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Synthesis of Hydroxylated Biphenyl Derivatives Bearing an α,β-Unsaturated Ketone as a Lead Structure for the Development of Drug Candidates against Malignant Melanoma

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A small collection of C_2-symmetric hydroxylated biphenyl derivatives featuring an α,β-unsaturated ketone as a lead structure was prepared, and the capacity of these compounds to act as antiproliferative agents against four human malignant melanoma cell lines was assayed. The prodrug approach was applied in order to improve the delivery of compounds into the cell by modulation of the phenolic hydroxy protecting group. The hydroxylated biphenyl structure bearing an α,β-unsaturated ketone and a phenolic-O-prenylated chain was found to facilitate the delivery of the molecule and interactions with biological targets. Four compounds showed antiproliferative activity resulting in IC_{50} values in the range of 1.2 to 2.8 μM.

Introduction

The search for natural products having antitumoral activity has been rapidly increasing, likely due to the structural diversity and distinct mechanism of action that natural occurring compounds exert in biological systems.[1] In fact, concomitant inhibition of multiple pathways required for tumor progression has recently been established as an innovative strategy to improve targeted therapies in cancer treatment.[2] Cutaneous malignant melanoma (MM) is the most lethal form of skin cancer that arises from uncontrolled proliferation of melanocytes that are cells producing pigments.[3] Despite noteworthy advances in the field, heterogeneity and complexity of MM due to several distinct genotypes and phenotypes, make this kind of tumor the most aggressive form of skin cancer.[4] Therefore, the design of efficient therapies represents a formidable challenge.

It is generally acknowledged that a healthy diet provides beneficial effect as preventive therapy against cancer. Dietary components of spices and food as curcumin, cinnamon, quercetin, resveratrol, lycopene and epigallocathechins interfere with the main molecular pathways of melanoma genesis[5] identifying suitable molecular framework on which design new drug candidates.[6,7] Although curcumin, the main component of *Curcuma longa*, shows to be extremely safe in animal and humans even at very high doses, it has not yet been approved as a therapeutic agent due to the poor solubility, stability at physiological level and low bioavailability (Figure 1).[8] The structure of curcumin, [(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione], consists of a diferuloylmethane framework that exhibits keto-enol tautomerism in solution.[7] The two phenyl unit of curcumin confer lipophilicity to the molecule whereas the flexible alkylated linker can adopt different conformations contributing to activate effective interactions with a large number of proteins of pathological relevance.[9] Curcumin contains an α,β-unsaturated Michael acceptor pharmacophore that is well recognised in the design of new antimelanoma drugs in virtue of the emerging role of this moiety in interacting with nucleophiles present in the cancer cells through a Michael addition reaction.[10,11]

Analogues of curcumin were prepared by independent research groups and the antiproliferative and apoptotic activities against a wide set of malignancies were assayed identifying lead structures.[7] Most of the curcumin analogues presents modifications to the flexible unsaturated 1,3-keto-enol moiety which is believed to be responsible for the poor physiological stability, the poor adsorption and the fast metabolism of the molecule.

Our group prepared an analogue of curcumin, compound **1** (Figure 1) that showed antiproliferative and pro apoptotic activities against MM and neuroblastoma cells that were ten times stronger than those of curcumin, displaying high selectivity toward cancer cells.[12,13] Intracellular concentration of compound **1** reached 600 pmol per 10^6 cells (about 270 nM) after two hours treatment, then degradation of the compound occurred.[13]

The mechanism underlying the cell growth inhibitory action of compound **1** (compound **D6** in the references) was well investigated.[12–14] In comparison with other analogues of

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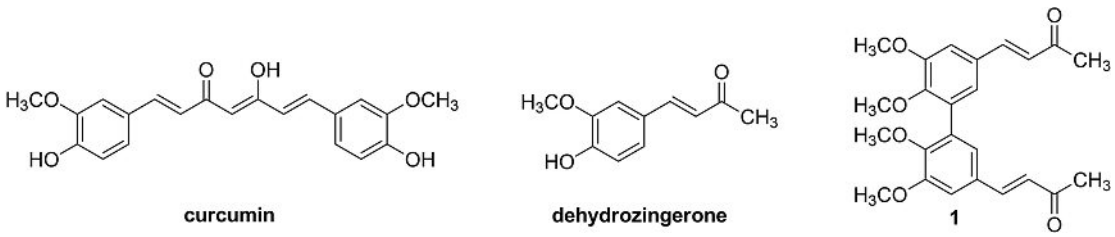


Figure 1. Chemical structures of curcumin, dehydrozingerone, and compound **1**.

curcumin, the novelty of the molecular framework of compound **1** is a combination of a hydroxylated biphenyl unit and an α,β-unsaturated carbonyl group with terminal methyl group that reminds the curcumin structure split in two parts and linked to the aromatic rings. The preparation of the molecular framework relied on a Claisen-Schmidt condensation between a ketone and an aldehyde under basic conditions.

Hydroxylated biphenyl unit is embedded in many structures of bioactive natural products, some of them of high biological relevance like ellagitannins and vancomycin, others, structurally less sophisticated, are natural occurring dimers of 4-substituted-2-methoxy phenols.[15] Hydroxylated biphenyls derivatives have been considered privileged structures due to their unique pharmacophore able of providing useful ligands for more than one type of receptor.[15] The scaffold shows structural characteristics such as flexibility combined with partial rigidity, tuneable and fully adaptable in virtue of the presence of certain functional groups.[16] As result, hydroxylated biphenyl provides an ideal molecular framework for structural modifications in the development of drug candidates.

Structurally, compound **1**, can be also related to two molecules of dehydrozingerone (Figure 1). Dehydrozingerone, known as feruloylmethane, is isolated from rhizomes of ginger (*Zingiber officinale* Roscoe), identified as a half structural analogue of curcumin and one of its degradation compounds at neutral and basic pH conditions.[17] Dehydrozingerone is stable in organic and aqueous solutions and share many structural and pharmacological features with curcumin.[18] The interesting results achieved with compound **1** against MM encouraged us to pursue further structural tuning through simple synthetic methods in order to discover more efficient drug candidates. The substitution in compound **1** of the α,β-unsaturated ketone at 5,5′ positions with an unsaturated β-diketo enol ester gave comparable results to that of curcumin in term of 50% inhibitory concentration (IC_{50}) against a set of MM cells and identified the α,β-unsaturated methyl ketone moiety of compound **1** as the most effective Michael acceptor pharmacophore in the series of the compounds investigated.[19]

With the aim of searching for an effective drug-like Michael acceptor with potential antimelanoma activity, in the present study, we prepared a collection of derivatives of compound **1**. All compounds possess a common α,β-unsaturated ketone moiety bearing in a hydroxylated biphenyl structure in which the phenolic hydroxy group was substituted with functionalities able to modify lipophilicity of the molecule with the aim to increase membrane permeability and bioavailability of the compound. The principal aim of the work was to prepare compounds that share the same core structure (hydroxylated biphenyl) but differ in patterns of substituents attached to the core structure. We applied the prodrug approach,[20,21] a successful tool in rational drug design, for improving bioactivity of compounds by proper transformation of the phenolic hydroxy group with a functionality that would increase bioavailability of the molecule or undergone *in vivo* biotransformation through chemical or enzymatic cleavage, thus modulating pharmacokinetic properties and/or favouring the delivery of the active compound with a higher yield. Further, all compounds were assayed *in vitro* on a set of human MM cell lines and their IC_{50} determined.

Results and Discussion

Chemistry

We started from the experimental evidence that compound **1**, a tetra-OMe biphenyl (**D6** in references 12–14) showed antiproliferative and apoptotic activity in malignant melanoma cells with negligible effect on normal cells.[13] A collection of eighteen derivatives of **1**, compounds **2**–**19**, were prepared through straightforward synthetic procedures. Compounds **2**–**13** possess a hydroxylated biphenyl bearing an α,β-unsaturated ketone as lead structure where phenolic hydroxy group was transformed with aliphatic group/chain with the aim to improve antimelanoma activity in comparison with that assayed in compound **1** (Figure 2). Compounds **14**–**19** are the corresponding monomers of hydroxylated biphenyls derivatives **1**, **4**, **5** and **11**–**13**. We applied the prodrug approach[20,21] and tuned the structure of compound **1** in such a way that the hydroxyl groups at the 2,2′ positions were transformed with a hydrolysable carrier (carrier-linker prodrug) as for compounds **4**, **6**, **7**, **9** and monomer **18** or with a functional group that could provide synergistic action after hydrolysis (mutual prodrugs) as for compounds **8**.[22] Biphenols **2**, **3** and **5** and monomer **19** were prepared as representative of compound **1** with increased hydrophilicity and biphenyl **10** as the most lipophilic compound. Concerning oxo-prenylated ethers **11**–**13** and **15**–**17** we though to increase bioavailability and selectivity of the compounds because oxo-prenylated phenols are able to interact with different and selected cell receptors and signal transductors (ex. Ras proteins)

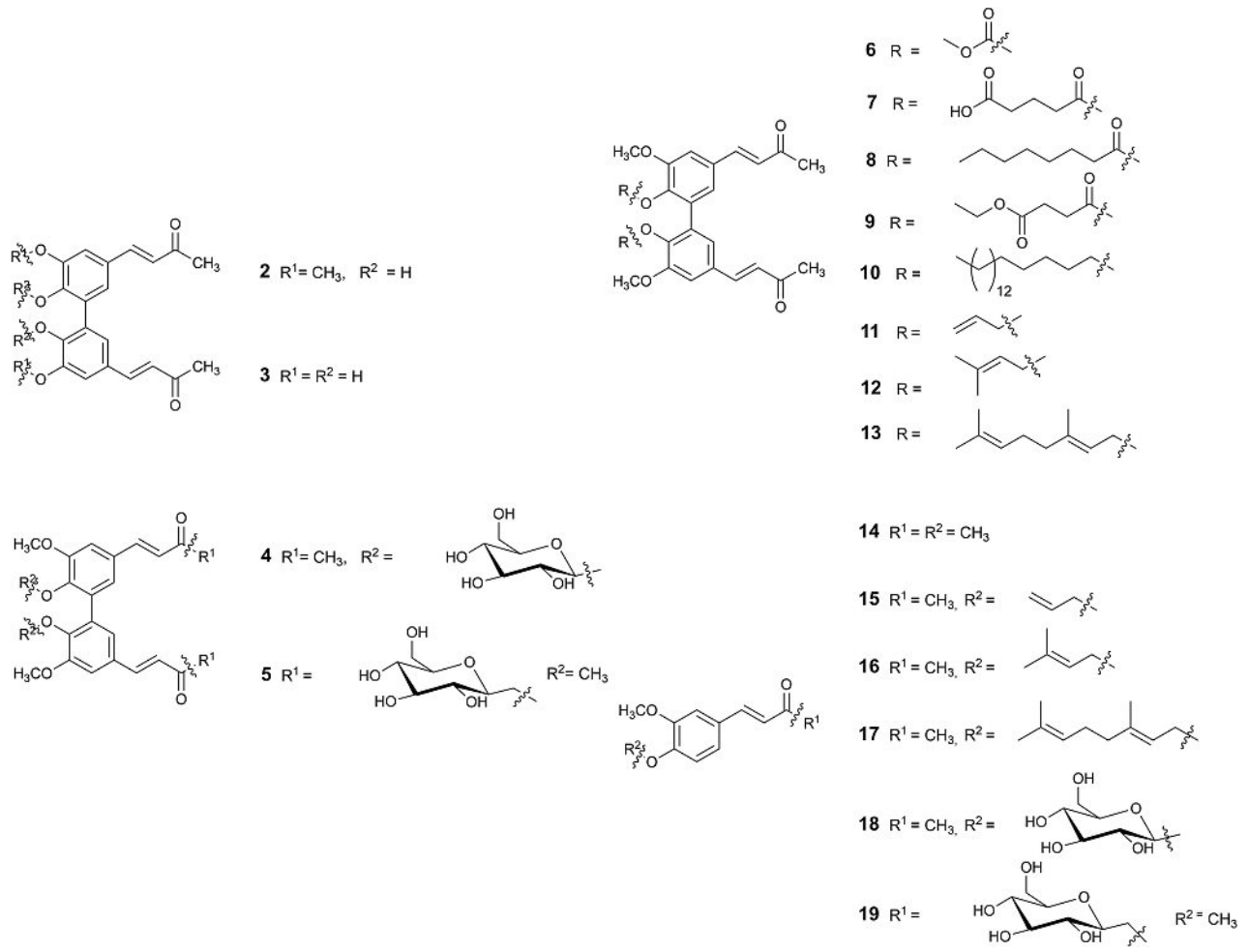
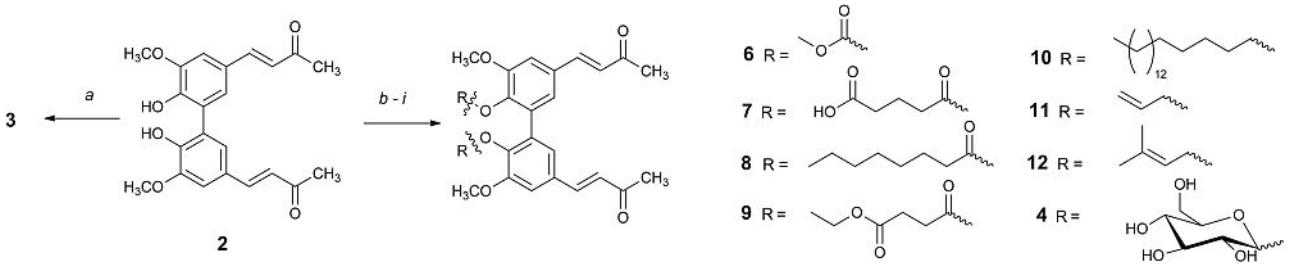


Figure 2. Chemical structures of compounds **2**–**19**.

accounting for their ability to modulate key metabolic processes in pathological disorders.[23]

Similarly, as in **1**, the presence of a C_2-symmetry axis in compounds **2**–**13** allows having the two aromatic rings indistinguishable, this structural feature provides an increase in reaction selectivity. The scarce water solubility is one hallmark of compound **1** when it was diluted in physiological medium[12] thus, free phenolic hydroxy groups at 2,2′-positions of compound **1** were obtained by Claisen-Schmidt reaction with dehydrodivanillin and acetone in the presence of Li(OH) to give compound **2** in almost 80% yield.[7] The compound was the starting material for the synthesis of compounds **4** and **6**–**12** (Scheme 1).



Scheme 1. (*a*) compound **3**: BBr_3 in CH_2Cl_2, −60 °C for 1 h; (*b*) compound **6**: methyl chloroformate, Et_3N at rt in CH_2Cl_2 for 1 h; (*c*) compound **7**: glutaric anhydride, DMAP at reflux in THF for 12 h; (*d*) compound **8**: octanoyl chloride, Et_3N at rt in CH_2Cl_2 for 4 h; (*e*) compound **9**: ethyl-3-(chloroformyl)propionate, Et_3N at rt in CH_2Cl_2 for 96 h. (*f*) compound **10**: 1-bromooctadecane, K_2CO_3, 18-crown-6 ether in CH_3CN, reflux for 12 h; (*g*) compound **11**: allyl bromide, K_2CO_3 in acetone, reflux for 12 h; (*h*) compound **12**: 3,3′-dimethylallyl bromide, K_2CO_3 in acetone, reflux for 12 h; (*i*) compound **4**: 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, Ag_2CO_3 at rt in pyridine for 12 h, then, after purification of the product, CH_3OH/CH_3ONa at rt for 10 min.

Catechol units were achieved from compound **2** with a large excess of equivalents of demethylating reagent at −60 °C for 1 h giving compound **3** in 89% yield. The browning of compound **3** after few days from the preparation was likely a consequence of the easy oxidation of the compound, therefore storage of compound **3** under nitrogen atmosphere was mandatory. While compound **2**, C_2-dimer of dehydrozingerone, showed scarce solubility in water and in physiological solution at concentrations > 2 mM, compounds **3** and **4** were completely soluble in water up to 30 mM, accordingly to their Log*P* (Table 1).

Then, we looked upon for molecular variations in developing a series of derivatives of **1** having different functional group/unit linked to the phenolic hydroxy group with the aim to improve bioavailability. First of all, the choice fell on easily hydrolysable leaving groups as acetals, carbonates and esters. Different hydrolysis rate would be expected for these classes of compounds releasing compound **2** at different compartments of the cell.[24]

Phenolic hydroxy groups of compound **2** were protected with a glycosylated unit starting from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and silver carbonate under basic conditions and further hydrolysis of acetyl groups following a known procedure that allowed to achieve compound **4** as β-anomer selectively.[25]

Methyl chloroformate with diol **2** in the presence of trimethylamine as base, gave dicarbonate **6** in 91% yield. With the aim to introduce an ester leaving group in an aliphatic chain with different lipophilicity and bioactivity, compounds **7**–**9** were prepared. Compound **7**, achieved by reaction of compound **2** with glutaric anhydride under basic conditions possesses, for each aromatic ring, an ester and a carboxylic acid functionality between three-methylene carbon chain. Seven-methylene carbon chain represents the octanoic acid portion of diester **8** whereas compound **9**, prepared by reaction with ethyl-3-(chloroformyl)propionate, possesses two-methylene carbon chain that links two ester functionalities. Although compounds **7**–**9** contains ester groups embedded onto the same hydroxylated biphenyl-α,β-unsaturated ketone moiety, compound **7** possesses a terminal carboxylic groups for each aromatic ring that make the molecule less lipophilic (Log*P* 2.29) than that of ester **9** (Log*P* 3.64) whereas ester **8** having a medium-chain fatty ester, is highly lipophilic (Log*P* 7.60). According to the pro-drug approach, compound **8**, after ester hydrolysis, should provide biphenyl **2** and octanoic acid (*i.e.* caprylic acid), two molecules with remarkably reduced lipophilicity in comparison to **8**.

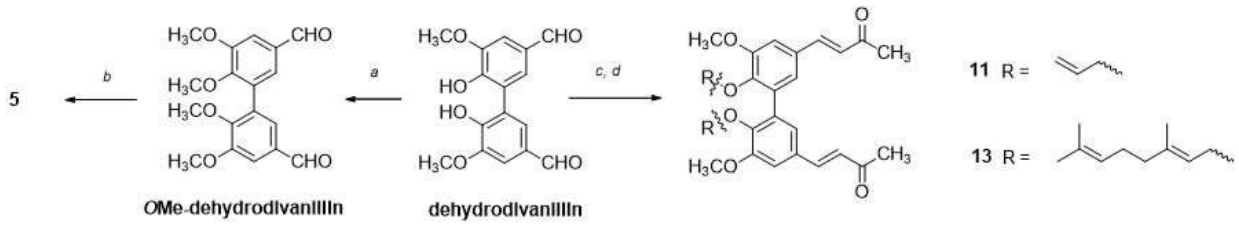
In compound **9**, the phenolic hydroxy is protected by a small chain featuring two ester functionalities that would facilitate the delivery of the molecule through the lipophilic cell membrane. Compound **9** can also exert the role of linker due to the easy hydrolyse of the ethyl ester group by esterases[26] producing a carboxylic acid group at the end of each small aliphatic chain. It would not be ruled out the expected amphiphilic properties of compound **9** when hydrolysis is applied. In the attempt to achieve another analogues of compound **1** with amphiphilic properties, a glycosylated unit was introduced at the end of the α,β-unsaturated ketone chain. Claisen-Schmidt condensation of *O*Me-dehydrodivanillin with *O-per*-acetylated-β-*C*-glucopyranosyl ketone in the presence of pyrrolidine as base and further deacetylation, gave compound **5** in 73% overall yield (Scheme 2).

The presence of two glycosylated units at the end of the α,β-unsaturated ketone chain provided in compound **5** a significant increase of hydrophilicity in comparison with that evaluated for compounds **4** bearing the glycosylated units at the phenolic hydroxy groups (Table 1).

The pro-drug approach was also applied in the synthesis of compound **9** in order to improve delivery or selectivity of the molecule in physiological environment under *in vivo* biotrans-

Table 1. Values of the logarithm of the partition coefficient of compounds **1**–**19** for *n*-octanol/water (Log*P*), estimated by ChemBioDraw13 Ultra 13.

Compound	Log*P*	Compound	Log*P*	Compound	Log*P*	Compound	Log*P*
1	2.70	**6**	3.24	**11**	4.08	**16**	2.76
2	2.17	**7**	2.29	**12**	5.17	**17**	3.87
3	1.65	**8**	7.60	**13**	8.36	**18**	−0.57
4	−1.51	**9**	3.64	**14**	1.53	**19**	−0.93
5	−2.22	**10**	> 8.50	**15**	2.22		



Scheme 2. (a) *O*Me-dehydrodivanillin: from dehydrodivanillin, ref. 26; (b) compound **5**: *per-O*-acetylated-β-*C*-glucopyranosyl ketone, pyrrolidine at rt in CH_2Cl_2, 48 h, then, after purification of the product, CH_3OH/CH_3ONa at rt for 10 min; (c) compound **11**: allyl bromide, K_2CO_3 in acetone, reflux, 12 h, then, after purification of the product, 1 N NaOH solution in acetone at rt for 12 h. (d) compound **13**: geranyl bromide, K_2CO_3 in acetone, reflux, 12 h, then, after purification of the product, 1 N NaOH solution in acetone at rt for 12 h.

formation through chemical or enzymatic cleavage.[20,21] The highest lipophilicity was achieved with compound **10** prepared at room temperature by Williamsons ether reaction of **2** with 1-bromooctadecane in the presence of 18-crown-6 ether as catalyst.

Different prenylated chains units were introduced at the phenolic hydroxy group of compound **2** by Williamson ether reaction, under basic conditions and in the presence of the corresponding prenylated bromide, as organo halide. Compounds **11** and **12** which contains an allylic and 3,3′-dimethyl allylic ether chain, respectively, were prepared in a range of 60–65% yield starting from **2** (Scheme 1). An alternative procedure was applied to compounds **11** and **13** that entailed Williamson ether reaction of dehydrodivanillin with the corresponding prenylated bromide, then the aldehyde group of the product was transformed in a α,β-unsaturated methyl ketone moiety by Claisen-Schmidt condensation in the presence of acetone under basic conditions (Scheme 2). Monomers **14**–**17** were prepared with the same synthetic procedures described in literature for these compounds that entailed protection of the phenolic hydroxy group of dehydrozingerone with the corresponding organohalide under basic conditions.[27,28] According to the reaction stoichiometry, monomers **18** and **19** were prepared with the same procedures applied for the synthesis of the corresponding dimers **4** and **5**, respectively (Scheme 3).

Remarkable different lipophilicity was estimated between the series of the monomer and dimers, in the latter, the difference was more evident as the number of carbon atoms in the prenylated chain increases.

Biological evaluation

We explored the bioactivity changes in compounds **2**–**19** induced by transformation of the functional phenolic hydroxy group underlying cell growth arrest in four MM cells lines, labelled LCP, LCM, CN and M14. We chose these four cell lines from a larger panel, for the following reasons: CN was one of the cell lines already demonstrated to be sensitive to compound **1**[12] LCP and LCM were a couple of cell lines deriving from different stage tumor lesions of the same melanoma patient, being LCP derived from the primitive lesion and LCM from a lymph node metastasis; M14 was a commercially available melanoma cell lines and can be considered as a melanoma reference *in vitro* model.[29] In our previous work[12] compound **1** showed an average IC_{50} of 1.8 ± 0.9 μM, calculated on the basis of results obtained from 72 h proliferation assays on five cell lines, including CN. Here, in a preliminary experiment, the antiproliferative activity of compounds **2**–**19** was calculated as IC_{50} value on the basis of proliferation assays performed treating cells up to 72 h. Subsequently, compounds with IC_{50} value below 7.0 μM on all the cells lines (compounds **6**–**9** and **11**, **12**) were tested after 24 h treatments (Table 2). Moreover, our most active compounds **11** and **12** were also tested on BJ normal fibroblast cell line, as a non-tumor cells control, in order to assess their selectivity.

With the aim to improve the antiproliferative activity of compound **1** (IC_{50} 1.0–2.7 μM),[12] we modified its physical-chemical properties by introducing at the 2,2′-positions of the biphenyl structure different groups/chains by tuning lipophilicity, leaving group and ability to undergone chemical/enzymatic hydrolysis in the cell. Moreover, it should be kept in mind the crucial role that lipophilicity plays in designing oncological drugs because facilitates cell membrane access of the compound in the required critical concentration.[30]

C_2-symmetry is a powerful tool in organic synthesis because when this element is present in a substrate, a control of the production of isomers occurs improving the selectivity of the reaction. The presence of a C_2-symmetry axis in compounds **2**–**13** is a useful structural element also from a biological point of view because identical interspecific interactions can be activated between each symmetrical portion of the molecule and target proteins, thus providing an increased selectivity and control in the molecule-protein interaction.

The hydrophilic compounds **2** and **3** featured with two and four free phenolic hydroxy groups, respectively, affected melanoma cells after 72 h with different antiproliferative activity (Table 2). Compound **2**, with guaiacyl units and similar to compound **1**, was more effective than **3** against LCP, LCM and CN cell lines with IC_{50} ranging from 6 ± 0.8 to 34 ± 3.2 μM. Previously, we found that compound **2** was effective breaking-chain activator in bulk lipid autoxidation and showed strong cytotoxicity against rat pheochromocytoma (PC12) cells, a slow-growing neuroendocrine tumor cell line.[31,27]

Glycosylation of compound **2** was detrimental for antiproliferative activity on all melanoma cell lines. Derivatives **4** and **5** with a *O*-glycoside unit linked at the phenolic hydroxy group and a *C*-glycoside unit at the end of the aliphatic chain, respectively, both gave value of $IC_{50} > 100$ μM after 72 h of treatment. Likely, the high hydrophilicity of **4** and **5** hindered complete bioavailability of the compounds into the cell membrane. Same results were obtained with the corresponding monomers **18** and **19** evidencing the key role of lipophilicity of the molecule in the delivery through the cell membrane.

Scheme 3. compound **18**: (*a*) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, Ag_2CO_3 at rt in pyridine for 18 h, then, after purification of the product, CH_3OH/CH_3ONa solution at rt for 10 min; compound **19**: (b) *per*-*O*-acetylated-β-*C*-glucopyranosyl ketone, pyrrolidine at rt in CH_2Cl_2, 72 h, then, after purification of the product, MeOH/MeONa at rt for 10 min.

Table 2. Cytotoxicity (IC_{50} in μM) of compounds **2**–**19**.

Compound	LCP IC_{50}		LCM IC_{50}		CN IC_{50}		M14 IC_{50}	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
2	n.d.	6.0 ± 0.8	n.d.	34 ± 3.2	n.d.	27 ± 1.8	n.d.	56 ± 5.1
3	n.d.	79 ± 8.3	n.d.	> 100	n.d.	55 ± 4.5	n.d.	38 ± 2.6
4	n.d.	> 100	n.d.	> 100	n.d.	> 100	n.d.	> 100
5	n.d.	> 100	n.d.	> 100	n.d.	> 100	n.d.	> 100
6	7.5 ± 1.1	1.3 ± 0.7	> 10	1.6 ± 0.4	> 10	1.8 ± 0.2	3.6 ± 0.1	2.5 ± 0.4
7	> 10	4.1 ± 0.8	> 10	3.7 ± 0.7	> 10	3.7 ± 0.8	> 10	6.2 ± 1.1
8	> 10	2.1 ± 0.5	7.0 ± 1.5	1.9 ± 0.3	6.4 ± 1.4	1.8 ± 0.2	4.4 ± 1.4	1.6 ± 0.9
9	> 10	4.0 ± 0.7	7.5 ± 1.0	2.2 ± 0.3	> 10	3.5 ± 0.4	4.6 ± 1.2	3.6 ± 0.9
10	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
11	6.6 ± 1.0	1.6 ± 0.1	7.9 ± 1.8	2.4 ± 0.6	7.9 ± 1.5	1.6 ± 0.2	2.4 ± 0.9	1.2 ± 0.2
12	6.7 ± 1.3	2.4 ± 0.6	8.8 ± 2.5	2.8 ± 0.8	5.6 ± 1.3	1.6 ± 0.1	6.5 ± 1.0	1.2 ± 0.1
13	n.d.	15 ± 3.2	n.d.	8.5 ± 1.1	n.d.	7.8 ± 2.7	n.d.	5.5 ± 1.7
14	n.d.	> 100	n.d.	> 100	n.d.	> 100	n.d.	> 100
15	n.d.	36 ± 4.2	n.d.	32 ± 3.8	n.d.	32 ± 3.7	n.d.	34 ± 2.6
16	n.d.	37 ± 5.0	n.d.	39 ± 3.5	n.d.	32 ± 4.1	n.d.	24 ± 2.9
17	n.d.	29 ± 2.7	n.d.	43 ± 3.8	n.d.	31 ± 2.9	n.d.	24 ± 2.1
18	n.d.	> 100	n.d.	> 100	n.d.	> 100	n.d.	> 100
19	n.d.	> 100	n.d.	> 100	n.d.	> 100	n.d.	> 100

IC_{50} values are the mean ± SD of three independent measurements, each performed in triplicate. The cell lines LCP, LCM, CN, M14 are primary human MM cell lines, as described in detail in the experimental section. n.s. = not soluble; n.d. = not determined.

Enzyme-catalysed hydrolysis of the methyl carbonate ester **6** would provide release of the biphenyl portion in the cell as experienced when bioactive methyl phenols carbonates were screened on M14 cell line, as result, higher antiproliferative activity was observed in comparison with the parent phenol.[6] In our work, compound **6** was more effective in the growth inhibition of M14 cell line (IC_{50} = 3.6 ± 0.1 μM) than in the other cell lines after 24 h. As proof of the hydrolysis of carbonate functionality, compound **6** was more effective against LCP, LCM and CN cell lines after 72 h of treatment, reaching IC_{50} values below 2 μM. It is likely that during this time, compound **6** or the corresponding hydrolysed forms reached more sensitive cell compartments in comparison to compound **2** that is the complete hydrolysed form of **6**. A similar trend was observed when cells were treated with compounds with hydrolysable esters (*i.e.* compounds **7**–**9**), the antiproliferative effect improved on all cell lines after 72 h giving IC_{50} values in the range of 1.6 ± 0.9 and 6.2 ± 1.1 μM. In comparison with IC_{50} of compound **2** achieved after 72 h, it is reasonable to suppose a synergistic effect of the hydrolysable chain of compounds **7**–**9** with the hydroxylated biphenyl portion providing an increase of the antiproliferative activity.

Although IC_{50} value is strictly dependent on the cell type, significant antiproliferative activity was observed after 24 h of treatment of LCM, CN and M14 cell lines with compound **8**, the activity improved after 72 h on all melanoma cell lines. A remarkable increased lipophilicity was estimated for compound **8** (Table 1), likely crucial in exerting an efficient delivery of the compound through the lipophilic cell membrane. Differences in the invasiveness of tumor cells could derive, among different advantageous features acquired during tumor transformation, from the activity of secreted and membrane-associated enzymes.[24] Notably, an octanoyl ester function is present in compound **8**. It is well recognised the role of medium chain fatty acids, in particular octanoic acid (*i.e.* caprylic acid),[22] in exerting antiproliferative activity against skin cancer *in vitro*[32] and, *in vivo*, in activating endogenous host peptides targeted to enhance intestinal epithelial immunological barrier.[33] In this work we did not investigated the real role of the octanoyl chain, but we observed the improvement of the antiproliferative activity of compound **8** in comparison with esters **6**, **7** and **9** after 72 h of treatment.

An excessive increase in lipophilicity was detrimental to bioavailability. Data achieved from compound **10** having a too long lipophilic aliphatic ether chain, were excluded from Table 1 since not reliable due to the scarce solubility of the molecule in water even at low concentrations.

We assayed compounds **11**–**13**, having a small, hindered and long *O*-prenylated chain, respectively, because there is convincing evidence that natural and synthetic oxyprenylated phenylpropanoids assume an important role in inhibiting some malignant cells.[34] Several mechanisms of action have been attributed to prenylated aromatic phenols.[23,35] Generally, it was demonstrated that oxyprenylated phenols are able to interact with different and selected cell receptors and signal transductors (ex. Ras proteins) accounting for their ability to modulate key metabolic processes in pathological disorders. Likely, their effect is immediately exerted on membrane cell due to the high affinity with the phospholipidic portion, favouring bioavailability of the compound.[36] In breast cancer, some oxyprenylated ferulic acids with 3,3′-dimethyl allyl and geranyl moiety at the phenolic hydroxy group, were successfully assayed for their binding affinities to MT1 melatonin receptors[37] and the antiproliferative and antimigratory properties were detected at μM concentration levels. In our work, allyl and 3,3′-dimethyl allyl *O*-prenylated compounds, **11** and **12** respectively, showed comparable antiproliferative activity assessed after 72 h in a range between 1.2 ± 0.1 and 2.8 ± 0.8 μM

and in higher level in comparison with that showed by compound **13** bearing a *O*-geranyl chain (IC_{50} 5.5 ± 1.7–15 ± 3.2 μM). It would not be ruled out that the higher lipophilicity of compound **13** (Log*P* 8.36) in comparison to compounds **11** and **12** (Log*P* 4.08 and 5.17, respectively) could likely facilitate membrane penetration but hinder diffusion of compound **13** inside the cell. After 24 h, compounds **11** and **12** showed interesting antiproliferative activity on all MM cell lines although M14 cell line appeared more sensitive to compound **11** even after 24 h treatment (IC_{50} 2.4 ± 0.9). In a lower order of magnitude, antiproliferative activity was observed after 72 h with the corresponding monomers **15**–**17** accounting for IC_{50}, homogeneously distributed on all MM cells lines, between 24 ± 2.1–43 ± 3.8 μM for all three compounds. When the phenolic hydroxy group was protected with a methyl group (*i.e.* compound **14**), the activity dropped significantly. Overall, the last results confirmed the beneficial influence that a small phenolic-*O*-prenylated chain exerts in an α,β-unsaturated Michael acceptor in enhancing antiproliferative activity of the molecule. A comparison of IC_{50} between monomers **15**–**17** and dimers **11**–**13** evidenced the key role of the hydroxylated biphenyl core in enhancing antiproliferative activity and in modulating the physical-chemical properties of the molecule.

Interestingly, compounds **8**, **11** and **12** showed comparable antiproliferative activity after 72 h on all MM cells lines even though differences between *O*-prenylated compound **11** and ester **8** were observed in LCP and M14 cell lines after 24 h of treatment. The effect could be due to the different rate of hydrolysis of the protecting group or different selectivity when the compounds are in the presence of cell specific enzymes or membrane transporters. Moreover, the activity of compounds **11** and **12** was much lower on BJ fibroblasts, the healthy, non-tumor cell line used as control. BJ cells were given the same treatments as MM cells, and showed to be much less affected by them. Indeed, compounds **11** and **12** showed significantly higher IC_{50} values after 72 h of treatments (6.4 ± 0.2 μM and 6.0 ± 0.6 μM, respectively) when compared to the mean IC_{50} value of all MM cell lines (1.7 ± 0.5 μM for **11** and 2.0 ± 0.7 μM for **12**) ($p = 0.000024$ and $p = 0.0006299$ respectively). The cytotoxicity ratio between cancer and healthy cells was 0.27 for **11** and 0.33 for **12**. These last evidences suggest a selectivity of antiproliferative activity against tumor cells for these two compounds. Such a difference should be related to the higher cell division activity of tumor cells, and might be exploited in future anticancer therapies designing. Selective antitumor activity was one of the major features of compound **1**,[12,13] and the present results show that it has been retained by compounds **11** and **12**.

Conclusions

We have prepared a small collection of C_2-symmetry hydroxylated biphenyls derivatives bearing a α,β-unsaturated ketone, compounds **2**–**13**, structurally related to compound **1**, known for selective and effective antiproliferative activity on MM cells.

The synthesis of compounds **2**–**19** was carried out in order to improve delivery of compounds into the cell by modulation of the phenolic hydroxy protecting group. The prodrug approach was applied in the synthesis of biphenyls **4**, **6**–**9** and monomer **18**. Different functional groups were introduced by straightforward methods giving ethers, esters, carbonate and acetal derivatives that influenced the physical-chemical properties of the molecule, mainly its lipophilicity and the capacity of hydrolysis into the cell or the selectivity toward tumor targets. By tuning the physical-chemical properties, we were able to identify the core molecular scaffold characterized by an hydroxylated biphenyl core bearing an α,β-unsaturated methyl ketone protected at the phenolic hydroxy group with a small *O*-prenylated chain, compounds **11** and **12**. Although the water solubility and the selective antiproliferative activity on MM cells of these two compounds showed to be comparable to those of compound **1**, this work evidenced the importance of a phenolic-*O*-prenylated chain in the structure of compound **1**, likely crucial for delivery of the molecule and interactions with the biological targets. Further studies devoted to shed light in the mechanism of compound **11** and **12** will be object of a next work.

Experimental Section

Materials and general remarks

Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used without further purification. Melting points were determined on a Büchi 530 apparatus and are uncorrected. All ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ (if not otherwise indicated) solution with a Varian VXR 5000 spectrometer at 399.94 MHz and 75.42 MHz respectively. Chemical shifts are given in ppm (δ); multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or dd (double of doublets). Elemental analyses were performed using an elemental analyser Perkin-Elmer model 240 C. Acetone was freshly distilled from $CaCl_2$. Flash chromatography was carried out with silica gel 60 (230–400 mesh, Kieselgel, EM Reagents) eluting with appropriate solution in the stated v:v proportions. Analytical thin-layer chromatography (TLC) was performed with 0.25 mm thick silica gel plates (Polygram®Sil G/UV_{254}, Macherey-Nagel). All reactions were monitored by TLC performed on 0.2 mm thick silica gel plates (60 F254 Merck). The purity of all new compounds was judged to be > 98% by ^{1}H NMR and ^{13}C NMR spectral determination.

Compounds **2** and **14** were prepared according to Marchiani *et al.*[7] whereas compounds **15**–**17** were prepared as described by Tatsuzaki *et al.*[28] Dehydrodivanillin and *O*Me-dehydrodivanillin (*i.e.*2,2′,3,3′-tetramethoxy-5,5′-diformyl-1,1′-biphenyl **23**, were obtained as described by Pisano *et al.*[12] whereas *per*-*O*-acetylated β-*C*-glucopyranosyl ketone was prepared following the procedure described by Llantén *et al.*[25]

Lipophilicity of compounds **1**–**19** was estimated by ChemBioDraw Ultra 13.0 software using the logarithm of the partition coefficient for *n*-octanol/water (Log*P*) and listed in Table 1.

Syntheses

(3E,3′E)-4,4′-(5,5′,6,6′-tetrahydroxy-[1,1′-biphenyl]-3,3′-diyl)bis (but-3-en-2-one) (3)

To a solution of **2** (0.6 g, 1.57 mmol) in dichloromethane (20 mL) at −60 °C under nitrogen was added boron tribromide (1.71 g, 6.95 mmol) dropwise. The solution was stirred at −60 °C for 1 h, washed with water (100 mL) and extracted with ethyl acetate (2× 20 mL). The organic solution was dried over sodium sulphate, rotoevaporated and washed with dichloromethane (2×10 mL) to give **3** as a yellow solid. (0.47 g, 89%): mp = 220–222 °C; ^{1}H NMR (CD_3OD) δ 2.34 (s, 6H), 4.90 (bs, 4H), 6.58 (d, *J* = 16.4 Hz, 2H), 7.07 (d, *J* = 2.0 Hz, Ar, 2H), 7.11 (d, *J* = 2 Hz, Ar, 2H), 7.56 (d, *J* = 16.4 Hz, 2H); ^{13}C NMR (CD_3OD) δ 25.61, 112.16, 123.64, 124.41, 125.89, 125.92, 145.45, 146.19, 146.35, 200.15. Anal. Calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12; Found: C, 67.83; H, 5.16.

(3E,3′E)-4,4′-(5,5′-dimethoxy-6,6′-bis (((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)-[1,1′-biphenyl]-3,3′-diyl)bis (but-3-en-2-one) (4)

Compound **22** (1 g, 0.96 mmol) was stirred in sodium methoxide/ methanol solution (0.02 g, 0.38 mmol in 10 ml) for 10 min. The reaction mixture was neutralized using a Dowex Marathon C (H+ form), filtrated and concentrated *in vacuo* to obtain **4** as a yellow solid. (0.61 g, 90%): mp = 125–126 °C; $[\alpha]_D^{20}$ 10.7 (c 0.2, MeOH); ^{1}H NMR (CD_3OD) δ 2.36 (s, 6H), 3.10–3.17 (series of m, 4H), 3.23–3.34 (series of m, 4H), 3.61 (dd, *J* = 4.8, 11.6 Hz, 2H), 3.72 (dd, *J* = 2.0, 11.6 Hz, 2H), 3.93 (s, 6H), 5.23 (bs, 2H), 6.78 (d, *J* = 16.4 Hz, 2H), 7.29 (s, Ar, 2H), 7.47 (bs, Ar, 2H), 7.65 (d, *J* = 16.4 Hz, 2H); ^{13}C NMR δ (CD_3OD) 26.02, 55.42, 60.94, 69.91, 74.19, 76.28, 76.93, 101.55, 111.22, 125.54, 125.86, 129.91, 144.38, 144.54, 152.30, 163.12, 200.18. Anal. Calcd. for $C_{34}H_{42}O_{16}$: C, 57.79; H, 5.99; Found: C, 57.80; H, 5.96.

(5,5′,6,6′-tetramethoxy-[1,1′-biphenyl]-3,3′-diyl)bis (1-((2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)but-3-en-2-one) (5)

Compound **24** (0.17 g, 0.15 mmol) was stirred in sodium methoxide/methanol solution (0.003 g, 0.06 mmol in 10 ml) for 10 min. The reaction mixture was neutralized using a Dowex Marathon C (H + form), filtrated and concentrated *in vacuo* to obtain **5** as a brown solid. (0.11 g, 95%): mp = 140–141 °C; $[\alpha]_D^{20}$ 19.1 (c 0.25, MeOH); ^{1}H NMR (CD_3OD) δ 2.88 (dd, *J* = 9.2, 16.4 Hz, 2H), 3.09–3.18 (series of m, 4H), 3.22 (m, 2H), 3.36 (m, 2H), 3.58–3.64 (series of m, 10H), 3.74 (m, 4H), 3.95 (s, 6H), 6.85 (d, *J* = 16.0 Hz, 2H), 7.10 (s, Ar, 2H), 7.32 (s, Ar, 2H), 7.62 (d, *J* = 16.0 Hz, 2H); ^{13}C NMR δ (CD_3OD) 42.96, 55.16, 59.80, 61.31, 70.21, 73.72, 76.08, 78.25, 80.15, 111.12, 123.93, 125.41, 130.16, 132.41, 143.36, 148.92, 153.01, 199.80.Anal. Calcd. for $C_{36}H_{46}O_{16}$: C, 58.85; H, 6.31; Found: C, 58.90; H, 6.37.

3,3′-dimethoxy-5,5′-bis ((E)-3-oxobut-1-en-1-yl)-[1,1′-biphenyl]-2,2′-diyl dimethyl dicarbonate (6)

To a solution of **2** (0.9 g, 2.35 mmol) and triethylamine (0.51 g, 5.04 mmol) in dry dichloromethane (10 mL) methyl chloroformate (0.55 g, 5.92 mmol) was added dropwise, at room temperature under nitrogen. The solution was stirred at room temperature for 1 h, washed with water (100 mL) and extracted with dichloromethane (2×20 mL). The crude was dried over sodium sulphate, rotoevaporated and washed with diethyl ether to give **6** as a white solid (1.01 g, 91%): mp = 182–183 °C; ^{1}H NMR δ 2.37 (s, 6H), 3.78 (s, 6H), 3.92 (s, 6H), 6.64 (d, *J* = 16.0 Hz, 2H), 7.06 (d, *J* = 2.0 Hz, Ar, 2H), 7.16 (d, *J* = 2.0 Hz, Ar, 2H), 7.45 (d, *J* = 16.0 Hz, 2H); ^{13}C NMR δ 27.62, 55.66, 56.25, 111.14, 123.07, 127.81, 130.75, 133.03, 139.47, 142.16, 151.97, 152.90, 197.92. Anal. Calcd. for $C_{26}H_{26}O_{10}$: C, 62.65; H, 5.26; Found: C, 62.70; H, 5.24.

5,5′-((3,3′-dimethoxy-5,5′-bis ((E)-3-oxobut-1-en-1-yl)-[1,1′-biphenyl]-2,2′-diyl)bis(oxy))bis (5-oxopentanoic acid) (7)

To a solution of **2** (2 g, 5.23 mmol) and glutaric anhydride (1.32 g, 11.57 mmol) in tetrahydrofuran (100 mL) was added *N*, *N*-dimethylaminopyridine (DMAP) (0.25 g, 2.04 mmol) and trimethylamine (3 mL, 21.52 mmol). The solution was stirred at reflux for 12 h, washed with hydrochloric acid (10% solution) (100 mL) and extracted with ethyl acetate (2×20 mL). The crude was dried over sodium sulphate, rotoevaporated and purified by flash chromatography using a 1:1 mixture of petroleum: acetone as eluent, to give **7** as a yellow solid. (1.96 g, 61%): mp = 90–91 °C; ^{1}H NMR δ 1.88 (m, 4H), 2.28 (t, *J* = 7.6 Hz, 4H), 2.37 (s, 6H), 2.43 (t, *J* = 6.8 Hz, 4H), 3.87 (s, 6H), 6.68 (d, *J* = 16.0 Hz, 2H), 7.03 (d, *J* = 2.0 Hz, Ar, 2H), 7.15 (d, *J* = 2.0 Hz, Ar, 2H), 7.45 (d, *J* = 16.0 Hz, 2H); ^{13}C NMR δ 19.72, 27.57, 32.46, 32.63, 56.10, 110.89, 123.03, 127.64, 131.38, 132.88, 139.27, 142.45, 151.70, 171.15, 178.37, 198.55.Anal. Calcd. for $C_{32}H_{43}O_{12}$: C, 62.95; H, 5.61; Found: C, 62.73; H, 5.64.

3,3′-dimethoxy-5,5′-bis ((E)-3-oxobut-1-en-1-yl)-[1,1′-biphenyl]-2,2′-diyl dioctanoate (8)

To a solution of **2** (0.9 g, 2.35 mmol) and triethylamine (0.51 g, 5.04 mmol) in dry dichloromethane (10 mL) octanoyl chloride (0.96 g, 5.92 mmol) was added dropwise, at room temperature under nitrogen. The solution was stirred at room temperature for 4 h, washed with water (100 mL) and extracted with dichloromethane (2×20 mL). The organic solution was dried over sodium sulphate, rotoevaporated and purified by flash chromatography using a 2:1 mixture of petroleum : ethyl acetate, as eluent, to give **8** as a white solid (1.31 g, 90%): mp = 112–113 °C; ^{1}H NMR δ 0.87 (t, *J* = 6.8 Hz, 6H), 1.21–1.27 (series of m, 16.0 Hz), 1.52 (m, 4H), 2.35 (t, *J* = 7.6 Hz, 4H), 2.38 (s, 6H), 3.89 (s, 6H), 6.67 (d, *J* = 16.0 Hz, 2H), 7.05 (d, *J* = 2.0 Hz, Ar, 2H), 7.15 (d, *J* = 2 Hz, Ar, 2H), 7.47 (d, *J* = 16.0 Hz, 2H); ^{13}C NMR δ 14.06, 22.59, 24.85, 27.59, 28.79, 28.92, 31.62, 33.79, 56.10, 110.72, 123.11, 127.51, 131.64, 132.63, 139.61, 142.33, 151.87, 171.07, 197.97.Anal. Calcd. for $C_{38}H_{50}O_8$: C, 71.90; H, 7.94; Found: C, 71.79; H, 7.84.

O,O′-(3,3′-dimethoxy-5,5′-bis ((E)-3-oxobut-1-en-1-yl)-[1,1′-biphenyl]-2,2′-diyl)diethyl disuccinate (9)

To a solution of **2** (0.35 g, 0.92 mmol) and triethylamine (0.21 g, 2.07 mmol) in dry dichloromethane (8 mL), ethyl-3-(chloroformyl) propionate (1.2 g, 7.29 mmol) was added, at room temperature under nitrogen. The solution was stirred at reflux for 4 days, washed with water (100 mL) and extracted with dichloromethane (2× 20 mL). The crude was dried over sodium sulphate, rotoevaporated and purified by flash chromatography using a 1:1 mixture of petroleum : ethyl acetate, as eluent, to give **9** as a white solid (0.45 g, 85%): mp = 97–98 °C; ^{1}H NMR δ 1.21 (t, *J* = 6.8 Hz, 6H), 2.37 (s, 6H), 2.55 (t, *J* = 6.8 Hz, 4H), 2.70 (t, *J* = 6.8 Hz, 4H), 3.88 (s, 6H), 4.10 (q, *J* = 6.8 Hz, 4H), 6.66 (d, *J* = 16.0 Hz, 2H), 7.01 (d, *J* = 2.0 Hz, Ar, 2H), 7.15 (d, *J* = 2.0 Hz, Ar, 2H), 7.48 (d, *J* = 16.0 Hz, 2H); ^{13}C NMR δ 14.16, 27.52, 28.67, 28.96, 56.19, 60.66, 110.67, 122.26, 127:67,

131.19, 132.89, 139.29, 142.44, 151.79, 171.77, 198.15.Anal. Calcd. for $C_{32}H_{38}O_{10}$: C, 65.97; H, 6.57; Found: C, 66.02; H, 6.54.

(3E,3′E)-4,4′-(5,5′-dimethoxy-6,6′-bis (octadecyloxy)-[1,1′-biphenyl]-3,3′-diyl)bis(but-3-en-2-one) (10)

To a solution of compound **2** (0.2 g, 0.52 mmol) and potassium carbonate (0.28 g, 2.13 mmol) and 18-crown-6 ether (0.014 gr. 0.05 mmol) in dry acetonitrile (15 mL) 1-bromooctadecane (0.7 g, 2.15 mmol) was added dropwise at room temperature under nitrogen. The solution was stirred at reflux for 12 h, acidified with hydrochloric acid (10% solution), extracted with diethyl ether (2× 50 mL) and dried over sodium sulphate. The crude product was then purified by silica chromatography using a 2:1 mixture of petroleum : acetone, as eluent, to give **10** as a yellow solid (0.37 g. 80%): mp = 51–52 °C; ^{1}H NMR δ 0.87 (t, *J* = 6.4 Hz, 6H), 1.07–1.27 (series of m, 64 H), 2.35 (s, 6H), 3.80 (t, *J* = 6.4 Hz, 4H), 3.91 (s. 6H), 6.64 (d, *J* = 16.4 Hz, 2H), 7.08 (d, *J* = 2.0 Hz, Ar, 2H), 7.11 (d, *J* = 2.0 Hz, Ar, 2H), 7.44 (d, *J* = 16.4 Hz, 2H). ^{13}C NMR δ 14.06, 22.65, 25.74, 27.43, 29.30, 29.32, 29.60, 29.61, 29.63, 29.68, 30.06, 31.89, 55.92, 73.42, 110.72, 124.57, 126.23, 129.39, 132.67, 143.17, 148.62, 153.25, 198.06. Anal. Calcd. for $C_{58}H_{94}O_6$: C, 78.50; H, 10.68; Found: C, 78.82; H, 10.72.

(3E,3′E)-4,4′-(6,6′-bis (allyloxy)-5,5′-dimethoxy-[1,1′-biphenyl]-3,3′-diyl)bis (but-3-en-2-one) (11)

Starting from dehydrodivanillin, compound 20:

To a solution of **20** (0.2 g, 0.52 mmol) in acetone (10 mL), aqueous 1 N solution of sodium hydroxide (1.6 mL) was added and the mixture was stirred at room temperature for 12 h. Water was added and, acidified with hydrochloric acid (10% solution) and extracted with dichloromethane. The crude material was purified by flash chromatography using a 3:1 mixture of petroleum : ethyl acetate, as eluent, to give **11** as a yellow solid (0,15 g, 65%): mp 116–118 °C; ^{1}H NMR δ 2.31 (s, 6H), 3.88 (s, 6H), 4.35 (m, 4H), 5.01 (m, 4H), 5.71 (m, 2H), 6.61 (d, *J* = 16.0 Hz, 2H), 7.05 (d, *J* = 2.0 Hz, Ar, 2H), 7.07 (d, *J* = 2.0 Hz, Ar, 2H), 7.42 (d, *J* = 16.0 Hz, 2H);^{13}C NMR δ 27.46, 55.92, 74.05, 110.74, 117.26, 124.31, 126.34, 129.68, 132.60, 133.96, 143.11, 147.98, 153.05, 198.21. Anal. Calcd for $C_{28}H_{30}O_6$ C, 72,71; H, 6,54; Found: C, 72.21; H, 6.82.

Starting from compound 2

To a solution of compound **2** (0.15 g, 0.39 mmol) and potassium carbonate (0.12 g, 0.87 mmol) in dry acetone (10 mL), allyl bromide (0.1 g, 0.87 mmol) was added dropwise at room temperature under nitrogen. The solution was stirred at reflux for 12 h, acidified with hydrochloric acid (10% solution), extracted with dichloromethane (2×50 mL) and dried over sodium sulphate. The crude product was then purified by silica chromatography using a 2:1 mixture of petroleum : ethyl acetate, as eluent, to give **11** (0.12 g, 65%).

(3E,3′E)-4,4′-(5,5′-dimethoxy-6,6′-bis((3-methylbut-2-en-1-yl) oxy)-[1,1′-biphenyl]-3,3′-diyl)bis(but-3-en-2-one) (12)

To a solution of compound **2** (1 g, 2.64 mmol) and potassium carbonate (1.1 g, 7.8 mmol) in dry acetone (100 mL), 3,3′-dimethylallyl bromide (1 g, 6.71 mmol) was added dropwise at room temperature under nitrogen. The solution was stirred at reflux for 12 h, acidified with hydrochloric acid (10% solution), extracted with dichloromethane (2×50 mL) and dried over sodium sulphate. The crude product was then purified by silica chromatography using a 2:1 mixture of petroleum : ethyl acetate, as eluent, to give **12** as a yellow oil (0.8 g, 60%); ^{1}H NMR δ 1.41 (s, 6H), 1.57 (s, 6H), 2.31 (s, 6H), 3.89 (s, 6H), 4.31 (d, *J* = 7.2 Hz, 2H), 5.17 (t, *J* = 1.6 Hz, 2H), 6.61 (d, *J* = 16.4 Hz, 2H), 7.06 (d, *J* = 2.0, Hz, Ar, 2H); 7.08 (d, *J* = 2.0, Hz, Ar, 2H); 7.43 (d, *J* = 16.4 Hz, 2H), ^{13}C NMR δ 17.62, 25.72, 27.45, 55.94, 69.37, 110.57, 120.27, 124.60, 126.19, 129.49, 132.99, 138.20, 143.29, 148.14, 153.33, 198.22, Anal. Calcd for $C_{32}H_{38}O_6$: C, 74.11; H, 7.39; Found: C, 74.69; H, 7.34.

(3E,3′E)-4,4′-(6,6′-bis(((E)-3,7-dimethylocta-2,6-dien-1-yl) oxy)-5,5′-dimethoxy-[1,1′-biphenyl]-3,3′-diyl)bis(but-3-en-2-one) (13)

To a solution of **21** (0.4 g, 0.75 mmol) in acetone (8 mL) aqueous 1 N solution of sodium hydroxide (0.5 mL) was added and the mixture was stirred at room temperature for 12 h. Water was added and the solution was then acidified with hydrochloric acid (10% solution) and extracted with dichloromethane. The crude material was purified by flash chromatography using a 3:1 mixture of petroleum : ethyl acetate, as eluent, to give **13** as a yellow solid (0,04 g, 33%): mp 166–168 °C; ^{1}H NMR δ 1.45 (s, 6H), 1.54 (s, 6H), 1.66 (s, 6H), 1.86–1.98 (series of m, 8H), 2.35 (s, 6H), 3.93 (s, 6H), 4.38 (d, *J* = 6.8 Hz, 4H), 5.01 (m, 2H), 5.21 (m, 2H), 6.63 (d, *J* = 16.4 Hz, 2H), 7.09 (d, *J* = 2.0 Hz, Ar, 2H), 7.12 (d, *J* = 2.0 Hz, Ar, 2H), 7.46 (d, *J* = 16.4 Hz, 2H); ^{13}C NMR δ 16.13, 17.63, 25.65, 26.26, 27.44, 39.52, 55.96, 69.53, 110.54, 119.89, 123.87, 124.61, 126.24, 129.52, 131.59, 133.02, 141.43, 143.28, 148.29, 153.35, 198.26. Anal. Calcd for $C_{42}H_{54}O_6$ C, 77,03; H, 8,31; Found: C, 77.21; H, 8.52.

(E)-4-(4-(((2S,4R,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)-3-methoxyphenyl)but-3-en-2-one (18)

Compound **25** (1 g, 1.93 mmol) was stirred in sodium methoxide/ methanol solution (0.02 g, 0.38 mmol in 20 ml) for 10 min. The reaction mixture was neutralized using a Dowex Marathon C (H+ form), filtrated and concentrated *in vacuo* to obtain **18** as a yellow solid. (0.61 g, 95%): mp = 210–211 °C; $[\alpha]_D^{20}$ 23.1 (c 0.5, MeOH); ^{1}H NMR (CD_3OD) δ 2.36 (s, 3H), 3.30–3.65 (series of m, 4H), 3.71 (dd, *J* = 5.2, 17.2 Hz, 1H), 3.86 (m, 1H), 3.90 (s, 3H), 4.90 (s, 4H), 4.98 (d, *J* = 7.2 Hz, 1H), 6.70 (d, *J* = 16 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, Ar, 1H), 7.21 (dd, *J* = 1.6, 8.4 Hz, Ar, 1H), 7.26 (d, *J* = 1.6 Hz, Ar, 1H), 7.59 (d, *J* = 16 Hz, 1H); ^{13}C NMR δ (CD_3OD) 25.86, 55.36, 61.07, 69.87, 73.39, 76.45, 76.89, 100.76, 111.28, 116.01, 122.50, 125.14, 129.18, 144.19, 148.93, 149.65, 199.91.Anal. Calcd. for $C_{17}H_{22}O_7$: C, 60.35; H, 6.55; Found: C, 60.39; H, 6.66

(E)-4-(3,4-dimeth-oxyphenyl)-1-((2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymeth-yl)tetrahydro-2H-pyran-2-yl)but-3-en-2-one (19)

Compound **26** (0.83 g, 1.54 mmol) was stirred in sodium methoxide/methanol solution (0.017 g, 0.32 mmol in 10 ml) for 10 min. The reaction mixture was neutralized using a Dowex Marathon C (H + form), filtrated and concentrated *in vacuo* to obtain **19** as a brown solid. (0.54 g, 95%): mp = 152–154 °C; $[\alpha]_D^{20}$ −13.1 (c 0.25, MeOH); ^{1}H NMR (CD_3OD) δ 2.88 (dd, *J* = 9.2, 15.6 Hz, 1H), 3.12 (dd, *J* = 2.4, 15.6 Hz, 1H), 3.16 (d, *J* = 9.6, Hz, 1H), 3.24 (m, 1H), 3.33 (m, 2H), 3.60 (dd, *J* = 5.2, 12 Hz, 1H), 3.75 (m, 2H), 3.86 (s, 3H), 3.87 (s, 3H), 6.81 (d, *J* = 16.0 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, Ar, 1H), 7.21 (dd, *J* = 2.0, 8.4 Hz, Ar, 1H), 7.25 (d, *J* = 2.0 Hz, 1H), 7.61 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR δ (CD_3OD) 42.73, 48.34, 54.91, 61.20, 70.11, 73.58, 76.01, 78.15, 80.05, 110.08, 111.05, 123.11, 123.81, 127.37, 143.85, 149.13,

151.50, 199.61.Anal. Calcd. for $C_{18}H_{24}O_8$: C, 58.69; H, 6.57; Found: C, 58.80; H, 6.59.

6,6′-Bis-allyloxy-5,5′-dimethoxy-biphenyl-3,3′-dicarbaldehyde (20)

To a solution of dehydrodivanillin (0.68 g, 2.25 mmol) and potassium carbonate (0.8 g, 5.78 mmol) in dry acetone (70 mL), allyl bromide (0.7 g, 5.78 mmol) was added dropwise, at room temperature under nitrogen. The solution was stirred at reflux for 12 h, washed with water (100 mL) and extracted with ether (2×20 mL). The crude, was dried over sodium sulphate to give **20** as a yellow solid (0.4 g, 50%): mp = 86–88 °C; ^{1}H NMR δ 3.94 (s, 6H), 4.46 (d, *J* = 5.6 Hz, 4H), 4.96–5.00 (series of m. 4H), 5.19–5.22 (series of m. 2H), 7.42 (d, *J* = 2.0 Hz, Ar, 2H), 7.47 (d, *J* = 2.0 Hz, Ar, 2H), 9.87 (s, 2H); ^{13}C NMR δ 55.99, 69.61, 109.68, 123.76, 128.27, 131.81, 132.49, 141.84, 151.59, 153.69, 191.03. Anal. Calcd for $C_{22}H_{22}O_6$: C, 69.10; H, 5.80; Found: C, 69.15; H, 5.92.

6,6′-bis(((E)-3,7-dimethylocta-2,6-dien-1-yl) oxy)-5,5′-dimethoxy-[1,1′-biphenyl]-3,3′-dicarbaldehyde (21)

To a solution of dehydrodivanillin (0.52 g, 1.42 mmol) and potassium carbonate (0.59 g, 4.26 mmol) in dry acetone (50 mL) dropwise geranyl bromide (1.0 g, 4.55 mmol) was added, at room temperature under nitrogen. The solution was stirred at reflux for 12 h, washed with water (100 mL) and extracted with diethyl ether (2× 20 mL). The crude, was dried over sodium sulphate and then purified by silica chromatography using a 2:1 mixture of petroleum : ethyl acetate, as eluent, to give **21** as a yellow oil (0.71 g, 72%): ^{1}H NMR δ 1.45 (s, 6H), 1.54 (s, 6H), 1.63 (s, 6H), 1.88–1.97 (series of m, 8H), 3.96 (s, 6H), 4.46 (d, *J* = 7.2 Hz, 4H), 4.96–5.00 (series of m, 2H), 5.19–5.22 (series of m, 2H); 7.42 (d, *J* = 2.0 Hz, Ar, 2H), 7.47 (d, *J* = 2.0 Hz, Ar, 2H), 9.87 (s, 2H); ^{13}C NMR δ 16.14, 17.62, 25.62, 26.21, 39.47, 55.99, 69.61, 109.68, 119.63, 123.76, 128.27, 131.63, 131.81, 132.49, 141.84, 151.59, 153.69, 191.03. Anal. Calcd. for $C_{36}H_{46}O_6$: C, 75,23; H, 8,07; Found: C, 75.69; H, 8.34.

(3E,3′E)-4,4′-(5,5′-dimethoxy-6,6′-bis (((2S,3R,4S,5S,6R)-3,4,5-triacetoxy-6-(acetoxymethyl) tetrahydro-2H-pyran-2-yl)oxy)-[1,1′-biphenyl]-3,3′-diyl)bis (but-3-en-2-one (22)

Silver carbonate (3.52 g, 12.8 mmol) was added to a stirred solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (3 g, 7.29 mmol) and **2** (0.62 g, 1.62 mmol) in pyridine (30 mL) at room temperature under shaded conditions. After stirring for 12 h, the solution was diluted with ethyl acetate (30 mL), washed with hydrochloric acid (10% solution) (100 mL), dried over anhydrous sodium sulphate and rotoevaporated. The residue was purified by flash chromatography using a 1:2 mixture of petroleum : ethyl acetate as eluent, to give **22** as a yellow solid. (1.01 g, 60%): mp = 187–188 °C; $[\alpha]_D^{20}$ −11.5 (c 0.5, $CHCl_3$); ^{1}H NMR δ 1.92 (s, 6H), 1.93 (s, 6H), 1.95 (s, 6H), 1.96 (s, 6H), 2.34 (s, 6H), 3.54 (m, 2H), 3.86, (m, 2H), 3.91 (s, 6H), 4.01 (m, 2H), 4.80–5.15 (series of m, 8H), 6.61 (d, *J* = 16 Hz, 2H), 6.97 (bs, Ar, 2H), 7.10 (d, *J* = 2 Hz, Ar, 2H), 7.39 (d, *J* = 16 Hz, 2H); ^{13}C NMR δ 19.62, 19.64, 19.76, 19.81, 26.25, 55.88, 61.65, 68.32, 70.14, 71.45, 71.61, 72.46, 100.23, 111.35, 124.8, 126.92, 131.02, 142.36, 144.47, 152.47, 168.87, 169.06, 169.37, 169.76, 197.01; Anal. Calcd. for $C_{50}H_{58}O_{24}$: C, 57.58; H, 5.61; Found: C, 57.60; H, 5.66.

(S,R,S,R,R,3E,3′E)-4,4′-(5,5′,6,6′-tetramethoxy-[1,1′-biphenyl]-3,3′-diyl)bis (1-((2S,3R,4R,5S,6R)-3,4,5-triacetoxy-6-(acetoxymethyl) tetrahydro-2H-pyran-2-yl)but-3-en-2-one) (24)

To a solution of *per-O*-acetylated β-*C*-glucopyranosyl ketone (0.51 g, 1.32 mmol) and 2,2′,3,3′-tetramethoxy-5,5′-diformyl-1,1′-biphenyl **23** (*O*Me-dehydrodivanillin) (0.2 g, 0.62 mmol) in 2 mL dry dichloromethane, pyrrolidine (0.018 g, 0.24 mmol) was added under nitrogen. The reaction was stirred at room temperature until the starting material was consumed as evidenced by TLC (48 h). The reaction mixture was neutralized with hydrochloric acid (10% solution) and the residue diluted in dichloromethane. The organic extracts were combined, dried over sodium sulphate, filtered and evaporated. The crude product was purified by column chromatography using a 2:3 mixture of petroleum : acetone as eluent, to give **24** as a yellow solid. (0.49 g, 77%): mp = 130–131 °C; $[\alpha]_D^{20}$ −16.3 (c 0.1, $CHCl_3$); ^{1}H NMR δ 1.96 (s, 6H), 1.97 (s, 6H), 1.98 (s, 6H), 1.99 (s, 6H), 2.63 (dd, *J* = 3.2, 16.8 Hz, 2H), 2.98 (dd, *J* = 8.4, 16.8 Hz, 2H), 3.69 (s, 6H), 3.70 (m, 2H), 3.92 (s, 6H), 3.98 (dd, *J* = 2.4, 12.4 Hz, 2H), 4.09 (m, 2H), 4.23 (dd, *J* = 4.8, 12.4 Hz, 2H), 4.94 (t, *J* = 9.2 Hz, 2H), 5.04 (t, *J* = 9.2 Hz, 2H), 5.19 (t, *J* = 9.2 Hz, 2H), 6.62 (d, *J* = 16 Hz, 2H), 7.04 (d, *J* = 2.0 Hz, Ar, 2H), 7.19 (d, *J* = 2 Hz, Ar, 2H), 7.47 (d, *J* = 16 Hz, 2H); ^{13}C NMR δ 20.59, 20.61, 20.65, 20.71, 42.49, 55.92, 60.86, 61.98, 68.44, 71.64, 74.07, 74.13, 75.68, 111.02, 124.19, 125.52, 129.59, 132.29, 143.35, 149.23, 153.01, 169.56, 169.97, 170.23, 170.61, 195.95; Anal. Calcd. for $C_{52}H_{62}O_{24}$: C, 58.31; H, 5.84; Found: C, 58.37; H, 5.86.

(2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-(2-methoxy-4-((E)-3-oxobut-1-en-1-yl)phenoxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (25)

Silver carbonate (4.5 g, 16.31 mmol) was added to a stirred solution of tetra-*O*-acetylglucopyranosyl bromide (3.67 g, 8.91 mmol) and dehydrozingerone (0.78 g, 4.05 mmol) in pyridine (30 mL) at room temperature under shaded conditions. After stirring for 18 h, the solution was diluted with ethyl acetate (30 mL), washed with hydrochloric acid (10% solution) dried over anhydrous sodium sulphate and rotoevaporated. The residue was purified by flash chromatography using a 1:1 mixture of petroleum : ethyl acetate as eluent, to give **25** as a brown solid. (1.88 g, 90%): mp = 189–190 °C; $[\alpha]_D^{20}$ −15.3 (c 0.5, $CHCl_3$); ^{1}H NMR δ 1.93 (s, 3H), 1.94 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.33 (s, 3H), 3.70 (m, 1H), 3.80 (s, 3H), 4.11 (dd, *J* = 2, 12 Hz, 1H), 4.24 (dd, *J* = 3.6, 12 Hz, 1H), 4.90–5.25 (series of m, 4H), 6.56 (d, *J* = 16 Hz, 1H), 6.98–7.08 (series of m, Ar, 3H), 7.40 (d, *J* = 2 Hz, Ar, 1H); ^{13}C NMR δ 20.51, 20.54, 20.62, 20.95, 27.38, 56.01, 61.83, 68.25, 71.04, 72.02, 72.41, 100.12, 111.41, 119.32, 121.93, 126.49, 130.81, 142.79, 147.95, 150.71, 169.24, 169.35, 170.15, 170.46, 198.17; Anal. Calcd. for $C_{25}H_{30}O_{12}$: C, 57.47; H, 5.79; Found: C, 57.42; H, 5.76.

(2R,3R,4R,5S,6S)-2-(acetoxymethyl)-6-((E)-4-(3,4-dimethoxyphenyl)-2-oxobut-3-en-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (26)

To a solution of *per-O*-acetylated β-*C*-glucopyranosyl ketone (1.35 g, 3.47 mmol) and veratraldehyde (0.57 g, 3.47 mmol) in 10 mL dry dichloromethane, pyrrolidine (0.05 g, 0.64 mmol) was added under nitrogen. The reaction was stirred at room temperature until the starting material was consumed as evidenced by TLC (72 h). The reaction mixture was neutralized with hydrochloric acid (10% solution) and the residue diluted in dichloromethane. The organic extracts were combined, dried over sodium sulphate, filtered and evaporated. The crude product was purified by column chromatography using a 1:1 mixture of petroleum : acetone as eluent, to give

26 as a light yellow solid. (1.3 g, 70%): mp = 146–148 °C; $[\alpha]_D^{20}$ −25.3 (c 0.1, $CHCl_3$); ^{1}H NMR δ 1.91 (s, 3H), 1.92 (s, 3H), 1.94 (s, 3H), 1.95 (s, 3H), 2.58 (dd, *J* = 2.8, 16 Hz, 1H), 2.83 (dd, *J* = 8, 16 Hz, 1H), 3.68 (m, 1H), 3.83 (s, 3H), 3.85 (s, 3H), 3.93 (dd, *J* = 2, 12 Hz, 1H), 4.01 (m, 1H), 4.17 (dd, *J* = 5.2, 12.4 Hz, 1H), 4.89 (t, *J* = 9.6 Hz, 1H), 5.04 (t, *J* = 9.6 Hz, 1H), 5.19 (t, *J* = 9.6 Hz, 1H), 6.54 (d, *J* = 16 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, Ar, 1H), 6.99 (d, *J* = 2 Hz, Ar, 1H), 7.05 (dd, *J* = 2, 8.4 Hz, 1H), 7.41 (d, *J* = 16 Hz, 1H); ^{13}C NMR δ 20.09, 21.04, 42.81, 56.22, 56.36, 62.11, 68.85, 72.13, 74.92, 75.68, 75.89, 110.14, 111.6, 123.4, 124.33, 127.82, 143.81, 149.81, 152.13, 170.24, 171.85, 196.16; Anal. Calcd. for $C_{26}H_{32}O_{12}$: C, 58.20; H, 6.01; Found: C, 58.17; H, 6.06.

Biological procedures

Cell lines and cell cultures

Malignant melanoma cell lines used in this study [LCP-mel (LCP), LCM-mel (LCM), CN-mel (CN) and M14] were primary tumor cell lines derived from tumor biopsy samples of malignant melanoma patients. They have been all kindly provided by the Institute Dermopatico dell'Immacolata (IDI) in Rome. Cell lines had been all previously genetically characterized.[38] LCP was derived from a primitive tumor, while LCM from a lymph node metastasis of the same melanoma patient. Both cell lines carry a $BRAF^{V600R}$ mutation and a $p16^{CD4N2A}$ exon 2 deletion (LCP) or a $p16^{CD4N2A}$ exons 1–2 deletion (LCM). CN cell line was derived from a melanoma lymph node metastasis and carries a $NRAS^{Q61R}$ mutation. M14 cell line was derived from a melanoma cutaneous metastasis, it was established in 1975[29] and it is also commercially available (ATCC). It carries a $BRAF^{V600E}$ mutation and an impaired locus CDKN2A ($p16^{455insC/del26\ IVS1+2T>C}$).

A healthy donor human fibroblasts cell line (BJ) was used as normal cells control. It has been purchased from ATCC (ATCC® CRL-2522).

Melanoma cells and fibroblasts were both grown in RPMI culture medium with stable glutamine, supplemented with 10% Fetal Bovine Serum (FBS) and penicillin/streptomycin (1 U/mL) (complete medium) in a humidified atmosphere with 5% CO_2, at 37 °C.

Cell proliferation assay

Cell proliferation assays were carried out for the 18 compounds, following the procedure previously described.[39] Briefly, cells were plated in 96-well plates in complete medium at the density of 3.0×10^3 cells per well and incubated in a humidified atmosphere with 5% CO_2, at 37 °C. After 24 h, medium was removed and replaced on days 1 and 3 by only fresh medium (control) or by medium supplemented with increasing concentrations of the freshly prepared solution of compounds **2**–**19**). After treatments cell viability was determined on day 2 (24 h) or day 4 (72 h) by MTT test.[40] Briefly 20 μL of MTT (5 mg/mL) were added to each well. After an incubation of 3 h at 37 °C the medium was removed and formazan crystals were dissolved with 100 μL DMSO per well, for 10 min at room temperature with gentle mix. Absorbance was measured at 570 nm using a microplate reader (Sunrise™ Absorbance Reader – TECAN). Percentage of cell growth was calculated by normalizing the absorbance of treated cells to that of the corresponding control. All the experiments were performed in triplicate and repeated at least three times.

Statistical analysis

Relative IC_{50} values were determined by nonlinear regression of variable slope (four parameters) model by Graph Pad Prism version 7.00 for Windows, Graph Pad Software, La Jolla California USA, www.graphpad.com. The average IC_{50} values, ± standard deviation (SD) were calculated based on the results obtained from three independent experiments of proliferation assay. The statistical significance of differential findings between experimental groups and controls was determined by Student's t-test. These findings were considered significant if P values were <0.001.

Supporting information

The Supporting Information contains ^{1}H NMR and ^{13}C NMR spectra of compounds **3**–**13** and **18**, **19**.

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Conflict of Interest

The authors declare no conflict of interest.

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