s, 2-H), 8.07 (1H, s, 8-H). HRMS (ES, M + H): *m*/*z* 452.3029 (calc. for $C_{26}H_{38}N_5O_2$: 452.3020).

4.2. Cytotoxicity assays

Four human tumour cell lines were used: NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma) from DSMZ (Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium containing 10% FBS and 2 mM glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin, at 37 °C, in a humidified air incubator containing 5% CO_2. The cell line was plated at an appropriate density (1.0×10^4 cells per well) in 96-well plates and allowed to attach for 24 h. Cells were then treated for 48 h with the different diluted compound solutions. Afterwards, sulforhodamine B assay[17a] was performed as follows: cold trichloroacetic acid (TCA 10%, 100 μL) was used in order to bind the adherent cells and further incubated for 60 min at 4 °C. After the incubation period, the plates were washed with deionised water and dried and sulforhodamine B solution (SRB 0.1% in 1% acetic acid, 100 μL) was then added to each plate well and incubated for 30 min at room temperature. The plates were washed with acetic acid (1%) in order to remove the unbound SRB and then air dried; the bound SRB was solubilised with Tris (10 mM, 200 μL) and the absorbance was measured at 540 nm using an ELX800 microplate reader (Bio-Tek Instruments, Inc.; Winooski, VT, USA). The results were expressed as GI_{50} values; compound concentration that inhibited 50% of the net cell growth. Ellipticine was used as positive control.

For hepatotoxicity evaluation, a cell culture was prepared from a freshly harvested porcine liver obtained from a local slaughterhouse, according to an established procedure[17b] and it was designed as PLP2. Cultivation of the cells was continued with direct monitoring every two to three days using a phase contrast microscope. Before confluence was reached, cells were subcultured and plated in 96-well plates at a density of 1.0×10^4 cells per well, and cultivated in DMEM medium with 10% FBS, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Cells were treated with different concentrations of the compounds and SRB assay was performed as previously described. The results were expressed as GI_{50} values; sample concentration that inhibited 50% of the net cell growth. Ellipticine was used as positive control.

For each compound, two independent experiments were performed, each one carried out in duplicate. The results are expressed as mean values and standard deviation (SD).

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