Synthesis of Hydroxylated Biphenyls Derivatives Bearing an α,β-Unsaturated Ketone as Lead Structure for the Development of New Drug Candidates Against Malignant Melanoma

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Abstract: A small collection of C_2-symmetry hydroxylated biphenyls derivatives featured with a α,β-unsaturated ketone as lead structure was prepared and the capability of such compounds to act as antiproliferative agents against four human malignant melanoma cell lines was assayed. The prodrug approach was applied in order to improve delivery of compounds into the cell by modulation of the phenolic-OH protective group. The hydroxylated biphenyl structure bearing an α,β-unsaturated ketone and a phenolic-*O*-prenylated chain would facilitated the delivery of the molecule and interactions with the biological targets. Four compounds showed antiproliferative activity resulting in IC_{50} value in the range 1.2 - 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

44 pharmacophore that is well recognised in the design of new antimelanoma drugs in virtue of the emerging role of this moiety in
45 interacting with nucleophiles present in the cancer cells through a Michael addition reaction.[10, 11]
46 Analogues of curcumin were prepared by independent research groups and the antiproliferative and apoptotic activities
47 against a wide set of malignancies were assayed identifying lead structures.[7] Most of the curcumin analogues presents
48 modifications to the flexible unsaturated 1,3-keto-enol moiety which is believed to be responsible for the poor physiological
49 stability, the poor adsorption and the fast metabolism of the molecule.
50 Our group prepared an analogue of curcumin, compound **1** (Figure 1) that showed antiproliferative and pro apoptotic
51 activities against MM and neuroblastoma cells that were ten times stronger than those of curcumin, displaying high selectivity
52 toward cancer cells.[12, 13] Intracellular concentration of compound **1** reached 600 pmoles/10^6 cells (about 270 nM) after two hours
53 after treatment, then degradation of the compound occurred.[13]
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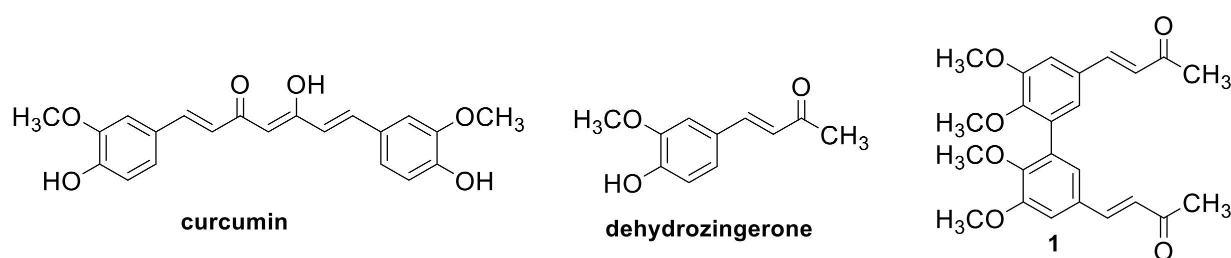

55
56 **Figure 1.** Chemical structures of curcumin, dehydrozingerone and compound **1**
57
58 The mechanism underlying the cell growth inhibitory action of compound **1** (compound **D6** in the references) was well
59 investigated.[12-14] In comparison with other analogues of curcumin, the novelty of the molecular framework of compound **1** is a
60 combination of a hydroxylated biphenyl unit and an α,β-unsaturated carbonyl group with terminal methyl group that reminds the
61 curcumin structure split in two parts and linked to the aromatic rings. The preparation of the molecular framework relied on a
62 Claisen-Schmidt condensation between a ketone and an aldehyde under basic conditions.
63 Hydroxylated biphenyl unit is embedded in many structures of bioactive natural products, some of them of high biological
64 relevance like ellagitannins and vancomycin, others, structurally less sophisticated, are natural occurring dimers of 4-substituted-
65 2-methoxy phenols.[15] Hydroxylated biphenyls derivatives have been considered privileged structures due to their unique
66 pharmacophore able of providing useful ligands for more than one type of receptor.[15] The scaffold shows structural characteristics
67 such as flexibility combined with partial rigidity, tuneable and fully adaptable in virtue of the presence of certain functional groups.[16]
68 As result, hydroxylated biphenyl provides an ideal molecular framework for structural modifications in the development of drug
69 candidates.
70 Structurally, compound **1**, can be also related to two molecules of dehydrozingerone (Figure 1). Dehydrozingerone,
71 known as feruloylmethane, is isolated from rhizomes of ginger (*Zingiber officinale* Roscoe), identified as a half structural analogue
72 of curcumin and one of its degradation compounds at neutral and basic pH conditions.[17] Dehydrozingerone is stable in organic
73 and aqueous solutions and share many structural and pharmacological features with curcumin.[18] The interesting results achieved
74 with compound **1** against MM encouraged us to pursue further structural tuning through simple synthetic methods in order to
75 discover more efficient drug candidates. The substitution in compound **1** of the α,β-unsaturated ketone at 5,5' positions with an
76 unsaturated β-diketo enol ester gave comparable results to that of curcumin in term of 50% inhibitory concentration (IC_{50}) against
77 a set of MM cells and identified the α,β-unsaturated methyl ketone moiety of compound **1** as the most effective Michael acceptor
78 pharmacophore in the series of the compounds investigated.[19]
79 With the aim of searching for an effective drug-like Michael acceptor with potential antimelanoma activity, in the present
80 study, we prepared a collection of derivatives of compound **1**. All compounds possess a common α,β-unsaturated ketone moiety
81 bearing in a hydroxylated biphenyl structure in which the phenolic -OH group was substituted with functionalities able to modify
82 lipophilicity of the molecule with the aim to increase membrane permeability and bioavailability of the compound. The principal
83 aim of the work was to prepare compounds that share the same core structure (hydroxylated biphenyl) but differ in patterns of
84 substituents attached to the core structure. We applied the prodrug approach[20, 21], a successful tool in rational drug design, for
85 improving bioactivity of compounds by proper transformation of the phenolic -OH group with a functionality that would increase

86 bioavailability of the molecule or undergone *in vivo* biotransformation through chemical or enzymatic cleavage, thus modulating
87 pharmacokinetic properties and/or favouring the delivery of the active compound with a higher yield. Further, all compounds were
88 assayed *in vitro* on a set of human MM cell lines and their IC_{50} determined.

89

90

91 Results and discussion

92

93 Chemistry

94 We started from the experimental evidence that compound **1**, a tetra-OMe biphenyl (**D6** in references 12-14) showed
95 antiproliferative and apoptotic activity in malignant melanoma cells with negligible effect on normal cells.[13] A collection of eighteen
96 derivatives of **1**, compounds **2**-**19**, were prepared through straightforward synthetic procedures. Compounds **2**-**13** possess a
97 hydroxylated biphenyl bearing an α,β-unsaturated ketone as lead structure where phenolic –OH group was transformed with
98 aliphatic group/chain with the aim to improve antimelanoma activity in comparison with that assayed in compound **1** (Figure 2).
99 Compounds **14**-**19** are the corresponding monomers of hydroxylated biphenyls derivatives **1**, **4**, **5** and **11**-**13**. We applied the
100 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
101 transformed with a hydrolisable carrier (carrier-linker prodrug) as for compounds **4**, **6**, **7**, **9** and monomer **18** or with a functional
102 group that could provide synergistic action after hydrolysis (mutual prodrugs) as for compounds **8**.[22] Biphenols **2**, **3** and **5** and
103 monomer **19** were prepared as representative of compound **1** with increased hydrophilicity and biphenyl **10** as the most lipophilic
104 compound. Concerning oxo-prenylated ethers **11**-**13** and **15**-**17** we though to increase bioavailability and selectivity of the
105 compounds because oxo-prenylated phenols are able to interact with different and selected cell receptors and signal transductors
106 (ex. Ras proteins) accounting for their ability to modulate key metabolic processes in pathological disorders.[23]

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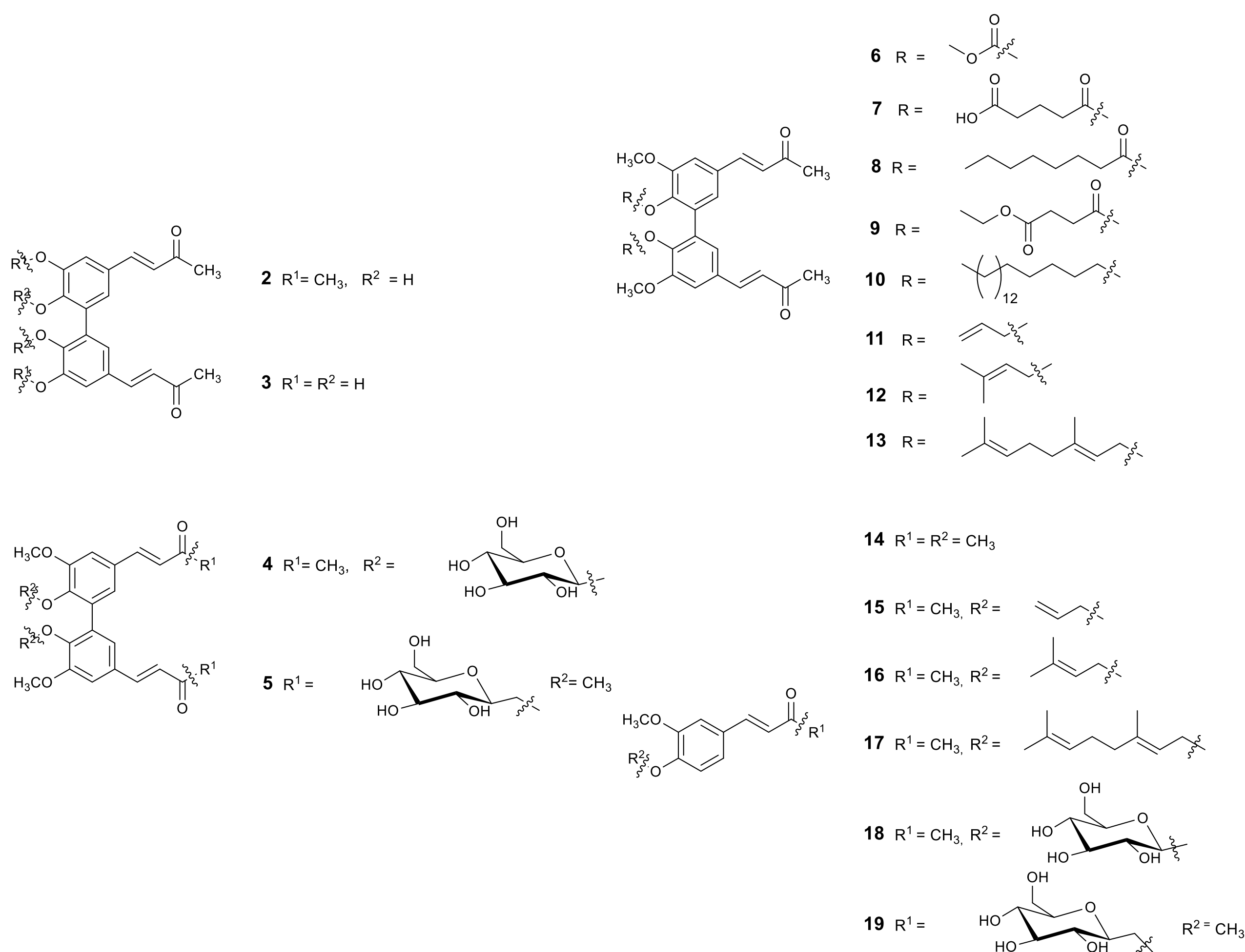
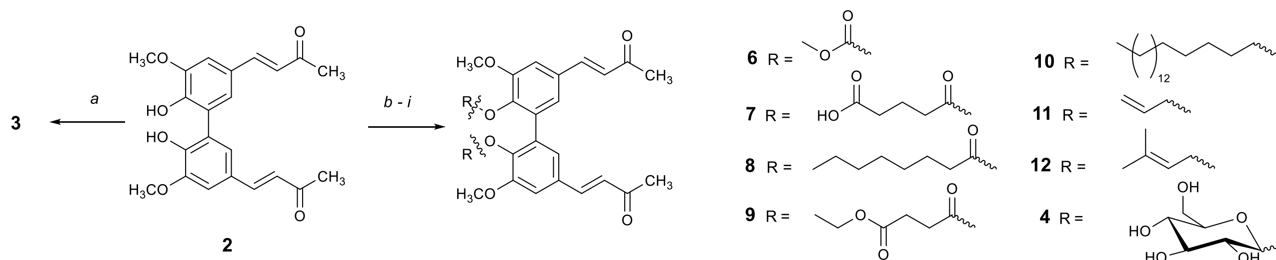


Figure 2. Chemical structures of compounds **2**-**19**.

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 –OH 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. Reagents and conditions: (*a*) compound **3**: BBr_3 in CH_2Cl_2, -60 °C for 1h; (*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'.

127 Catechol units were achieved from compound **2** with a large excess of equivalents of demethylating reagent at -60 °C
128 for 1 h giving compound **3** in 89% yield. The browning of compound **3** after few days from the preparation was likely a consequence
129 of the easy oxidation of the compound, therefore storage of compound **3** under nitrogen atmosphere was mandatory. While
130 compound **2**, C_2-dimer of dehydrozingerone, showed scarce solubility in water and in physiological solution at concentrations >
131 2mM, compounds **3** and **4** were completely soluble in water up to 30 mM, accordingly to their LogP (Table 1).
132

133

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

135

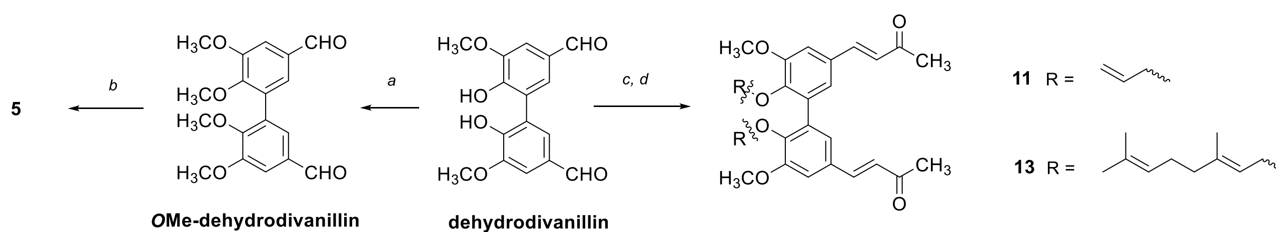
Compounds	LogP	Compounds	LogP	Compounds	LogP	Compounds	LogP
1	2.70	**6**	3.24	**11**	4.08	**16**	2.76
2	2.17	**7**	2.29	**12**	5.17	**17**	3.87
3	1.65	**8**	7.60	**13**	8.36	**18**	-0.57
4	-1.51	**9**	3.64	**14**	1.53	**19**	-0.93
5	-2.22	**10**	>8.50	**15**	2.22		

Then, we looked upon for molecular variations in developing a series of derivatives of **1** having different functional group/unit linked to the phenolic -OH 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 -OH 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 (LogP 2.29) than that of ester **9** (LogP 3.64) whereas ester **8** having a medium-chain fatty ester, is highly lipophilic (LogP 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 -OH 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*-acetilated-β-*C*-glucopyranosyl ketone in the presence of pyrrolidine as base and further deacetylation, gave compound **5** in 73% overall yield (Scheme 2).



Scheme 2.Reagents and conditions: (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'; (c) compound **11**: allyl bromide, K_2CO_3 in acetone, reflux, 12 h, then, after purification of the product, 1N 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, 1N NaOH solution in acetone at rt for 12 h.

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 –OH 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* biotransformation 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 -OH 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-

180 unsaturated methyl ketone moiety by Claisen-Schmidt condensation in the presence of acetone under basic conditions (Scheme
181 2). Monomers **14**-**17** were prepared with the same synthetic procedures described in literature for these compounds that entailed
182 protection of the phenolic -OH group of dehydrozingerone with the corresponding organohalide under basic conditions.[27, 28]
183 According to the reaction stoichiometry, monomers **18** and **19** were prepared with the same procedures applied for the synthesis
184 of the corresponding dimers **4** and **5**, respectively (Scheme 3).

185

H_3CO, HO, O, CH_3 — *a* → **18**

dehydrozingerone

H_3CO, H_3CO, CHO — *b* → **19**

veratraldehyde

186

187 **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,
188 CH_3OH/CH_3ONa solution at rt for 10'; compound **19**: (b) *per-O*-acetylated-β-*C*-glucopyranosyl ketone, pyrrolidine at rt in CH_2Cl_2, 72 h, then, after purification
189 of the product, MeOH/MeONa at rt for 10'

190

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

193

194 Biological evaluation

195

196 We explored the bioactivity changes in compounds **2**-**19** induced by transformation of the functional phenolic -OH group
197 underlying cell growth arrest in four MM cells lines, labelled LCP, LCM, CN and M14. We chose these four cell lines from a larger
198 panel, for the following reasons: CN was one of the cell lines already demonstrated to be sensitive to compound **1**[12] LCP and
199 LCM were a couple of cell lines deriving from different stage tumor lesions of the same melanoma patient, being LCP derived
200 from the primitive lesion and LCM from a lymph node metastasis; M14 was a commercially available melanoma cell lines and can
201 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 ±
202 0.9 μM, average calculated on the basis of results obtained from 72h proliferation assays on five cell lines, including CN. Here, in
203 a preliminary experiment, the antiproliferative activity of compounds **2**-**19** was calculated as IC_{50} value on the basis of proliferation
204 assays performed treating cells up to 72h. Subsequently, compounds with IC_{50} value below 7.0 μM on all the cells lines
205 (compounds **6**-**9** and **11**, **12**) were tested after 24h treatments (Table 2). Moreover, our most active compounds **11** and **12** were
206 also tested on BJ normal fibroblast cell line, as a non-tumor cells control, in order to assess their selectivity.
207 With the aim to improve the antiproliferative activity of compound **1** (IC_{50} 1.0-2.7 μM)[12], we modified its physical-chemical
208 properties by introducing at the 2,2'-positions of the biphenyl structure different groups/chains by tuning lipophilicity, leaving group
209 and ability to undergone chemical/enzymatic hydrolysis in the cell. Moreover, it should be kept in mind the crucial role that
210 lipophilicity plays in designing oncological drugs because facilitates cell membrane access of the compound in the required critical
211 concentration.[30]

212

213
214 **Table 2.** Cytotoxicity (IC_{50} in µM) of compounds **2**-**19**. IC_{50} values represent mean value (±SD) of three independent measurements each performed in
215 triplicate. The cell lines LCP, LCM, CN, M14 are primary human MM cell lines, as described in detail in the experimental section.
216

Compounds	**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

217 n.s. = not soluble n.d.= not determined

218 C2-symmetry is a powerful tool in organic synthesis because when this element is present in a substrate, a control of
219 the production of isomers occurs improving the selectivity of the reaction. The presence of a C_2-symmetry axis in compounds **2**-
220 **13** is a useful structural element also from a biological point of view because identical interspecific interactions can be activated
221 between each symmetrical portion of the molecule and target proteins, thus providing an increased selectivity and control in the
222 interaction molecule-protein.
223 The hydrophilic compounds **2** and **3** featured with two and four free phenolic –OH groups, respectively, affected
224 melanoma cells after 72h with different antiproliferative activity (Table 2). Compound **2**, with guaiacyl units and similar to compound
225 **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
226 found that compound **2** was effective breaking-chain activator in bulk lipid autoxidation and showed strong cytotoxicity against rat
227 pheochromocytoma (PC12) cells, a slow-growing neuroendocrine tumor cell line.[31, 27]
228 Glycosylation of compound **2** was detrimental for antiproliferative activity on all melanoma cell lines. Derivatives **4** and **5**
229 with a *O*-glycoside unit linked at the phenolic -OH group and a *C*-glycoside unit at the end of the aliphatic chain, respectively, both
230 gave value of IC_{50} >100 μM after 72 h of treatment. Likely, the high hydrophilicity of **4** and **5** hindered complete bioavailability of
231 the compounds into the cell membrane. Same results were obtained with the corresponding monomers **18** and **19** evidencing the
232 key role of lipophilicity of the molecule in the delivery through the cell membrane.
233 Enzyme-catalysed hydrolysis of the methyl carbonate ester **6** would provide release of the biphenyl portion in the cell as
234 experienced when bioactive methyl phenols carbonates were screened on M14 cell line, as result, higher antiproliferative activity
235 was observed in comparison with the parent phenol.[6] In our work, compound **6** was more effective in the growth inhibition of M14
236 cell line (IC_{50} = 3.6 ± 0.1 μM) than in the other cell lines after 24h. As proof of the hydrolysis of carbonate functionality, compound
237 **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
238 during this time, compound **6** or the corresponding hydrolysed forms reached more sensitive cell compartments in comparison to
239 compound **2** that is the complete hydrolysed form of **6**. A similar trend was observed when cells were treated with compounds
240 with hydrolysable esters (*i.e.* compounds **7**-**9**), the antiproliferative effect improved on all cell lines after 72h giving IC_{50} values in
241 the range of 1.6 ± 0.9 and 6.2 ± 1.1 μM. In comparison with IC_{50} of compound **2** achieved after 72h, it is reasonable to suppose a
242 synergistic effect of the hydrolysable chain of compounds **7**-**9** with the hydroxylated biphenyl portion providing an increase of the
243 antiproliferative activity.
244 Although IC_{50} value is strictly dependent on the cell type, significant antiproliferative activity was observed after 24h of
245 treatment of LCM, CN and M14 cell lines with compound **8**, the activity improved after 72h on all melanoma cell lines. A remarkable
246 increased lipophilicity was estimated for compound **8** (Table 1), likely crucial in exerting an efficient delivery of the compound
247 through the lipophilic cell membrane. Differences in the invasiveness of tumor cells could derive, among different advantageous
248 features acquired during tumor transformation, from the activity of secreted and membrane-associated enzymes.[24] Noteworthy,
249 an octanonyl ester function is present in compound **8**. It is well recognised the role of medium chain fatty acids, in particular
250 octanoic acid (*i.e.* caprylic acid)[22], in exerting antiproliferative activity against skin cancer *in vitro*[32]and, *in vivo*, in activating
251 endogenous host peptides targeted to enhance intestinal epithelial immunological barrier.[33] In this work we did not investigated
252 the real role of the octanoyl chain, but we observed the improvement of the antiproliferative activity of compound **8** in comparison
253 with esters **6**, **7** and **9** after 72 h of treatment.
254 An excessive increase in lipophilicity was detrimental to bioavailability. Data achieved from compound **10** having a too
255 long lipophilic aliphatic ether chain, were excluded from Table 1 since not reliable due to the scarce solubility of the molecule in
256 water even at low concentrations.
257 We assayed compounds **11**-**13**, having a small, hindered and long *O*-prenylated chain, respectively, because there is
258 convincing evidence that natural and synthetic oxyprenylated phenylpropanoids assume an important role in inhibiting some
259 malignant cells.[34] Several mechanisms of action have been attributed to prenylated aromatic phenols.[23, 35].Generally, it was
260 demonstrated that oxyprenylated phenols are able to interact with different and selected cell receptors and signal transductors
261 (ex. Ras proteins) accounting for their ability to modulate key metabolic processes in pathological disorders. Likely, their effect is
262 immediately exerted on membrane cell due to the high affinity with the phospholipidic portion, favouring bioavailability of the
263 compound.[36] In breast cancer, some oxyprenylated ferulic acids with 3,3'-dimethyl allyl and geranyl moiety at the phenolic -OH
264 group, were successfully assayed for their binding affinities to MT1 melatonin receptors[37] and the antiproliferative and
265 antimigratory properties were detected at μM concentration levels. In our work, allyl and 3,3'-dimethyl allyl *O*-prenylated
266 compounds, **11** and **12** respectively, showed comparable antiproliferative activity assessed after 72h in a range between 1.2 ±

267 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 ±
268 1.7 – 15 ± 3.2 μM). It would not be ruled out that the higher lipophilicity of compound **13** (LogP 8.36) in comparison to compounds
269 **11** and **12** (LogP 4.08 and 5.17, respectively) could likely facilitate membrane penetration but hinder diffusion of compound **13**
270 inside the cell. After 24h, compounds **11** and **12** showed interesting antiproliferative activity on all MM cell lines although M14 cell
271 line appeared more sensitive to compound **11** even after 24h treatment (IC_{50} 2.4 ± 0.9). In a lower order of magnitude,
272 antiproliferative activity was observed after 72h with the corresponding monomers **15**-**17** accounting for IC_{50}, homogeneously
273 distributed on all MM cells lines, between 24 ± 2.1 – 43 ± 3.8 μM for all three compounds. When the phenolic -OH group was
274 protected with a methyl group (*i.e.* compound **14**), the activity dropped significantly. Overall, the last results confirmed the
275 beneficial influence that a small phenolic-*O*-prenylated chain exerts in an α,β-unsaturated Michael acceptor in enhancing
276 antiproliferative activity of the molecule. A comparison of IC_{50} between monomers **15**-**17** and dimers **11**-**13** evidenced the key role
277 of the hydroxylated biphenyl core in enhancing antiproliferative activity and in modulating the physical-chemical properties of the
278 molecule.

279 Interestingly, compounds **8**, **11** and **12** showed comparable antiproliferative activity after 72h on all MM cells lines even
280 though differences between *O*-prenylated compound **11** and ester **8** were observed in LCP and M14 cell lines after 24h of
281 treatment. The effect could be due to the different rate of hydrolysis of the protecting group or different selectivity when the
282 compounds are in the presence of cell specific enzymes or membrane transporters. Moreover, the activity of compounds **11** and
283 **12** was much lower on BJ fibroblasts, the healthy, non-tumor cell line used as control. BJ cells were given the same treatments
284 as MM cells, and showed to be much less affected by them. Indeed, compounds **11** and **12** showed significantly higher IC_{50} values
285 after 72h 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
286 (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
287 and healthy cells was 0.27 for **11** and 0.33 for **12**. These last evidences suggest a selectivity of antiproliferative activity against
288 tumor cells for these two compounds. Such a difference should be related to the higher cell division activity of tumor cells, and
289 might be exploited in future anticancer therapies designing. Selective antitumor activity was one of the major features of compound
290 **1** [12,13], and the present results show that it has been retained by compounds **11** and **12**.

291

292 **Conclusions**

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

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

307

308 **Experimental**

309 *Material and general remarks*

310 Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used
311 without further purification. Melting points were determined on a Büchi 530 apparatus and are uncorrected. All ^1H NMR and
312 ^{13}C NMR spectra were recorded in $CDCl_3$ (if not otherwise indicated) solution with a Varian VXR 5000 spectrometer at 399.94

313 MHz and 75.42 MHz respectively. Chemical shifts are given in ppm (δ); multiplicities are indicated by s (singlet), d (doublet), t
314 (triplet), q (quartet), m (multiplet) or dd (double of doublets). Elemental analyses were performed using an elemental analyser
315 Perkin-Elmer model 240 C. Acetone was freshly distilled from $CaCl_2$. Flash chromatography was carried out with silica gel 60
316 (230-400 mesh, Kiesgel, EM Reagents) eluting with appropriate solution in the stated v:v proportions. Analytical thin-layer
317 chromatography (TLC) was performed with 0.25 mm thick silica gel plates (Polygram®Sil G/UV_{254}, Macherey-Nagel). All
318 reactions were monitored by TLC performed on 0.2 mm thick silica gel plates (60 F254 Merck). The purity of all new
319 compounds was judged to be >98% by ^{1}H NMR and ^{13}C NMR spectral determination.
320 Compounds **2** and **14** were prepared according to Marchiani *et al.* [7] whereas compounds **15**-**17** were prepared as described
321 by Tatsuzaki *et al.*[28] Dehydrodivanillin and *O*Me-dehydrodivanillin (*i.e.*2,2',3,3'-tetramethoxy-5,5'-diformyl-1,1'-bipheny (**23**),
322 were obtained as described by Pisano *et al.*[12] whereas *per-O*-acetylated β-*C*-glucopyranosyl ketone was prepared following
323 the procedure described by Llantén *et al.*[25].
324 Lipophilicity of compounds **1**-**19** was estimated by ChemBioDraw Ultra 13.0 software using the logarithm of the partition
325 coefficient for *n*-octanol/water (LogP) and listed in Table 1.

326

327 Chemical synthesis

328 *(3E,3'E)-4,4'-(5,5',6,6'-tetrahydroxy-[1,1'-biphenyl]-3,3'-diyl)bis(but-3-en-2-one)* ***(3)***

329 To a solution of **2** (0.6 g, 1.57 mmol) in dichloromethane (20 mL) at -60 °C under nitrogen was added boron tribromide
330 (1.71 g, 6.95 mmol) dropwise. The solution was stirred at -60 °C for 1h, washed with water (100 mL) and extracted with ethyl
331 acetate (2 x 20 mL). The organic solution was dried over sodium sulphate, rotoevaporated and washed with dichloromethane
332 (2 x 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
333 (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
334 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;
335 Found: C, 67.83; H, 5.16.

336

337 (*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)-*
338 *[1,1'-biphenyl]-3,3'-diyl)bis(but-3-en-2-one)* ***(4)***

339 Compound **22** (1 g, 0.96 mmol) was stirred in sodium methoxide/methanol solution (0.02 g, 0.38 mmol in 10 ml) for
340 10 min. The reaction mixture was neutralized using a Dowex Marathon C (H+ form), filtrated and concentrated *in vacuo* to
341 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-
342 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,
343 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)
344 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,
345 200.18. Anal. Calcd. for $C_{34}H_{42}O_{16}$: C, 57.79; H, 5.99; Found: C, 57.80; H, 5.96.

346

347 *(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-*
348 *pyran-2-yl)but-3-en-2-one)* ***(5)***

349 Compound **24** (0.17 g, 0.15 mmol) was stirred in sodium methoxide/methanol solution (0.003 g, 0.06 mmol in 10 ml)
350 for 10 min. The reaction mixture was neutralized using a Dowex Marathon C (H+ form), filtrated and concentrated *in vacuo* to
351 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,
352 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),
353 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,

354 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.
355 for $C_{36}H_{46}O_{16}$: C, 58.85; H, 6.31; Found: C, 58.90; H, 6.37.

356

357 *3,3'-dimethoxy-5,5'-bis((E)-3-oxobut-1-en-1-yl)-[1,1'-biphenyl]-2,2'-diyl dimethyl dicarbonate **(6)***

358 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
359 chloroformate (0.55 g, 5.92 mmol) was added dropwise, at room temperature under nitrogen. The solution was stirred at room
360 temperature for 1 h, washed with water (100 mL) and extracted with dichloromethane (2 x 20 mL). The crude was dried over
361 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
362 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,
363 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,
364 151.97, 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.

365

366 *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)***

367 To a solution of **2** (2 g, 5.23 mmol) and glutaric anhydride (1.32 g, 11.57 mmol) in tetrahydrofuran (100 mL) was
368 added *N*, *N*-dimethylaminopyridine (DMAP) (0.25 g, 2.04 mmol) and trimethylamine (3 mL, 21.52 mmol). The solution was
369 stirred at reflux for 12 h, washed with hydrochloric acid (10% solution) (100 mL) and extracted with ethyl acetate (2 x 20 mL).
370 The crude was dried over sodium sulphate, rotoevaporated and purified by flash chromatography using a 1:1 mixture of
371 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* =
372 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,
373 *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,
374 132.88, 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,
375 5.64.

376

377 *3,3'-dimethoxy-5,5'-bis((E)-3-oxobut-1-en-1-yl)-[1,1'-biphenyl]-2,2'-diyl dioctanoate **(8)***

378 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
379 chloride (0.96 g, 5.92 mmol) was added dropwise, at room temperature under nitrogen. The solution was stirred at room
380 temperature for 4 h, washed with water (100 mL) and extracted with dichloromethane (2 x 20 mL). The organic solution was
381 dried over sodium sulphate, rotoevaporated and purified by flash chromatography using a 2:1 mixture of petroleum : ethyl
382 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
383 (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* =
384 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,
385 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
386 $C_{38}H_{50}O_{8}$: C, 71.90; H, 7.94; Found: C, 71.79; H, 7.84.

387

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

389 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-
390 (chloroformyl)propionate (1.2 g, 7.29 mmol) was added, at room temperature under nitrogen. The solution was stirred at reflux
391 for 4 days, washed with water (100 mL) and extracted with dichloromethane (2 x 20 mL). The crude was dried over sodium
392 sulphate, rotoevaporated and purified by flash chromatography using a 1:1 mixture of petroleum : ethyl acetate, as eluent, to
393 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),
394 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,

395 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,
396 131.19, 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,
397 6.54.

398

399 *(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)***

400 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
401 (0.014 gr. 0.05 mmol) in dry acetonitrile (15 mL) 1-bromooctadecane (0.7 g, 2.15 mmol) was added dropwise at room
402 temperature under nitrogen. The solution was stirred at reflux for 12 h, acidified with hydrochloric acid (10% solution), extracted
403 with diethyl ether (2 x 50 mL) and dried over sodium sulphate. The crude product was then purified by silica chromatography
404 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
405 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,
406 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,
407 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,
408 153.25, 198.06.Anal. Calcd. for $C_{58}H_{94}O_{6}$: C, 78.50; H, 10.68; Found: C, 78.82; H, 10.72.

409

410 *(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)***

411 Starting from dehydrodivanillin, compound **20**:

412 To a solution of **20** (0.2 g, 0.52 mmol) in acetone (10 mL), aqueous 1N solution of sodium hydroxide (1.6 mL) was added and
413 the mixture was stirred at room temperature for 12 h. Water was added and, acidified with hydrochloric acid (10% solution) and
414 extracted with dichloromethane. The crude material was purified by flash chromatography using a 3:1 mixture of petroleum :
415 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
416 (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,
417 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,
418 153.05, 198.21. Anal. Calcd for $C_{28}H_{30}O_{6}$ C, 72,71; H, 6,54; Found: C, 72.21; H, 6.82.

419

420 Starting from compound **2**

421 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
422 mL), allyl bromide (0.1 g, 0.87 mmol) was added dropwise at room temperature under nitrogen. The solution was stirred at
423 reflux for 12 h, acidified with hydrochloric acid (10% solution), extracted with dichloromethane (2 x 50 mL) and dried over
424 sodium sulphate. The crude product was then purified by silica chromatography using a 2:1 mixture of petroleum : ethyl
425 acetate, as eluent, to give **11** (0.12g, 65%).

426

427 *(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)***

428 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),
429 3,3'-dimethylallyl bromide (1 g, 6.71 mmol) was added dropwise at room temperature under nitrogen. The solution was stirred
430 at reflux for 12 h, acidified with hydrochloric acid (10% solution), extracted with dichloromethane (2 x 50 mL) and dried over
431 sodium sulphate. The crude product was then purified by silica chromatography using a 2:1 mixture of petroleum : ethyl
432 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
433 (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,
434 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,
435 138.20, 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.

436

437 *(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)*
438 ***(13)***

439 To a solution of **21** (0.4 g, 0.75 mmol) in acetone (8 mL) aqueous 1N solution of sodium hydroxide (0.5 mL) was
440 added and the mixture was stirred at room temperature for 12 h. Water was added and the solution was then acidified with
441 hydrochloric acid (10% solution) and extracted with dichloromethane. The crude material was purified by flash chromatography
442 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
443 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
444 (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,
445 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,
446 131.59, 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.

447

448 *(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*
449 ***(18)***

450 Compound **25** (1 g, 1.93 mmol) was stirred in sodium methoxide/methanol solution (0.02 g, 0.38 mmol in 20 ml) for
451 10 min. The reaction mixture was neutralized using a Dowex Marathon C (H+ form), filtrated and concentrated *in vacuo* to
452 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-
453 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
454 (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
455 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,
456 144.19, 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

457

458 *(E)-4-(3,4-dimethoxyphenyl)-1-((2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)but-3-en-2-*
459 *one* ***(19)***

460 Compound **26** (0.83 g, 1.54 mmol) was stirred in sodium methoxide/methanol solution (0.017 g, 0.32 mmol in 10 ml)
461 for 10 min. The reaction mixture was neutralized using a Dowex Marathon C (H+ form), filtrated and concentrated *in vacuo* to
462 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,
463 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,
464 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,
465 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,
466 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,
467 58.69; H, 6.57; Found: C, 58.80; H, 6.59.

468

469 *6,6'-Bis-allyloxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde* ***(20)***

470 To a solution of dehydrodivanillin (0.68 g, 2.25 mmol) and potassium carbonate (0.8 g, 5.78 mmol) in dry acetone
471 (70 mL), allyl bromide (0.7 g, 5.78 mmol) was added dropwise, at room temperature under nitrogen. The solution was stirred
472 at reflux for 12 h, washed with water (100 mL) and extracted with ether (2 x 20 mL). The crude, was dried over sodium sulphate
473 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
474 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,
475 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;
476 Found: C, 69.15; H, 5.92.

477

478 *6,6'-bis(((E)-3,7-dimethylocta-2,6-dien-1-yl)oxy)-5,5'-dimethoxy-[1,1'-biphenyl]-3,3'-dicarbaldehyde **(21)***

479 To a solution of dehydrodivanillin (0.52 g, 1.42 mmol) and potassium carbonate (0.59 g, 4.26 mmol) in dry acetone
480 (50 mL) dropwise geranyl bromide (1.0 g, 4.55 mmol) was added, at room temperature under nitrogen. The solution was
481 stirred at reflux for 12 h, washed with water (100 mL) and extracted with diethyl ether (2 x 20 mL). The crude, was dried over
482 sodium sulphate and then purified by silica chromatography using a 2:1 mixture of petroleum : ethyl acetate, as eluent, to give
483 **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),
484 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
485 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,
486 131.81, 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.

487

488 *(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)-*
489 *[1,1'-biphenyl]-3,3'-diyl)bis(but-3-en-2-one **(22)***

490 Silver carbonate (3.52g, 12.8 mmol) was added to a stirred solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl
491 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
492 stirring for 12 h, the solution was diluted with ethyl acetate (30 mL), washed with hydrochloric acid (10% solution) (100 mL),
493 dried over anhydrous sodium sulphate and rotoevaporated. The residue was purified by flash chromatography using a 1:2
494 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,
495 $CHCl_3$); ^{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),
496 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
497 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,
498 126.92, 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;
499 Found: C, 57.60; H, 5.66.

500

501 *(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-*
502 *(acetoxymethyl)tetrahydro-2H-pyran-2-yl)but-3-en-2-one) **(24)***

503 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'-
504 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
505 mmol) was added under nitrogen. The reaction was stirred at room temperature until the starting material was consumed as
506 evidenced by TLC (48 h). The reaction mixture was neutralized with hydrochloric acid (10% solution) and the residue diluted
507 in dichloromethane. The organic extracts were combined, dried over sodium sulphate, filtered and evaporated. The crude
508 product was purified by column chromatography using a 2:3 mixture of petroleum : acetone as eluent, to give **24** as a yellow
509 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,
510 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,
511 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
512 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
513 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,
514 132.29, 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:
515 C, 58.37; H, 5.86.

516

517 *(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*
518 *triacetate **(25)***

519 Silver carbonate (4.5 g, 16.31 mmol) was added to a stirred solution of tetra-*O*-acetylglucopyranosyl bromide (3.67
520 g, 8.91 mmol) and dehydrozingerone (0.78 g, 4.05 mmol) in pyridine (30 mL) at room temperature under shaded conditions.
521 After stirring for 18 h, the solution was diluted with ethyl acetate (30 mL), washed with hydrochloric acid (10% solution) dried
522 over anhydrous sodium sulphate and rotoevaporated. The residue was purified by flash chromatography using a 1:1 mixture
523 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, $CHCl_3$);
524 ^{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,
525 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
526 (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,
527 119.32, 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 $C_{25}H_{30}O_{12}$:
528 C, 57.47; H, 5.79; Found: C, 57.42; H, 5.76.

529

530 *(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*
531 *triacetate **(26)***

532 To a solution of *per-O*-acetylated β-*C*-glucopyranosyl ketone (1.35 g, 3.47 mmol) and veratraldehyde (0.57 g, 3.47
533 mmol) in 10 mL dry dichloromethane, pyrrolidine (0.05 g, 0.64 mmol) was added under nitrogen. The reaction was stirred at
534 room temperature until the starting material was consumed as evidenced by TLC (72 h). The reaction mixture was neutralized
535 with hydrochloric acid (10% solution) and the residue diluted in dichloromethane. The organic extracts were combined, dried
536 over sodium sulphate, filtered and evaporated. The crude product was purified by column chromatography using a 1:1 mixture
537 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, $CHCl_3$);
538 ^{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
539 (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
540 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
541 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,
542 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.
543 for $C_{26}H_{32}O_{12}$: C, 58.20; H, 6.01; Found: C, 58.17; H, 6.06.

544

545 Biological procedures

546 Cell lines and Cell cultures

547 Malignant melanoma cell lines used in this study [LCP-mel (LCP), LCM-mel (LCM), CN-mel (CN) and M14] were primary
548 tumor cell lines derived from tumor biopsy samples of malignant melanoma patients. They have been all kindly provided by the
549 Institute Dermopatico dell'Immacolata (IDI) in Rome. Cell lines had been all previously genetically characterized.[38] LCP was
550 derived from a primitive tumor, while LCM from a lymph node metastasis of the same melanoma patient. Both cell lines carry a
551 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
552 from a melanoma lymph node metastasis and carries a NRAS ^{Q61R} mutation. M14 cell line was derived from a melanoma
553 cutaneous metastasis, it was established in 1975 [29] and it is also commercially available (ATCC). It carries a BRAF ^{V600E} mutation
554 and an impaired locus CDKN2A (p16 $^{455insC/del26\ IVS1+2T>C}$).
555 A healthy donor human fibroblasts cell line (BJ) was used as normal cells control. It has been purchased from ATCC
556 (ATCC® CRL-2522).
557 Melanoma cells and fibroblasts were both grown in RPMI culture medium with stable glutamine, supplemented with 10%
558 Fetal Bovine Serum (FBS) and penicillin/streptomycin (1 U / mL) (complete medium) in a humidified atmosphere with 5% CO_2, at
559 37 °C.

560

561 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 24h, 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 (24h) or day 4 (72h) 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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Keywords: cancer, drug discovery, Michael acceptor, molecular scaffold, natural products

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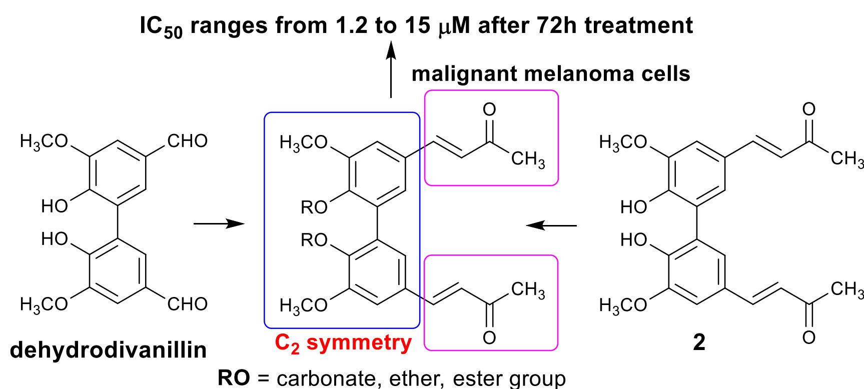
637 # Table of Contents



638

639 Cutaneous malignant melanoma is the most lethal form of skin cancer that arises from uncontrolled proliferation of melanocytes
640 that are cells producing pigments. We have prepared a small collection of hydroxylated biphenyls derivatives, compounds **2-13**,
641 structurally related to a class of naturally occurring compounds known for selective and effective antiproliferative activity on
642 malignant melanoma cells.