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Synthesis of *C*- and *O*-prenylated tetrahydroxystilbenes and *O*-prenylated cinnamates and their action towards cancer cells

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ABSTRACT

Synthesis of the naturally occurred *C*- and *O*-prenylated tetrahydroxystilbenes and *O*-prenylated cinnamates was carried out by decarbonylative Heck reaction and selenium dioxide catalysed oxidation, respectively. In the decarbonylative Heck synthetic route, fusion of benzoyl chloride and styrene derivatives was catalysed by an *N*-heterocyclic carbene system generated *in situ* by palladium acetate and 1,3-bis(2,6-diisopropylphenyl)imidazolinium chloride to form a *E*-tetrahydroxystilbene derivative. Formation of allyl ether was subsequently carried out by reaction of the deprotected OH in the A phenyl ring of the stilbene with 3,3-dimethylallyl bromide and a base (sodium hydride) to form *O*-prenylated tetrahydroxystilbene derivatives. [1,5]-Rearrangement of the isoprenyl unit from *O*- to *C*-position in the A ring was carried out at elevated temperature in the presence of magnesium silicate (Florisil) to form the corresponding *C*-prenylated tetrahydroxystilbene. Formation of *O*-prenylated cinnamate was first carried out by base catalysed allyl ether formation between 3,3-dimethylallyl bromide and hydroxycinnamic acid methyl ester. The methyl group of the isoprenyl unit was subsequently oxidized using selenium dioxide to form a terminal hydroxyl group. The prenylated tetrahydroxystilbenes and cinnamate synthesized in this study were novel derivatives of piceatannol and methyl 4-(3′-methylbut-2′-enyloxy)cinnamate isolated from propolis in Kangaroo Island, South Australia. The synthetic compounds were tested against K562 cancer cells and potent growth inhibitory activity was observed for *E*-1-[5-hydroxy-3-methoxy-2-(3-methyl-2-butenyl)phenyl]-2-[4-hydroxy-3-methoxyphenyl]ethene, IC_{50} = 0.10 μM.

1. Introduction

Stilbenes, C_6–C_2–C_6, and cinnamates, C_6–C_3, have been found in a wide range of plant sources, aromatherapy products and dietary supplements. The naturally occurring stilbenes and cinnamates exist in the *E* form and normally occur as hydroxylated compounds commonly found in coniferous plants such as fir tree *Abies* species, spruce *Picea* species, pine *Pinus* species, juniper *Juniperus* species, also, in rhubarb *Rheum* species and mulberry *Morus* species [1].

Over the years, stilbene compounds have attracted the attention of many researchers due to their wide range of biological activities including stimulation of protein synthesis process, increase retention of nitrogen and accelerate cellular growth and development [2]. Moreover, the stilbenes have shown to increase the concentration of α-lipoprotein and decrease the concentration of cholesterol in blood, stimulate development of bones and cartilages, increase blood clotting by blocking of blood clotting inhibitors, widen blood vessels, improve circulation and increase penetration through blood vessel walls [3–5]. Research has revealed that the *E* form exhibits more potent activity than the corresponding *Z* form across the biological screens including anticancer and anti-oxidant activities [6].

The most well-known and well-characterized stilbene is resveratrol, (*E*)-3,4′,5-trihydroxystilbene, a natural phytoalexin found in grapes, red wine, mulberries, peanuts and other food products, including a rare report of its occurrence in propolis [1]. Resveratrol is synthesized by plants using stilbene synthase enzyme [1]. Resveratrol has been reported to play a role in the prevention of heart diseases and diabetes, alteration of eicosanoid synthesis, modulation of lipid, lipoprotein metabolism and to be the main protagonist for the so-called “French paradox” [7]. Scientists have attributed this phenomenon to moderate consumption of anti-inflammatory and anti-oxidant polyphenolic compounds,

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such as piceatannol and resveratrol, in red wine [8,9]. Piceatannol, (*E*)-3,3′,4′,5-tetrahydroxystilbene, is another naturally occurring hydroxystilbene found in grapes, sugar cane, berries and peanuts [10–12]. Piceatannol, a 3-hydroxylated resveratrol, is synthesized in plants in response to fungal attack, ultraviolet exposure, and microbial infection [13]. Induction of piceatannol synthesis is also evident during the ripening of grapes and increases during the process of wine production due to β-glucosidase activity of bacteria [14]. Piceatannol has been shown to exert various pharmacological effects such as anticancer, anti-inflammatory, anti-oxidant, anti-aging, anti-diabetic and cardio-protective activities [15–17]. Furthermore, the potency of piceatannol usually surpasses the resveratrols [15,17–19].

In past decade, natural products containing prenyl groups have been recognized as valuable biologically active phytochemicals. Prenylation is a chemical or enzymatic addition of a hydrophobic isoprenoid side chain to an accepting molecule such as “flavonoid or polyhydroxy aromatic compound. From a pharmacological point of view, the prenylated compounds appear to exert more potent or higher efficacy than the parent compound. These natural products represent new leads for the development of novel drugs [20]. Furthermore, prenylated aromatic secondary metabolites play a critical role in the biosynthesis of a wide range of molecules exerting valuable pharmacological effects across phylogenetically different classes of living organisms, from bacteria to mammals and plants. The most commonly reported of these groups are prenylated cinnamic acids and their derivatives, while prenylated flavonoids are less common, and prenylated benzophenones are rare natural occurrence. Prenylated piceatannol derivatives are also examples of rare natural products.

Recently, our research group has discovered a series of novel *C*- and *O*-prenylated piceatannols from propolis in Kangaroo Island of South Australia [21]. We report here the total synthesis of these novel prenylated piceatannols. An *E*-tetrahydroxystilbene was first formed *via* decarbonylative Heck reaction of a benzoyl chloride and a styrene catalysed by an *N*-heterocyclic carbene generated *in situ* by palladium acetate and 1,3-bis(2,6-diisopropylphenyl)imidazolinium chloride. An allyl ether was formed at the *O*-3 position of the stilbene using 3,3-dimethylallyl bromide and NaH to form *O*-prenylated tetrahydroxystilbene. Rearrangement of the isoprenyl unit from *O*-3 to *C*-6 was carried out at elevated temperature (110 °C) in the presence of magnesium silicate (Florisil) to form the corresponding *C*-prenylated tetrahydroxystilbene. Synthesis of hydroxyl derivative of *O*-prenylated cinnamate was carried out first by base catalysed allyl ether formation between 3,3-dimethylallyl bromide and *para*-hydroxycinnamic acid methyl ester. The methyl group of the isoprenyl unit was subsequently oxidized using selenium dioxide to form a terminal hydroxyl group.

2. Chemistry

In this study we report total synthesis of the novel prenylated *E*-tetrahydroxystilbenes including *E*-1-[5-hydroxy-3-(3-methylbut-2-enyloxy)phenyl]-2-[4-hydroxy-3-methoxyphenyl]ethene (**14a**), *E*-1-[5-hydroxy-3-(3-methylbut-2-enyloxy)phenyl]-2-[3-hydroxy-4-methoxyphenyl]ethene (**14b**) and *E*-1-[5-hydroxy-3-methoxy-2-(3-methylbut-2-enyloxy)phenyl]-2-[4-hydroxy-3-methoxyphenyl]ethene (**17**) and their dihydro analogues including **15a**, **15b** and **18** as shown in Fig. 1. The prenylated stilbenes **14a** and **17** are naturally occurring piceatannol derivatives isolated from propolis in Kangaroo Island [21]. Synthesis of the *C*-prenylated hydroxystilbene was successively achieved first by decarbonylative Heck reaction to form an *E*-hydroxystilbene. A reaction employing an *N*-heterocyclic carbene system generated *in situ* from 1,3-bis(2,6-diisopropylphenyl)imidazolinium chloride with palladium acetate to catalyse a carbonylative cross-coupling of a benzoyl chloride with a styrene as shown in Scheme 2. The stilbene was then reacted with 3,3-dimethylallyl bromide to form *O*-prenylated tetrahydroxystilbene. The allyl group subsequently underwent a [1,5]-rearrangement to form the corresponding *C*-prenylated tetrahydroxystilbene as shown in Scheme 3. It is interesting to note that the [1,5]-rearrangement of the allyl group was rather unexpected. It is known that under Claisen rearrangement the allyl group migrates primarily to adjacent unsubstituted *ortho* position and to *para* position when both *ortho* positions are blocked. However, there are cases that the allyl group migrates to *para* position despite the availability of an unsubstituted *ortho* position. This behaviour is commonly seen in polyhydroxybenzenes or in the presence of a catalyst. In these circumstances the allyl group migrates to the most stable position. In current study, in the presence of magnesium silicate, the allyl group migrated from O-3 to C-6 position to form the *C*-prenylated tetrahydroxystilbene as shown in Scheme 3. Spectroscopic data of compound **17** was identical with one of the compounds isolated from Kangaroo Island propolis [21].

In this study, inexpensive and readily accessible starting material resorcylic acid (3,5-dihydroxybenzoic acid) **1** was used to prepare benzoyl chlorides (Scheme 1) as precursors for decarbonylative Heck reaction. It provides the option of introduction of various ether substituents or different protecting groups for selective introduction of isoprenyl units. As a result, three protected benzoyl chlorides were successfully formed. The main drawback in this synthetic route was undesirably low yield of mono-benzyl or mono-methyl ethers obtained due to formation of di-substituted compounds despite only one equivalent molar of benzyl bromide used in the reaction. This resulted in incomplete benzylation of resorcylic acid.

Preparation of the styrene intermediates **10a** and **10b** was accomplished by Wittig reaction. Treatments of protected vanillin or isovanillin with methyltriphenylphosphonium bromide in the presence of potassium *tert*-butoxide afforded styrenes **10a** or **10b**, respectively, at reasonably high yield (Scheme 1).

Decarbonylative coupling reactions are useful synthetic routes to form C–C or C–heteroatom bonds. The main advantage of decarbonylative Heck reaction is its outstanding *E* selectivity, whilst others generally give a mixture of *E*/*Z*-isomers [22]. Overall, moderate to high yields were achieved in all steps except for the removal of benzyl protecting group in *O*-prenylated hydroxystilbene. It appears that the *E*-conjugated system stabilizes the benzyl protecting group against removal. This led to hydrogenation of the double bond seen from the formation of compounds **15a**, **15b** and **18** (Schemes 2 and 3, Fig. 1). Different protecting groups other than benzyl group such as silyl ether protective groups could be used to avoid hydrogenation, thereby improving the yield of both *O*- and *C*-prenylated tetrahydroxystilbenes. Rearrangement of isoprenyl group from *O*- to *C*-position can also be improved by variation of reaction conditions and catalysts such as silica or alumina particles.

Esters of cinnamic acid and esters of substituted cinnamic acid are well-known compounds in propolis such as benzyl caffeate, cinnamyl caffeate, CAPE, isopentenyl cinnamates, isopentyl ferulate and benzyl ferulate which are isolated from poplar type propolis [23,24]. In Brazilian propolis, cinnamic acid derivatives are found to be *C*-prenylated cinnamic acid derivatives. Examples of these compounds are artepillin C, baccharin, drupanin, 3-prenyl-4-hydroxycinnamic acid (PHCA), 2,2-dimethyl-6-carboxyethenyl-8-prenyl-2H-1-benzopyran (DPB) and 2,2-dimethyl-6-carboxyeth enyl-2H-1-benzopyran (DCBEN) [25].

The prenylated hydroxylated cinnamic acid derivative (compound **21**) synthesized in this study is a novel compound first isolated from Kangaroo Island propolis. It is an *O*-prenylated cinnamic methyl ester with a terminal hydroxyl group attached to one

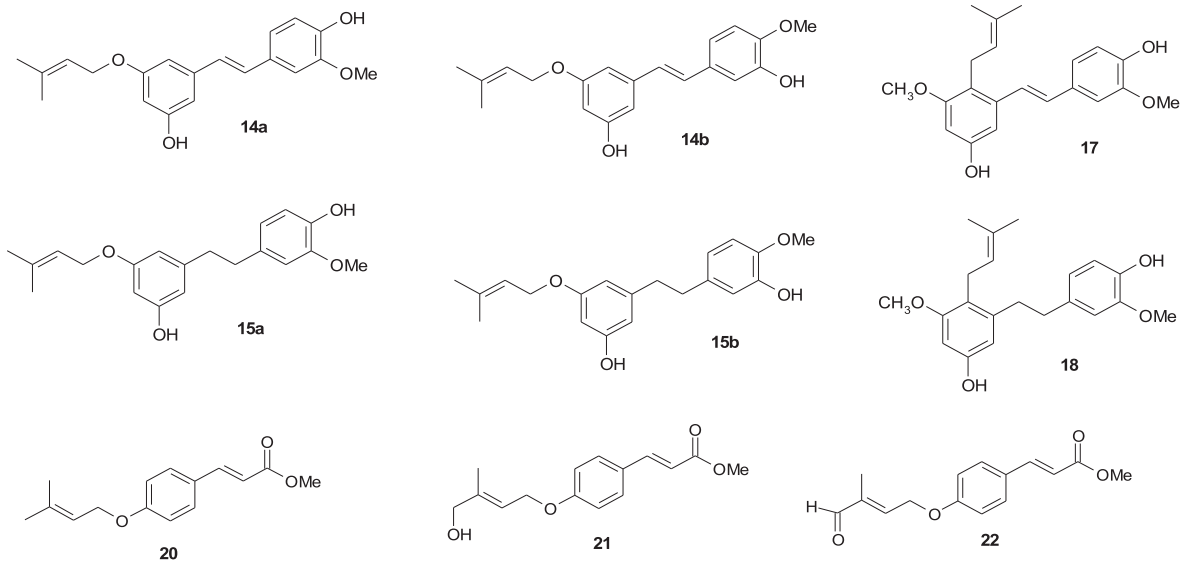


Fig. 1. Structures of prenylated tetrahydroxystilbenes and their dihydro analogues and prenylated cinnamates.

of the vinyl methyl of the isoprenyl unit (compound **21**, Fig. 1) and found in abundance in the propolis. This type of hydroxylation is not common in plants and could involve very specific enzymatic activity.

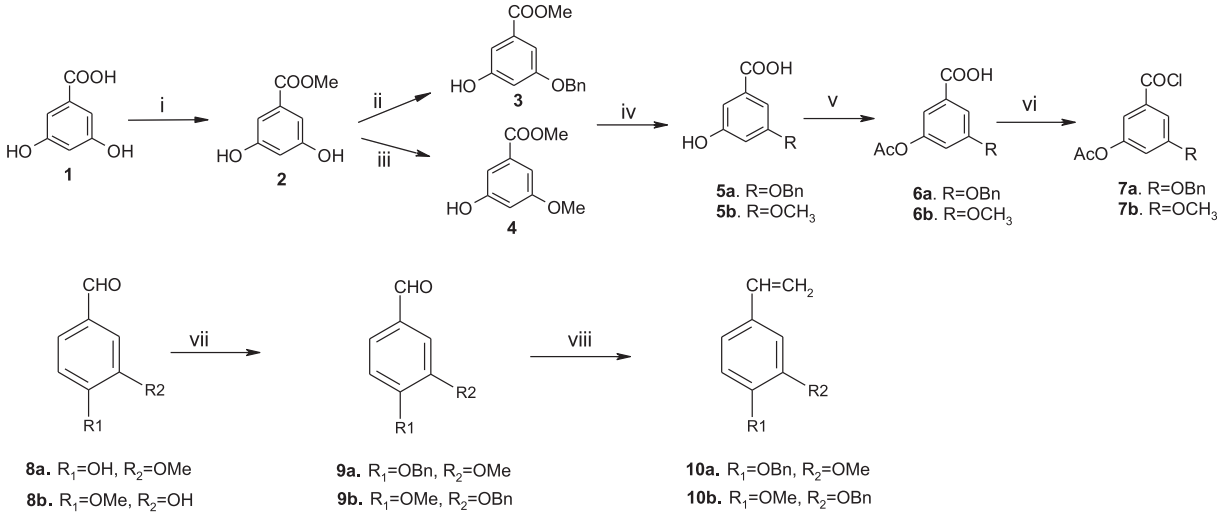
The parent compound (compound **20**, Fig. 1) has also been isolated in very small amount from the same propolis. This compound was first reported in 1968 from Australian native plant *Cotula australis* (Asteraceae).

3. Pharmacology

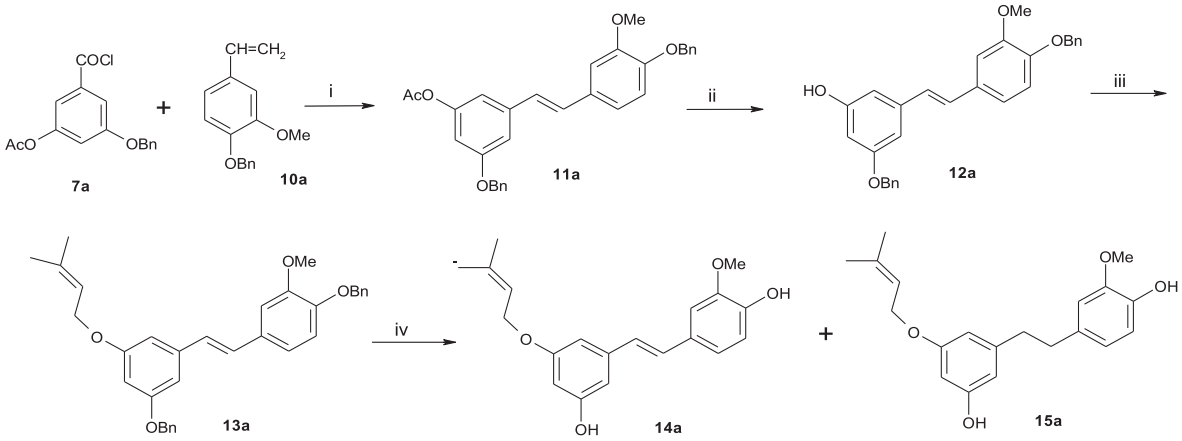
The determination of drug safety and cell toxicity measure is a critical step in pre-clinical drug discovery process. To test the toxicity of the final compounds towards the K562 leukemic cancer cell line, MTT cytotoxicity assay was performed.

The MTT antiproliferative assay against leukemia K562 cell line revealed that these prenylated tetrahydroxystilbenes inhibited cell growth in a concentration-dependent manner. The inhibitory potency is dependent on their structures. As shown in Table 1 compound **17** was a remarkably potent inhibitor with an IC_{50} value of 0.10 μM, followed by **18** (IC_{50} value of 10 μM), **14a** (IC_{50} value of 21.0 μM), **15b** (IC_{50} value of 31.6 μM), **14b** (IC_{50} value of 46.8 μM) and **15a** (IC_{50} value of 63.0 μM).

It is clear that the conjugated double bond, which bridges the two aromatic rings, is an important structural feature in the inhibition of cancer cell proliferation. Reduction of this bond of **17** to give **18** has dramatically reduced (about 100-fold) the potency of the compound. The role of the double bond is to keep the resorcinol and catechol rings in a co-planar orientation. It is well documented that a number of beneficial effects of stilbenes such as anti-oxidant properties, relate to the resonance effects [26–28]. In fact, the unpaired electrons are mainly distributed to the *O*-atom in *para* position, double bond, and B-benzene ring. Without the double bond the resonance effects between the two benzene rings is totally blocked and co-planarity is lost resulting in major changes in both electrostatic and steric properties. In addition, *C*-linkage of isoprenyl to aromatic moiety appears to enhance significantly the inhibitory potency of the compound in comparison with *O*-linkage



Scheme 1. Preparation of compounds **10a** and **10b**: (i) anhyd. MeOH, CH_3COCl, reflux under N_2, 2 h, **2**: 90%; (ii) DMF, NaH, N_2 atm, 1 equiv. $PhCH_2Br$, rt, 2 h, **3**: 42%; (iii) DMF, NaH, N_2 atm, MeI, rt, 2 h, **4**: 41%; (iv) MeOH, 1M NaOH, N_2 atm, rt, 2 h, **5a** and **5b**: 94%; (v) Ac_2O, pyridine, rt, 2 h, **6a** and **6b**: 94%; (vi) $SOCl_2$, reflux, 4 h, **7a** and **7b**: quantitative yield; (vii) DMF, NaH, 1.2 equiv. $PhCH_2Br$, rt, 3 h, **9a** and **9b**: 92%; (viii) THF, $CH_3P(Ph)_3Br$, N_2 atm, $(CH_3)_3COK$, rt, 2 h, **10a** and **10b**: 85%.



Scheme 2. Preparation of compound **15a**: (i) xylene, 5% $Pd(AcO)_2$, 1,3-bis-(2,6-diisopropylphenyl)imidazolinium chloride, *N*-ethylmorpholine, reflux under N_2 atm, 18–22 h, **11a**: 34%; (ii) MeOH–THF, 1 M NaOH, N_2 atm, rt, 3 h, **12a**: 44%; (iii) DMF, NaH, N_2 atm, C_5H_9Br, rt, 3 h, **13a**: 43%; (iv) 1,4-cyclohexadiene–EtOH, 10% Pd–C, reflux under N_2 atm, **14a**: 20%, **15a**: 15%.

of isoprenyl. Consequently, **14a** was considerably less active in comparison to **17** and **18**.

It is also noticeable that vanilloid moiety (3-methoxyl-4-hydroxyphenyl) appears to contribute to inhibitory activities of the stilbenes in this study. As a result, exchanging the methoxy and hydroxyl groups from vanilloid to isovanilloid moiety as shown in **14b** reduces the inhibition of **14a** by approximately three fold. The cell proliferation assay suggests the variation of the hydroxyl group pattern of the B phenyl ring, concerning number and position, resulted in the differences in activity shown. It is of further interest that hydroxyl group at the 4′-position contributes more to cytotoxic activity than the other hydroxyl group. Therefore, the 4′-position in stilbene provides the most acidic hydrogen and its removal from the 4′-position is highly preferred [26,29]. It is well accepted that phenol compounds are not active as anti-oxidants unless substitution at either the *ortho* or *para* position has increased the electron density at the hydroxy group and lowered the oxygen–hydrogen bond energy, in effect increasing the reactivity towards the lipid free radicals. Substitution in phenolic compounds at the *meta* position has a rather limited effect, and compounds like resorcinol with a hydroxyl group in the *meta* position are thus poor scavengers of free radicals compared to similar compounds with substitution at the *ortho* or *para* position. The presence of additional hydroxy groups, either at the *ortho* or *para* position, further increases the antioxidative activity of the compound as intramolecular hydrogen bonds stabilize the phenoxyl radicals [30].

The MTT antiproliferative assay against leukemia K562 cell line revealed significant variation in activity between the tested compounds. Among all the cinnamic acid derivatives tested, the aldehyde derivative **22** showed the highest activity (IC_{50} = 57.5 μM) then the hydroxylated compound **21** (IC_{50} = 70.8 μM) with the parent compound **20** showing lowest activity (IC_{50} = 97.7 μM).

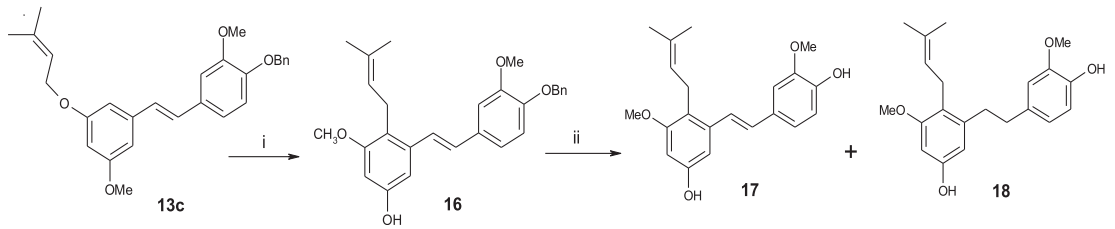
4. Conclusion

With careful selection of starting precursors of interest, a number of derivatives of prenylated mono-, di-, tri-, or polyhydroxystilbenes can be prepared for structure–activity relationship analysis. As described in the Introduction, natural products that contain prenyl groups are of particular interest in drug discovery and development. From a pharmacological point of view, the prenylated compounds appear to exert more potent or higher efficacy than their parent compounds. These natural products thus represent new leads for the development of novel drugs [20].

5. Experimental

5.1. General procedures

Commercially available reagents were used without purification unless otherwise stated. Anhydrous tetrahydrofuran (THF) was freshly distilled from sodium hydride under a nitrogen atmosphere. Anhydrous dimethylformamide (DMF) was distilled from calcium hydride under a nitrogen atmosphere. All air- and moisture-sensitive reactions were carried out under a dry nitrogen atmosphere in oven-dried glass-ware. Analytical thin-layer chromatography was carried out on aluminum backed plates coated with silica gel (Merck, 60, F254) and visualized under UV light at 254 nm. Chromatographic purification was carried out by short column vacuum chromatography using Merck silica gel (60H). Melting points were taken on a hot stage microscope and are uncorrected. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ on a Varian-Gemini 400MR (Palo Alto, CA, USA) with VNMRj 2.2C Varian software. TMS signal was used as reference. Low- and high-resolution mass spectra were measured by direct



Scheme 3. Preparation of compounds **17** and **18**: (i) Toluene, 10× equiv. Florisil, reflux under N_2 atm, 4 h, **16**: 59%; (ii) 1,4-cyclohexadiene–EtOH, 10% Pd–C, reflux under N_2 atm, **17**: 65%, **18**: 25%.

Table 1
Cytotoxicity activities of compounds **14a**–**b**, **15a**–**b**, **17**, **18** and **20**–**22**. Antiproliferative activity was determined by MTT assay as shown in Experimental part 5.2. All data are presented as mean values of two independent experiments. Coefficients of variation were <10%. IC_{50}: concentration of the tested compound that inhibits 50% of cell growth.

Compound	IC_{50} (μM)
14a	21.0
14b	46.8
15a	63.0
15b	31.6
17	0.10
18	10.0
20	97.7
21	70.8
22	57.5

infusion electrospray ionization tandem mass spectrometry and reported as mass to charge ratio (*m*/*z*) and related intensity (%). Low-resolution ESI-MS was recorded on a Thermo-Finnigan TSQ 7000 (LC-MS/MS system). High resolution ESI-MS was measured on a Bruker Daltonics Apex Ultra Fourier Transform Ion Cyclotron Resonance 7 T Mass Spectrometer.

Preparation of intermediates such as 3-acetoxy-5-benzyloxybenzoyl chloride (**7a**) and 3-acetoxy-5-methoxybenzoyl chloride (**7b**), 4-benzyloxy-3-methoxy-1-ethenylbenzene (**10a**) and 3-benzyloxy-4-methoxy-1-ethenylbenzene (**10b**) for the synthetic routes as described in Scheme 1, and methyl (*E*)-4-(3′-methylbut-2-enyloxy)cinnamate (**20**) for Scheme 4 were carried out by methods as described in literature. For details of these syntheses refer to Supplementary data.

Refer to Supplementary data for plotted ^{1}H NMR and ^{13}C NMR spectra of the key compounds including *E*-1-[5-hydroxy-3-(3-methylbut-2-enyloxy)-phenyl]-2-[3-hydroxy-4-methoxyphenyl] ethene (**14b**), 1-[5-hydroxy-3-(3-methylbut-2-enyloxy)-phenyl]-2-[4-hydroxy-3-methoxyphenyl]ethane (**15a**), 1-[5-hydroxy-3-(3-methylbut-2-enyloxy)-phenyl]-2-[3-hydroxy-4-methoxyphenyl] ethane (**15b**), 1-[5-hydroxy-3-methoxy-2-(3-methyl-2-butenyl) phenyl]-2-[4-hydroxy-3-methoxyphenyl]-ethane (**18**) and methyl (*E*)-4-(3′-carbonylbut-(*E*)-2′-enyloxy)cinnamate (**22**).

5.2. Preparation of E-1-[3-acetoxy-5-benzyloxyphenyl]-2-[4-benzyloxy-3-methoxyphenyl]-ethene (**11a**)

5.2.1. General method

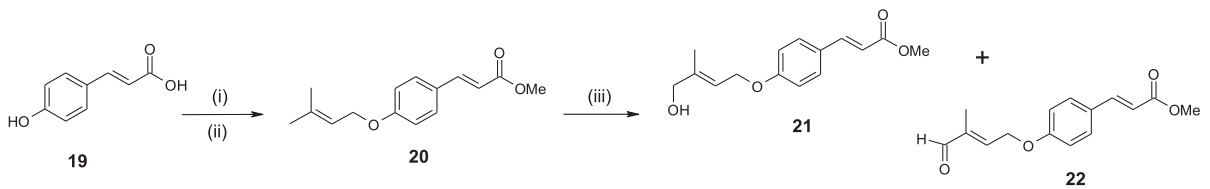
To a solution of 1,3-bis(2,6-diisopropylphenyl)imidazolinium chloride (0.093 g, 10%, 0.217 mmol) in xylene (3 mL) under nitrogen was added palladium acetate (0.048 g, 10%, 0.217 mmol). The mixture was stirred for 30 min at room temperature followed by the addition of the solution of **7a** (0.7 g, 2.17 mmol) in xylene (2 mL), *N*-ethylmorpholine (0.04 mL, 0.316 mmol) and the solution of styrene **10a** (0.627 g, 2.61 mmol) in xylene (3 mL). The resulting mixture was refluxed at 130 °C for 18–22 h and monitored by TLC. The solvent was evaporated under reduced pressure. The residue was purified on silica gel using hexane/ethyl acetate (3:1) as mobile phase to give **11a** as yellowish oil (0.43 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.45–7.28 (10 H, m, 2 × benzyl Ph), 7.06 (1H, d, *J* = 1.8 Hz, H-2′), 7.02 (1H, d, *J* = 16.4 Hz, H-α), 6.97 (1H, dd, *J* = 6.4, 1.8 Hz, H-6′), 6.89 (1H, d, *J* = 16.0 Hz, H-β), 6.87–6.85 (3H, m, H-2,5′,6), 6.62 (1H, t, *J* = 2.2 Hz, H-4), 5.18 (2H, s, benzyl CH_2), 5.07 (2H, s, benzyl CH_2), 3.94 (3H, s, OCH_3), 2.30 (3H, s, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.39 (CO), 159.78, 151.81, 149.79, 148.32, 139.82, 137.00, 136.57, 130.48, 129.78, 128.63 (2C), 128.58 (2C), 128.10, 127.88, 127.55 (2C), 127.24 (2C), 126.02, 119.98, 113.95, 112.02, 110.50, 109.48, 107.28, 71.00 (benzyl CH_2), 70.27 (benzyl CH_2), 56.02 (3′-OCH_3), 21.19 (CH_3CO); MS (ESI) *m*/*z* (%): 503 ($[M + Na]^+$, 98), 481 ($[M + H]^+$, 100), 412 (19), 408 (45), 241 (17); HRMS (ESI) (*m*/*z*): calcd for $C_{31}H_{28}O_5$ $[M + Na]^+$ 503.1829, found 503.1828.

5.3. Preparation of E-1-[3-acetoxy-5-benzyloxyphenyl]-2-[3-benzyloxy-4-methoxyphenyl]ethene (**11b**)

The title compound was prepared similar to that described for **11a** with the condensation of **7a** and **10b** to give **11b**. The product was recrystallised from a mixture of hexane/toluene (2:1) to afford yellowish needle crystals (0.4 g, 32%), mp 133–134 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.49–7.29 (10H, m, 2 × benzyl Ph), 7.07 (1H, d, *J* = 2.0 Hz, H-2′), 7.05 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′), 6.97 (1H, d, *J* = 16.2 Hz, H-α), 6.95 (1H, t, *J* = 1.8 Hz, H-6), 6.89 (1H, d, *J* = 8.4 Hz, H-5′), 6.84 (1H, t, *J* = 1.8 Hz, H-2), 6.82 (1H, d, *J* = 16.2 Hz, H-β), 6.61 (1H, t, *J* = 2.2 Hz, H-4), 5.19 (2H, s, benzyl CH_2), 5.07 (2H, s, benzyl CH_2), 3.90 (3H, s, OCH_3), 2.30 (3H, s, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.39 (CO), 159.76, 151.80, 149.90, 148.34, 139.83, 137.05, 136.58, 129.94, 129.78, 128.64 (2C), 128.60 (2C), 128.11, 127.94, 127.58 (2C), 127.38 (2C), 125.88, 120.59, 112.00, 111.91, 111.81, 110.52, 107.22, 71.16, 70.27, 56.06 (4′-OCH_3), 21.18 (CH_3CO); MS (ESI) *m*/*z* (%): 503 ($[M + Na]^+$, 98), 481 ($[M + H]^+$, 32), 393 (58), 323 (100); HRMS (ESI) (*m*/*z*): calcd for $C_{31}H_{28}O_5$ $[M + Na]^+$ 503.1829, found 503.1832.

5.4. Preparation of E-1-[3-acetoxy-5-methoxyphenyl]-2-[4-benzyloxy-3-methoxyphenyl]-ethene (**11c**)

The title compound was prepared similar to that described for **11a** with the condensation of **7b** and **10a** to give **11c**. The product was purified on silica gel using hexane/ethyl acetate (3:1) as mobile phase to give **11c** as off-white solid (0.45 g, 43%), mp 104–106 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.28 (5H, m, phenyl Ph), 7.07 (1H, d, *J* = 2.4 Hz, H-2′), 7.03 (1H, d, *J* = 16.2 Hz, H-α), 6.98 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′), 6.90 (1H, d, *J* = 16.8 Hz, H-β), 6.88–6.84 (3H, m, H-2,5′,6), 6.53 (1H, d, *J* = 2.0, H-4), 5.18 (2H, s, benzyl CH_2), 3.95 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 2.31 (3H, s, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.41 (CO), 160.58, 151.83, 149.78, 148.31, 139.76, 137.00, 130.49, 129.72, 128.58 (2C), 127.88, 127.24 (2C), 126.07, 119.97, 113.94, 111.73, 109.67, 109.46, 106.54, 71.01, 56.02 (3′-OCH_3), 55.50 (5-OCH_3), 21.18 (CH_3CO); MS (ESI) *m*/*z* (%): 405 ($[M + H]^+$, 57), 363 (93), 315 (62), 273 (100), 239 (54), 91 (30); HRMS (ESI) (*m*/*z*): calcd for $C_{25}H_{24}O_5$ $[M + H]^+$ 405.1696, found 405.1695.



Scheme 4. Preparation of compounds **20**–**22**: (i) anhyd. MeOH, CH_3COCl, N_2 atm, rt, 24 h; (ii) anhyd. MeOH, NaH, N_2 atm, C_5H_9Br, rt, 3 h, **20**: 26%; (iii). DCM, SeO_2, TBHP, rt, 48 h, **21**: 38%, **22**: 12%.

5.5. Preparation of E-1-[5-benzyloxy-3-hydroxyphenyl]-2-[4-benzyloxy-3-methoxyphenyl]-ethene (**12a**)

5.5.1. General method

To the solution of **11a** (0.04 g, 0.083 mmol) in a mixture of equal part of methanol, tetrahydrofuran and water (9 mL) at 0 °C under nitrogen was added sodium hydroxide (0.02 g, 0.35 mmol). The reaction mixture was warmed up to room temperature and stirred for further 3 h. The product was acidified with 0.1 M hydrochloric acid (5 mL) then extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 20% aqueous sodium chloride (10 mL), dried over sodium sulfate and evaporated under reduced pressure. The residue was purified on silica gel using hexane/ethyl acetate (2:1), followed by recrystallisation from a mixture of hexane–ethyl acetate (2:1) to afford **12a** as an off-white powder (0.016 g, 44%), mp 114–115 °C. ^{1}H NMR (400 MHz, Methanol-d_4) δ (ppm) 7.45–7.28 (10H, m, 2 × benzyl Ph), 7.06 (1H, d, J = 2.0 Hz, H-2′), 7.00 (1H, d, J = 16.2 Hz, H-α), 6.98 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 6.87 (1H, d, J = 8.4 Hz, H-5′), 6.86 (1H, d, J = 16.2 Hz, H-β), 6.71 (1H, t, J = 2.0 Hz, H-6), 6.59 (1H, t, J = 2.0 Hz, H-2), 6.38 (1H, t, J = 2.2 Hz, H-4), 5.18 (2H, s, benzyl CH_2), 5.07 (2H, s, benzyl CH_2), 4.82 (1H, s br, OH), 3.95 (3H, s, OCH_3); ^{13}C NMR (100 MHz, Methanol-d_4) δ (ppm) 160.29, 156.78, 149.76, 148.19, 139.92, 136.98, 136.84, 130.65, 129.20, 128.61 (2C), 128.57 (2C), 128.02, 127.88, 127.49 (2C), 127.26 (2C), 126.54, 119.94, 113.96, 109.45, 105.97, 105.69, 101.54, 71.02 (benzyl CH_2), 70.01 (benzyl CH_2), 56.03 (3′-OCH_3); MS (ESI) m/z (%): 461 ([M + Na]$^+$, 100), 439 ([M + H]$^+$, 18); HRMS (ESI) (m/z): calcd for $C_{29}H_{26}O_4$ [M + H]$^+$ 439.1904, found 439.1908.

5.6. Preparation of E-1-[5-benzyloxy-3-hydroxyphenyl]-2-[3-benzyloxy-4-methoxyphenyl]ethene (**12b**)

The title compound was prepared similar to that described for **12a** above with the use of **11b** to give **12b** as colorless solid (0.017 g, yield 46%), mp 117–119 °C. ^{1}H NMR (400 MHz, Methanol-d_4) δ (ppm) 7.49–7.30 (10H, m, 2 × benzyl Ph), 7.07 (1H, d, J = 2.0 Hz, H-2′), 7.06 (1H, dd, J = 8.2, 2.0 Hz, H-6′), 6.96 (1H, d, J = 16.2 Hz, H-α), 6.87 (1H, d, J = 8.2 Hz, H-5′), 6.79 (1H, d, J = 16.2 Hz, H-β), 6.99 (1H, t, J = 1.8 Hz, H-6), 6.57 (1H, t, J = 1.8 Hz, H-2), 6.38 (1H, t, J = 2.2 Hz, H-4), 5.19 (2H, s, benzyl CH_2), 5.07 (2H, s, benzyl CH_2), 3.90 (3H, s, OCH_3). ^{13}C NMR (ppm) (100 MHz, Methanol-d_4) δ 160.20, 158.30, 149.68, 148.25, 139.70, 137.41, 137.31, 130.62, 128.16, 128.07 (2C), 128.05 (2C), 127.52, 127.41 (3C), 127.15 (2C), 126.62, 120.35, 111.95 (2C), 105.67, 104.09, 101.11, 70.88 (benzyl CH_2), 69.56 (benzyl CH_2), 55.10 (4′-OCH_3); MS (ESI) m/z (%): 461 ([M + Na]$^+$, 100), 439 ([M + H]$^+$, 30); HRMS (ESI) (m/z): calcd for $C_{29}H_{26}O_4$ [M + H]$^+$ 439.1904, found 439.1907.

5.7. Preparation of E-1-[3-hydroxy-5-methoxyphenyl]-2-[4-benzyloxy-3-methoxyphenyl]ethene (**12c**)

The title compound was prepared similar to that described for **12a** with the use of **11c** to prepare **12c** as yellowish solid (0.019 g, yield 53%), mp 110–112 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.45–7.28 (5H, m, benzyl Ph), 7.07 (1H, d, J = 2.0 Hz, H-2′), 7.00 (1H, d, J = 16.6 Hz, H-α), 6.98 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.87 (1H, d, J = 16.8 Hz, H-β), 6.85 (1H, d, J = 8.4 Hz, H-5′), 6.62 (1H, t, J = 1.8 Hz, H-6), 6.57 (1H, t, J = 1.8 Hz, H-2), 6.31 (1H, t, J = 2.2 Hz, H-4), 5.17 (2H, s, benzyl CH_2), 4.94 (1H, s, OH), 3.94 (3H, s, OCH_3), 3.81 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 161.08, 156.86, 149.73, 148.16, 139.85, 136.96, 130.69, 129.11, 128.57 (2C), 127.90, 127.29 (2C), 126.61, 119.95, 113.97, 109.47, 105.72, 104.73, 100.72, 71.03, 56.03 (3′-OCH_3), 55.36 (5-OCH_3); MS (ESI) m/z (%): 385 ([M + Na]$^+$, 100), 363 ([M + H]$^+$, 18); 323 (40); HRMS (ESI) (m/z): calcd for $C_{23}H_{22}O_4$ [M + H]$^+$ 363.1591, found 363.1592.

5.8. Preparation of E-1-[5-benzyloxy-3-(3-methylbut-2-enyloxy) phenyl]-2-[4-benzyloxy-3-methoxyphenyl]ethene (**13a**)

5.8.1. General method

To the suspension of sodium hydride (60% dispersion in mineral oil, 0.005 g, 0.125 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) at 0 °C under nitrogen was added dropwise the solution of **12a** (0.04 g, 0.091 mmol) in *N*,*N*-dimethylformamide (3 mL) followed by the addition of 3,3-dimethylallyl bromide (0.011 mL, 0.091 mmol) dropwise [31]. The resulting mixture was stirred at room temperature for 2–3 h, quenched with cold distilled water (5 mL), acidified with cold 0.1 M hydrochloric acid (5 mL) and extracted with diethyl ether (3 × 10 mL). The combined organic layers were washed with distilled water (2 × 10 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure. The residue was purified on silica gel using hexane/ethyl acetate (2:1) as mobile phase to afford **13a** as yellowish oil (0.02 g, yield 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.46–7.28 (10H, m, 2 × benzyl Ph), 7.07 (1 H, d, J = 2.0 Hz, H-2′), 7.01 (1H, d, J = 16.4 Hz, H-α), 6.96 (1H, d, J = 8.0, 2.0 Hz, H-6′), 6.90 (1H, d, J = 16.4 Hz, H-β), 6.87 (1H, d, J = 8.4 Hz, H-5′), 6.73 (1H, t, J = 2.0 Hz, H-6), 6.68 (1H, t, J = 2.0 Hz, H-2), 6.48 (1H, t, J = 2.2 Hz, H-4), 5.52–5.49 (1H, m, H-2″), 5.17 (2H, s, benzyl CH_2), 5.07 (2H, s, benzyl CH_2), 4.52 (2H, d, J = 6.8 Hz, H-1″), 3.95 (3H, s, OCH_3), 1.80 (3H, d, J = 0.8 Hz, H-4″), 1.76 (3H, d, J = 0.8 Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 160.21, 160.12, 149.78, 148.14, 139.49, 138.35, 137.03, 136.94, 130.76 (C-β), 128.94, 128.59 (2C), 128.56 (2C), 127.94, 127.86, 127.54 (2C), 127.24 (2C), 126.97 (C-α), 119.87, 119.53, 113.98, 109.43, 105.38, 105.32, 101.14, 71.01 (benzyl CH_2), 70.09 (benzyl CH_2), 64.84 (C-1″), 56.03 (3′-OCH_3), 25.85 (C-4″), 18.22 (C-5″); MS (ESI) m/z (%): 529 ([M + Na]$^+$, 100), 507 ([M + H]$^+$, 30); HRMS (ESI) (m/z): calcd for $C_{34}H_{34}O_4$ [M + H]$^+$ 507.2530, found 507.2528.

5.9. Preparation of E-1-[5-benzyloxy-3-(3-methylbut-2-enyloxy) phenyl]-2-[3-benzyloxy-4-methoxyphenyl]ethene (**13b**)

The title compound was prepared similar to that described for **13a** with the use of **12b** to give **13b** as off-white solid (0.022 g, yield 47%), mp 88–90 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.49–7.32 (10H, m, 2 × benzyl Ph), 7.08 (1H, d, J = 2.0 Hz, H-2′), 7.06 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 6.97 (1H, d, J = 16.4 Hz, H-α), 6.89 (1H, d, J = 8.4 Hz, H-5′), 6.82 (1H, d, J = 16.4 Hz, H-β), 6.71 (1H, t, J = 1.6 Hz, H-6), 6.66 (1H, t, J = 1.6 Hz, H-2), 6.47 (1H, t, J = 2.0 Hz, H-4), 5.53–5.49 (1H, m, H-2″), 5.20 (2H, s, benzyl CH_2), 5.07 (2H, s, benzyl CH_2), 4.52 (2H, d, J = 6.4 Hz, H-1″), 3.91 (3H, s, OCH_3), 1.80 (3H, d, J = 0.8 Hz, H-4″), 1.75 (3H, d, J = 0.8 Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 161.04, 160.95, 150.51, 149.11, 140.24, 139.03, 137.81, 137.67, 130.91 (C-β), 129.57, 129.27 (4C), 128.66, 128.58, 128.22 (2C), 128.05 (2C), 127.50 (C-α), 121.10, 120.20, 112.48, 112.40, 105.95, 105.86, 101.60, 71.55 (benzyl CH_2), 70.47 (benzyl CH_2), 65.18 (C-1″), 56.36 (4′-OCH_3), 26.00 (C-4″), 18.33 (C-5″); MS (ESI) m/z (%): 529 ([M + Na]$^+$, 88), 507 ([M + H]$^+$, 18), 323 (100); HRMS (ESI) (m/z): calcd for $C_{34}H_{34}O_4$ [M + H]$^+$ 507.2530, found 507.2533.

5.10. Preparation of E-1-[3-(3-methylbut-2-enyloxy)-5-methoxyphenyl]-2-[4-benzyloxy-3-methoxyphenyl]ethene (**13c**)

The title compound was prepared similar to that described for **13a** with the use of **12c** to give **13c** as off-white solid (0.011 g, yield 46%), mp 66–69 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.28 (5H, m, benzyl Ph), 7.08 (1H, d, J = 2.0 Hz, H-2′), 7.02 (1H, d, J = 16.0 Hz, H-α), 6.98 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.90 (1H, d, J = 16.0 Hz, H-β),

6.87 (1H, d, $J = 8.4$ Hz, H-5′), 6.67 (1H, t, $J = 1.8$ Hz, H-6), 6.64 (1H, t, $J = 1.8$ Hz, H-2), 6.40 (1H, t, $J = 2.2$ Hz, H-4), 5.53–5.49 (1H, m, H-2″), 5.17 (2H, s, benzyl CH_2), 4.53 (2H, d, $J = 6.8$ Hz, H-1″), 3.96 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 1.81 (3H, d, $J = 0.8$ Hz, H-4″), 1.76 (3H, d, $J = 0.8$ Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.91, 160.23, 149.76, 148.14, 139.46, 138.35, 137.04, 130.78 (C-β), 128.90, 128.56 (2C), 127.87, 127.25 (2C), 127.02 (C-α), 119.86, 119.56, 113.98, 109.44, 104.96, 104.49, 100.37, 71.02 (benzyl CH_2), 64.82 (C-1″), 56.04 (3′-OCH_3), 55.36 (5-OCH_3), 25.86 (C-4″), 18.22 (C-5″); MS (ESI) m/z (%): 453 ([M + Na]$^+$, 85); HRMS (ESI) (m/z): calcd for $C_{28}H_{30}O_4$ [M + Na]$^+$ 453.2036, found 453.2036.

5.11. Preparation of E-1-[5-hydroxy-3-(3-methylbut-2-enyloxy) phenyl]-2-[4-hydroxy-3-methoxyphenyl]ethene (**14a**)

5.11.1. General method

To the solution of **13a** (0.02 g, 0.035 mmol) in absolute ethanol (8 mL), was added 1,4-cyclohexadiene (3 mL, 0.030 mmol) and palladium on charcoal (10%, 0.002 g). The reaction mixture was refluxed with stirring under nitrogen, for 1–2 h and monitored by TLC. The product was filtered and evaporated under reduced pressure to give a yellowish residue which was purified by preparative HPLC on a silica gel column, eluted with hexane/ethyl acetate (2:1) to afford **14a** as yellowish oil (0.0025 g, yield 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.02 (1H, d, $J = 2.0$ Hz, H-2′), 7.00 (1H, d, $J = 16.4$ Hz, H-α), 6.99 (1H, d, $J = 8.0$ Hz, H-5′), 6.91 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 6.85 (1H, d, $J = 16.4$ Hz, H-β), 6.64 (1H, t, $J = 1.6$ Hz, H-2), 6.56 (1H, t, $J = 1.6$ Hz, H-6), 6.33 (1H, t, $J = 2.0$ Hz, H-4), 5.53–5.48 (1H, m, H-2″), 4.52 (2H, d, $J = 6.8$ Hz, H-1″), 3.95 (3H, s, OCH_3), 1.82 (3H, d, $J = 1.0$ Hz, H-4″), 1.76 (3H, d, $J = 1.0$ Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 160.38, 156.70, 146.67, 145.69, 139.87, 138.37, 129.73, 129.29 (C-β), 126.15 (C-α), 120.63, 119.49, 114.54, 108.24, 105.58, 105.38, 101.29, 64.85 (C-1″), 55.92 (3′-OCH_3), 25.85 (C-4″), 18.22 (C-5″); MS (ESI) m/z (%): 325 ([M − H], 100); HRMS (ESI) (m/z): calcd for $C_{20}H_{22}O_4$ [M + H]$^+$ 327.1590, found 327.1588.

5.12. Preparation of E-1-[5-hydroxy-3-(3-methylbut-2-enyloxy) phenyl]-2-[3-hydroxy-4-methoxyphenyl]ethene (**14b**)

The title compound was prepared similar to that described for **14a** above with the use of **13b** to give **14b** as yellowish oil (0.003 g, yield 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.13 (1H, d, $J = 2.0$ Hz, H-2′), 6.98 (1H, d, $J = 16.0$ Hz, H-α), 6.97 (1H, dd, $J = 8.0$, 2.2 Hz, H-6′), 6.86 (1H, d, $J = 8.0$ Hz, H-5′), 6.86 (1H, d, $J = 16.0$ Hz, H-β), 6.64 (1H, t, $J = 2.0$ Hz, H-2), 6.57 (1H, t, $J = 2.0$ Hz, H-6), 6.33 (1H, t, $J = 2.0$ Hz, H-4), 5.60 (1H, s, OH), 5.52–5.48 (1H, m, H-2″), 4.77 (1H, s, OH), 4.52 (2H, d, $J = 6.8$ Hz, H-1″), 3.91 (3H, s, OCH_3), 1.82 (3H, d, $J = 0.8$ Hz, H-4″), 1.76 (3H, d, $J = 0.8$ Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 160.37, 156.69, 146.51, 145.75, 139.83, 138.36, 130.87, 128.92 (C-β), 126.78 (C-α), 119.51, 119.40, 111.84, 110.62, 105.66, 105.48, 101.33, 65.18 (C-1″), 56.36 (4′-OCH_3), 26.00 (C-4″), 18.33 (C-5″); MS (ESI) m/z (%): 349 ([M + Na]$^+$, 24); HRMS (ESI) (m/z) calcd for $C_{20}H_{22}O_4$ [M + Na]$^+$ 349.1410, found 349.1408.

Prenylated tetrahydroxystilbenes **15a** and **15b** were formed as side products as the result of saturation of the double bond of **13a** and **13b**, respectively. These compounds were purified and characterised as follows.

5.13. 1-[5-hydroxy-3-(3-methylbut-2-enyloxy)phenyl]-2-[4-hydroxy-3-methoxyphenyl]ethane (**15a**)

The title compound was obtained as a yellowish oil (0.002 g, 15%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.84 (1H, d, $J = 2.0$ Hz, H-2′), 6.69 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 6.62 (1H, d, $J = 8.0$ Hz, H-5′), 6.34 (1H, t, $J = 1.9$ Hz, H-6), 6.26 (1H, t, $J = 1.9$ Hz, H-2), 6.24 (1H, t, $J = 1.8$ Hz, H-4), 5.46–5.70 (1 H, m, H-2″), 5.46 (1H, s, OH), 4.68 (1H, s, OH), 4.46 (2H, d, $J = 6.8$ Hz, H-1″), 3.84 (3H, s, OCH_3), 2.85–2.76 (4H, m, CH_2CH_2), 1.80 (3H, d, $J = 0.4$ Hz, H-4″), 1.73 (3H, d, $J = 0.4$ Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 160.11, 156.44, 146.22, 144.46, 143.73, 138.23, 133.61, 120.95, 119.55, 114.15, 111.10, 107.89, 107.58, 99.62, 64.72 (C-1″), 55.84 (3′-OCH_3), 38.29 (CH_2CH_2), 37.23 (CH_2CH_2), 25.84 (C-4″), 18.18 (C-5″); MS (ESI) m/z (%): 351 ([M + Na]$^+$, 100); HRMS (ESI) (m/z): calcd for $C_{20}H_{24}O_4$ [M + Na]$^+$ 351.1566, found 351.1566.

5.14. 1-[5-hydroxy-3-(3-methylbut-2-enyloxy)phenyl]-2-[3-hydroxy-4-methoxyphenyl]ethane (**15b**)

The title compound was obtained as a yellowish oil (0.0027 g, 18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.79 (1H, d, $J = 2.0$ Hz, H-2′), 6.77 (1H, d, $J = 8.0$ Hz, H-5′), 6.66 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 6.35 (1H, t, $J = 2.0$ Hz, H-6), 6.27–6.25 (2H, m, H-2, 4), 5.56 (1H, s, OH), 5.50–5.46 (1H, m, H-2″), 4.74 (1H, s, OH), 4.46 (2H, d, $J = 6.8$ Hz, H-1″), 3.88 (3H, s, OCH_3), 2.80–2.78 (4H, m, CH_2CH_2), 1.80 (3H, d, $J = 1.2$ Hz, H-4″), 1.74 (3H, d, $J = 1.2$ Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 160.11, 156.42, 145.41, 144.80, 144.51, 138.18, 135.07, 119.73, 119.59, 114.59, 110.55, 107.81, 107.49, 99.64, 64.73 (C-1″), 55.00 (4′-OCH_3), 38.05 (CH_2CH_2), 36.89 (CH_2CH_2), 25.83 (C-4″), 18.18 (C-5″); MS (ESI) m/z (%): 351 ([M + Na]$^+$, 100); HRMS (ESI) (m/z) calcd for $C_{20}H_{24}O_4$ [M + Na]$^+$ 351.1566, found 351.1566.

5.15. Preparation of E-1-[5-hydroxy-3-methoxy-2-(3-methyl-2-butenyl)phenyl]-2-[4-benzyloxy-3-methoxyphenyl]ethene (**16**)

To a solution of **13c** (0.024 g, 0.061 mmol) in toluene (30 mL) was added 60–100 mesh Florisil (0.24 g, 10×). The reaction mixture was refluxed at 110 °C under nitrogen, for 4 h, then filtered and evaporated under reduced pressure to give a brownish residue which was purified on silica gel using hexane–ethyl acetate (2:1) to afford a yellowish solid. The product was recrystallised from a mixture of hexane/ethyl acetate (3:1) affording **16** as an off-white powder (0.012 g, 59%), mp 161–162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.46–7.28 (5H, m, benzyl Ph), 7.19 (1H, d, $J = 16.0$ Hz, H-α), 7.06 (1H, d, $J = 2.0$ Hz, H-2′), 6.97 (1H, dd, $J = 8.4$, 2.0 Hz, H-6′), 6.88 (1H, d, $J = 16.0$ Hz, H-β), 6.87 (1H, d, $J = 8.4$ Hz, H-5′), 6.66 (1H, t, $J = 2.4$ Hz, H-6), 6.40 (1H, t, $J = 2.4$ Hz, H-4), 5.18 (2H, s, benzyl CH_2), 5.13–5.09 (1H, m, H-2″), 4.64 (1H, s, OH), 3.94 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.42 (2H, d, $J = 6.4$ Hz, H-1″), 1.80 (3H, d, $J = 1.2$ Hz, H-4″), 1.68 (3H, d, $J = 1.2$ Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 158.56, 154.35, 149.76, 148.07, 138.11, 137.04, 131.21, 130.63 (C-β), 130.24, 128.56 (2C), 127.86, 127.22 (2C), 124.73 (C-α), 123.61, 120.78, 119.84, 113.99, 109.42, 103.88, 98.21, 71.03 (benzyl CH_2), 55.97 (3′-OCH_3), 55.68 (3-OCH_3), 25.77 (C-1″), 24.46 (C-4″), 17.98 (C-5″); MS (ESI) m/z (%): 453 ([M + Na]$^+$, 100), 431 ([M + H]$^+$, 75); HRMS (ESI) (m/z): calcd for $C_{28}H_{30}O_4$ [M + H]$^+$ 431.2217, found 431.2217.

5.16. Preparation of E-1-[5-hydroxy-3-methoxy-2-(3-methyl-2-butenyl)phenyl]-2-[4-hydroxy-3-methoxyphenyl]ethene (**17**)

The title compound was prepared similar to that described for **14a** with the use of **16** to give **17** as yellowish oil (0.013 g, 65%) after purification by silica gel column chromatography using hexane–ethyl acetate (2:1) and HPLC using hexane–isopropanol (2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.17 (1H, d, $J = 16.0$ Hz, H-α), 7.01 (1H, dd, $J = 2.0$ Hz, H-2′), 7.0 (1H, d, $J = 8.2$, 2.0 Hz, H-6′), 6.91 (1H, d, $J = 8.0$ Hz, H-5′), 6.87 (1H, d, $J = 16.0$ Hz, H-β), 6.66 (1H, t, $J = 2.4$ Hz, H-6), 6.36 (1H, t, $J = 2.4$ Hz, H-4), 5.66 (1H, s, OH), 5.15–5.09 (1H, m, H-2″), 4.64 (1H, s, OH), 3.94 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.42

(2H, d, J = 6.8 Hz, H-1″), 1.81 (3H, d, J = 1.0 Hz, H-4″), 1.68 (3H, d, J = 1.0 Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 158.57, 154.37, 146.68, 145.60, 138.18, 130.61, 130.44, 130.27, 124.28, 123.67, 120.73, 120.58, 114.55, 108.26, 103.88, 98.18, 55.88 (3′-OCH_3), 55.71 (3-OCH_3), 25.79 (C-1″), 24.48 (C-4″), 17.98 (C-5″); MS (ESI) m/z (%): 339 ([M − H], 100); HRMS (ESI) (m/z): calcd for $C_{21}H_{24}O_4$ [M + Na]$^+$ 363.1566, found 363.1566.

5.17. 1-[5-hydroxy-3-methoxy-2-(3-methyl-2-butenyl)phenyl]-2-[4-hydroxy-3-methoxyphenyl]ethane (**18**)

The title compound was formed as side product as the result of saturation of the double bond of **17**. The product was obtained as yellowish oil (0.005 g, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.86 (1H, d, J = 8.0 Hz, H-5′), 6.70 (1H, dd, J = 2.0 Hz, H-2′), 6.64 (1H, d, J = 8.0, 2.0 Hz, H-6′), 6.30 (1H, d, J = 2.4 Hz, H-6), 6.24 (1H, d, J = 2.4 Hz, H-4), 5.48 (1H, s, OH), 5.07–5.03 (1H, m, H-2″), 4.67 (1H, s, OH), 3.85 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.28 (2H, d, J = 6.4 Hz, H-1″), 2.82–2.73 (4H, m, CH_2CH_2), 1.74 (3H, d, J = 1.2 Hz, H-4″), 1.66 (3H, d, J = 1.2 Hz, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 158.63, 154.23, 146.23, 143.73, 142.01, 133.93, 130.59, 123.87, 120.92, 120.67, 114.21, 111.02, 107.96, 96.90, 55.84 (3′-OCH_3), 55.60 (3-OCH_3), 37.19, 35.49, 25.76 (C-1″), 24.38 (C-4″), 17.95 (C-5″); MS (ESI) m/z (%):381 ([M + K]$^+$26), 365 ([M + Na]$^+$, 100), 286 (20), 137 (28); HRMS (ESI) (m/z): calcd for $C_{21}H_{26}O_4$ [M + Na]$^+$ 365.1723, found 365.1723.

5.18. Preparation of methyl (E)-4-(4′-hydroxy-3′-methylbut-(E)-2′-enyloxy)cinnamate (**21**)

To a solution of dichloromethane (2 mL) in round bottom flask was added selenium dioxide (0.066 g) and *tert*-butylhydroperoxide (TBHP) (0.3 mL). The mixture was stirred at room temperature for half an hour then added a solution of **20** (0.135 g) in dichloromethane (1 mL). The resulting mixture was stirred at room temperature for 24 h. The product was extracted twice with dichloromethane (5 mL) and 20% NaCl solution (5 mL). The organic layers were combined, washed with water (5 mL), dried over $MgSO_4$, and evaporated under reduced pressure. The product was purified by vacuum column chromatography on silica gel. The column was eluted with a stepwise gradient of hexane/ethyl acetate (0, 20, 35, 40, 50, 60 and 100%; 2 × 50 mL each) to give **21** as white solid (0.055 g, yield 38%), mp 94–96 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.64 (1H, *dd*, J = 15.9 Hz), 7.46 (2H, *dd*, J = 8.8 Hz), 6.90 (2H, *dd*, J = 8.8 Hz), 6.30 (1H, *dd*, J = 15.9 Hz), 5.76 (1H, *bt*, J = 6.5 Hz), 4.62 (2H, *dd*, J = 6.5 Hz), 4.08 (2H, *s*), 3.79 (3H, *s*), 1.77 (3H, *s*); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.9, 160.5, 144.6, 140.5, 129.7, 127.1, 119.3, 115.2, 115.0, 67.6, 64.4, 51.6, 14.0; HRMS(ESI) (m/z): calcd for $C_{15}H_{18}O_4$ [M + Na]$^+$ 285.1097, found 285.1096.

In addition to the production of the title compound **21**, there was formation of minor aldehyde derivative as white solid (0.017 g, yield 12%), mp 110–112 °C determined as methyl (*E*)-4-(3′-carbonylbut-(*E*)-2′-enyloxy)cinnamate (**22**). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.49 (1H, *s*), 7.66 (1H, *dd*, J = 16 Hz), 7.50 (2H, *dd*, J = 8.8 Hz), 6.92 (2H, *dd*, J = 8.8 Hz), 6.67 (1H, *tm*, J = 5.6 Hz), 6.33 (1H, *dd*, J = 16 Hz), 4.91 (2H, *dd*, J = 5.6 Hz), 3.80 (3H, *s*), 1.85 (3H, *dd*, J = 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 193.9, 167.6, 159.7, 146.8, 144.2, 140.1, 129.8, 127.9, 115.8, 114.9, 64.8, 51.6, 9.7; HRMS (ESI) (m/z): calcd for $C_{15}H_{16}O_4$ [M + Na]$^+$ 283.0941, found 283.0941.

5.19. Cytotoxicity activity

K562 cell line was maintained and incubated. Cells were treated with the compounds at a range of concentrations (0.01–100 μM) for 72 h at 37 °C in a humidified incubator with 5% of carbon dioxide. Data is the average of two independent assays each performed in duplicate. Two concentrations were used for each compound in order to estimate the IC_{50} value. DMSO was used as vehicle at the final concentration of 0.5% and at this concentration showed no effect on cell growth (data not shown).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.02.017. These data include MOL files and InChiKeys of the most important compounds described in this article.

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