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PII: S0223-5234(13)00358-9

DOI: 10.1016/j.ejmech.2013.05.040

Reference: EJMECH 6218

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 5 March 2013

Revised Date: 20 May 2013

Accepted Date: 25 May 2013

Please cite this article as: M. Miliovsky, I. Svinyarov, Y. Mitrev, Y. Evstatieva, D. Nikolova, M. Chochkova, M.G. Bogdanov, A Novel One-pot Synthesis and Preliminary Biological Activity Evaluation of *cis*-Restricted Polyhydroxy Stilbenes Incorporating Protocatechuic acid and Cinnamic Acid Fragments, *European Journal of Medicinal Chemistry* (2013), doi: 10.1016/j.ejmech.2013.05.040.

**Research Highlights:**

- Polyhydroxylated *cis*-stilbenes are readily available via a novel one-pot synthetic procedure
- *trans*-Cinnamic acid fragment ensures *cis*-configuration of the stilbene backbone
- *cis*-Stilbenes possess a triple biological action
- Combination of two pharmacophoric fragments in one molecule evoke synergistic effect
- Dicarboxylic acid fragment might be important for tyrosinase inhibition

# A Novel One-pot Synthesis and Preliminary Biological Activity Evaluation of *cis*-Restricted Polyhydroxy Stilbenes Incorporating Protocatechuic acid and Cinnamic Acid Fragments

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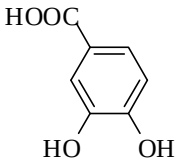
**Abstract**:

A series of new stilbenes **4a-e**, **5** were synthesized through a novel one-pot Perkin-like reaction between 6,7-dimethoxyhomophthalic anhydride and aromatic aldehydes, followed by treatment with $BBr_3$. This synthesis is straightforward and allows polyhydroxylated *cis*-stilbenes gathering two well-known pharmacophoric fragments to be obtained in good yields and for short reaction times. The structure of the newly synthesized compounds was established by spectroscopic methods ($^1$H NMR, $^{13}$C NMR, IR and HRMS) and the double bond configuration was unequivocally elucidated by means of gated decoupling $^{13}$C NMR spectra and 2D NOESY experiments. Preliminary differentiating screening of their radical scavenging, antibacterial, anti-fungal and tyrosinase inhibitory activity was further performed. The results obtained suggest that the tested compounds possess a triple biological action as potent radical scavengers, antifungal agents and tyrosinase inhibitors in micromolar concentration. The most promising bioactive compound amongst the others was **4a**, acting as excellent radical scavenger against DPPH$^\bullet$ radical ($IC_{50} \leq 10$ μM), antifungal agent suppressing the growth of *F. graminearum* (89% inhibition at 0.17 μmol/mL), and tyrosinase inhibitor showing higher activity than hydroquinone at 23 μM.

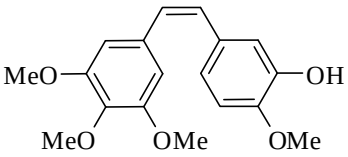
*Keywords*: homophthalic anhydride, polyhydroxy stilbenes, antioxidant activity, antifungal activity, tyrosinase inhibitors

## 1. Introduction

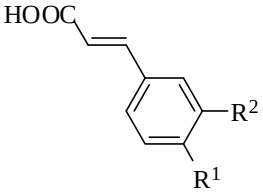
Stilbenoids are a class of natural compounds, formed by a particular branch of the flavonoid biosynthetic pathway. Being first isolated from plants in 1899, they attracted the scientific community attention due to their chemical diversity and wide range of biological effects [1]. Nowadays it is widely accepted, that stilbenoids play an important role as phytoalexins in plant resistance to fungal pathogens [2]. Recently, natural hydroxy stilbenes (a particular group of stilbenoids) such as resveratrol and combretastatin, as well as their synthetic analogues, become of a great interest to the medicinal chemists. These compounds have been proved to be antioxidant reagents, that inhibit cell apoptosis, suppress growing cancer cells, influence the activity of specific enzymes, effect the animal ageing and metabolisms of estrogens [3-12], etc. Although the hydroxy stilbenoids possess remarkable medical benefits, it is noteworthy that their bioavailability is very narrow. In fact they are only produced in nanogram scale in plants, in response to stress situations such as fungal infection or injury, and thus, it is hardly to be obtained in large quantities from their natural sources. To overcome this, various synthetic approaches for the synthesis of stilbenes have been developed including Colvin rearrangement to an alkyne, followed by selective semi-reduction [13], Suzuki cross-coupling [14,15], Heck [16], Wittig [17-18] and Perkin reactions [19-22], to name just a few. It is however rather important to be mentioned, that the formation of the carbon–carbon double bond between the two aromatic rings is the key step in all the synthetic approaches developed, but in most of the cases a mixture of *cis*- and *trans*- isomers is produced. Thus, additional work is required in order these isomeric mixtures to be separated, which lowers the products yields and limits the benefits of these approaches from an applied standpoint. Moreover, the classical methods such as Suzuki and Heck reactions usually require relatively long synthetic sequences and use of expensive catalyst and reagents. Therefore, the need from fast and highly efficient stereo specific synthetic methods for large-scale preparation of such compounds can be put forward.



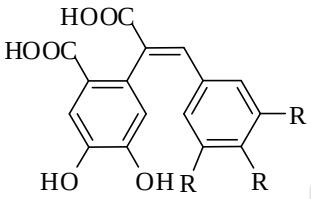

Protocatechuic acid




Combretastatin A-4




cinnamic acid: $R^1 = R^2 = H$
*p*-coumaric acid: $R^1 = OH$; $R^2 = H$
caffeic acid: $R^1 = R^2 = OH$




Target compounds
in this study
R = H or OH or *t*-Bu

In this context, herein we report a novel highly diastereoselective synthesis of polyhydroxy *cis*-stilbenes by means of one-pot procedure, including initial Perkin-like reaction between commercially available homophthalic anhydrides and aromatic aldehydes, followed by treatment with $BBr_3$. This straightforward procedure allows the synthesis of polyhydroxylated stilbenes in high yields and as single *cis*-isomers, which are structurally based on two independent pharmacophoric fragments, such as protocatechuic acid and cinnamic acid. It is noteworthy, that the incorporated *trans*-cinnamic acid fragment into the target compounds structure ensures *cis*-configuration of the stilbene backbone and prevents further isomerization along the carbon–carbon double bond. Furthermore, the combination of two different and independently acting fragments of well-known pharmacological profiles [23-29] into one covalently bonded hybrid molecule can evoke synergistic effect, which is a precondition of an enhanced biological activity to be expected. Therefore, in order the synergistic effect of this combination of pharmacophoric moieties to be assessed, the synthesized compounds were subjected to a preliminary *in vitro* study of their radical scavenging, antibacterial, antifungal and tyrosinase inhibitory activities.

## 2. Results and discussion

### *2.1. Chemistry*

Amongst the above mentioned synthetic approaches for the formation of carbon-carbon double bond, the Perkin reaction between benzaldehydes and phenylacetic acids in the presence of acetic

anhydride and a base proved to be useful in the synthesis of stilbenes [19-22]. Despite its utility, however, the classical Perkin reaction requires high temperature and prolonged reaction time. Moreover, in order polyhydroxylated stilbenes to be synthesized, one should follow multistep approach involving sequential protection, condensation, decarboxylation and deprotection steps. This sequence is necessary due to the extreme susceptibility of the hydroxyl function towards oxidation and polymerization, but results in a low overall product yields. To overcome these limitations, we developed a novel one-pot procedure for the synthesis of polyhydroxy stilbenes, including initial Perkin-like reaction between commercially available homophthalic anhydrides and aromatic aldehydes, followed by treatment with $BBr_3$. The synthetic strategy towards the target compounds **4** and their substitution pattern are presented in Scheme 1. The idea behind is coming from recent studies of ours [30-32], where we have shown, that homophthalic anhydride reacts with substituted benzaldehydes in the presence of 4-dimethylaminopyridine (DMAP) at room temperature to produce diastereomeric mixture of *cis*- and *trans*-3-aryl-3,4-dihydroisocoumarin-4-carboxylic acids (**3**) [30] and that in presence of a base these acids undergo rapid lactone ring opening reaction to form an equilibrium mixture of **3** and the corresponding dicarboxylic acid of type **4** [32]. It is noteworthy, that **4** were obtained as single diastereomers with *cis*-stilbene backbone, regardless the relative configuration of the starting compounds. Based on the above, we were initially interested in evaluating of the behavior of acids **3** in the presence of $BBr_3$ ($CH_2Cl_2$ solution) and to check the stability of the lactone ring under demethylation reaction conditions. So, we found that acid *trans*-**3a** (used as a model compound) undergo quantitative conversion to the hydroxystilbene acid **4a**. Considering **3** as intermediates in the reaction scheme towards the target compounds, we further assumed possible synthesis shortening by addition of $BBr_3$ solution to the anhydride-aldehyde reaction mixture, immediately after the corresponding acids **3** are formed.

Indeed, this approach proved successful, showing the possible formation of **4** without isolation of any intermediates. Following that procedure, aromatic aldehydes of different substitution pattern (**2a-e**) were initially reacted with 6,7-dimethoxyhomophthalic anhydride (**1**). On the one hand, this provides the protocatechuic acid pharmacophoric fragment as ring A at the end of the synthetic sequence, and on the other, it allows the influence of the substituents in ring B on the biological activity of compounds **4** to be studied. The diastereomeric mixtures of *cis*- and *trans*-**3**, produced from the above reaction in presence of DMAP/$CH_2Cl_2$, were further successfully

converted into **4** by direct addition of $BBr_3/CH_2Cl_2$ solution at room temperature. The liquid chromatography analysis of the reaction mixtures showed, that **3** were formed in 10-15 min in all cases, whereas the time needed for the formation of **4** depends on the number of groups, which have to be demethylated. To study this, individual reactions were performed for each compound, and the products yields were estimated after working-up the reaction mixtures on every 10 min. The results obtained showed that the least substituted **4a** (two hydroxyl groups) and the highly substituted **4d** (5 hydroxyl groups) were obtained in 10 and 60 min, respectively, i.e. the higher substituted nuclei, lead to an increase in reaction time. Summarizing, if one follows that one-pot procedure, polyhydroxylated *cis*-stilbenes are easily available from commercial starting materials in less than 90 min. The compounds synthesized in this way were purified by column chromatography and their structures were characterized by spectral methods ($^1$H and $^{13}$C NMR, IR, HRMS). The configuration of the stilbene double bond was estimated as describe in Section 2.2.

It is noteworthy, that when **1** reacts with 3,5-di-*tert*-butyl-4-hydroxybenazaldehyde (**2e**), *cis*-stilbene **5** was formed instead the expected 3,4-dihydroisocoumarin acid of type **3**. The latter can be attributed to the positive mesomeric effect of the hydroxyl group, which induces a lactone ring opening reaction. This chance, however, allowed the influence of the catechol moiety in the protocatechuic acid fragment on the biological effects to be assessed. On the other hand, it showed the possibility for diversification of the developed synthetic procedure, and now experiments on the reactions of homophthalic anhydrides with *p*-hydroxybenzaldehyde derivatives are in progress in our laboratory.

*2.2. Structure elucidation*

It is noteworthy, that the unique substitution pattern of the polyhydroxystilbene acids **4a-e** poses difficulties in their structural elucidation. In most of the cases, their proton NMR spectra consist mainly of singlets, and thus, the lack of proton-proton coupling constants data yields practically no information in respect to the double bond configuration. Very recently Zou and coworkers [21] found, that the vinyl protons in *E*-isomers of a series of *E*-2,3-diarylacrilic acids appear at lower field compared to the same protons in the *Z*-isomers, which was attributed to the field effect of the carboxylic group. Nevertheless, the lack of spectroscopic data for the opposite diastereomers suggests limited applicability of this approach for determination of the double

bond configuration of such systems, since the chemical shifts for the vinyl proton (H8) seem to be dependent on the substituents electronic effects (see Table 1). Moreover, the possible signal overlapping in some cases could lead to wrong data interpretation. To overcome this problem, an approach based on the heteronuclear coupling constants could be applied, since it is known, that the vicinal heteronuclear coupling constants exhibit Karplus type relationship between its value and the dihedral angle between the interacting nuclei [33]. This approach has already proved successful and accurate for configuration studies of similar compounds, even when the diastereomers with the opposite configuration are not available [32]. In order to elucidate the configuration of the herein synthesized compounds and to evaluate the substituents effect on their spectral properties, acids **4a**, **4b** and **4d** containing no, one and three hydroxylic groups into ring B, respectively, were chosen as model compounds. Gated decoupling $^{13}C$ NMR spectra for those compounds were recorded and the corresponding H8-C7 and H2-C1a coupling constants were extracted. The assignment of the two carbonyl signals was made using HSQC and HMBC spectra. The data obtained is summarized in Table 1.

The most important information gained from the above mentioned experiments is the observed low values of the coupling constants for H2-C1a and H8-C7, which were found in all cases in the range about 4 Hz and 7 Hz for, respectively. These results are in excellent agreement with the literature data for *cis*-orientation along the double bond of such a spin system [32,33] and allow *cis*-configuration of the stilbene moiety in compounds **4** to be assigned. It should be noted, that the opposite *trans*-orientation results in higher (14-16 Hz) heteronuclear coupling constants [33], which is obviously not the case here. Nevertheless, the assigned configuration for **4** was further independently estimated by means of 2D NOESY experiments. Using this NMR technique the spatial proximity between particular nuclei could be established. The performed analysis showed no cross-peaks between H8 and H5 which allowed the *trans*-orientation of H8 and aromatic ring A to be proved.

Regarding the relationship chemical shifts–substituents effect, it is noteworthy, that the presence of hydroxyl groups in ring B does not influence significantly the chemical shifts of the two carboxylic groups and the protons in ring A, thus making them useful probe for the configuration assignment of structural analogues. In all cases, the signal corresponding to H2 resonates at 7.45 ppm and that for H5 is around 6.35-6.40, which can be explained by the shielding effect of ring B, and the C-7 carbonyl has chemical shift in the interval between

168.25-168.74 ppm. This allowed us to attribute *cis*-configuration of the stilbene moiety in acids **4c**, **4e** and **5** just by comparing the chemical shifts for the selected signals.

In summary, although it was possible to prove the double bond configuration by NMR in this particular study, the full characterization of those systems is far from trivial and requires more sophisticated experiments, along with meticulous extraction and interpretation of all heteronuclear coupling constants.

### *2.3. Biological activity*

#### *2.3.1. DPPH$^{\bullet}$ scavenging activity*

The DPPH$^{\bullet}$ radical is one of the commercially available stable organic nitrogen radicals, which does not have to be generated prior to analysis. It is easy to perform, highly reproducible and comparable with other methods such as ABTS assay, reduction of superoxide anion and inhibition of lipid peroxidation [34], and has been successfully applied for evaluation of the antioxidant activity of both synthetic and natural antioxidants. Therefore, we applied this method to assess the radical scavenging activity of the synthesized polyhydroxy *cis*-stilbenes. The preliminary assay was performed at 3.3 μM concentration of each of the tested compounds, and the radical scavenging activity was evaluated by measuring *in vitro* the residual DPPH$^{\bullet}$. For comparison, caffeic acid (CA), protocatechuic acid (PCA) and their equimolar mixture (CA+PCA) were used as positive controls. The results obtained are presented in Fig. 1.

In general, the group of compounds shows higher DPPH$^{\bullet}$ radical scavenging activity than the standards used. The activity increases as follows: CA < **5** ≤ PCA ≈ **4a** ≈ (CA + PCA) ≤ **4b** < **4e** < **4c** < **4d**. As can be seen, **5** is the least active compound scavenging 18% of DPPH$^{\bullet}$ radicals, and this can be attributed to the blocked (methylated) catechol moiety into the protocatechuic acid fragment (ring A) and presence of not only one, but sterically hindered hydroxyl group into ring B. Considering compounds **4a-4d**, it can be seen that the presence of a single hydroxyl group at 4$^{th}$ position in ring B (**4b**) did not affect significantly the activity of **4a**, but the addition of second or third did it in a great value, and thus compounds **4c** and **4d** are respectively two- and four-folds of higher activity than the former. This suggests that in addition to the number of the hydroxyl groups, their distribution into the basic stilbene backbone is of a great importance for the hydrogen transfer ability and for the resulting radical stabilization. Interestingly, in case of **4c** a synergistic effect from the combination of two active fragments into one hybrid molecule could

be established. As it can be seen **4c** shows higher activity than CA, PCA and their equimolar mixture, and this could be attributed to the formation of intramolecular intermediates capable to scavenge $DPPH^{\bullet}$ radicals. It is noteworthy that the expected $IC_{50}$ (concentration needed to scavenge 50% of $DPPH^{\bullet}$) for some of the compounds studied should be lower than 3.3 μM, and for the rest lower than 10 μM. This makes them attractive targets for further detailed studies of their antioxidant activity, since it is well-known that one of the main characteristics responsible for the antioxidant activity of phenolic compounds is their ability to scavenge free radicals. The work in this direction is in progress and the results will be published in due course.

*2.3.2. Antimicrobial activity*

The antimicrobial activity of **4a-e**, **5** was screened against four bacterial strains – Gram-positive *Bacillus subtilis* (ATCC 6633), *Staphilococcus aureus* (ATCC 25923), Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeroginosa* (ATCC 10145), one yeast strain – *Candida albicans* (ATCC 10231) and three fungal strains – *Aspergillus niger* (A3), *Aspergillus flavus* (IMI 052104) and *Fusarium graminearum* (ATCC 24373). The analyses were performed as described in Sections 4.3.2 and 4.3.3, and the results obtained for the antifungal activity are presented in Table 2.

Regarding the antibacterial activity, compounds **4a-e** can be considered not to be active against the four bacterial strains in concentration 50 μg/mL. Exception is compound **5**, which shows 44% of the inhibitory effect of the standard Amikacine against *B. Subtilis* at the same concentration. By contrary, the whole group of compounds can be considered as potent antifungal agents. Their activity against the yeast strain *C. albicans* is 34-48% of the inhibitory effect of the standard Nystatin in concentration 50 μg/mL and is in the same range for *A. niger* and *A. flavus*. Considering that narrow range, the apparent antifungal activity can be attributed to some specific interactions between the *cis*-stilbenes and the fungal cells, since it does obviously not depend on the number of the hydroxyl groups. However, the presence of *t*-Bu substituents slightly increases the activity, and thus **4e** and **5** can be considered as the most active amongst the others in the series. It is noteworthy the higher antifungal activity of the tested compounds against *F. graminearum*, and in particular, that of **4a** which inhibit significantly its growth at concentration 0.17 μmol/mL. This is of a great importance from an industrial standpoint since *F. graminearum* is known as a plant pathogen [35] that causes various diseases, such as gibberella

ear rot in corn and fusarium head blight or scab in wheat and other grains. Moreover, the major mycotoxins produced by *F. graminearum* – deoxynivalenol and zearalenone – have been found to be toxigenic in humans and animals [36,37]. Consequently, the investigation of the antifungal activity of other derivatives of **4** against that microorganism should be further considered.

*2.3.3. Tyrosinase inhibitory activity*

Phenolic compounds, such as hydroxycinnamic acids and their derivatives, have been reported to act as promising tyrosinase inhibitors [38-43]. Considering the synthesized *cis*-stilbenes as cinnamic acid analogues, their tyrosinase inhibitory activities were examined using mushroom tyrosinase and *L*-tyrosine as a substrate. This preliminary assay was performed at 23 μM concentration of each of the tested compounds and the monophenolase inhibitory activity was evaluated by measuring the dopachrome product *in vitro* as reported previously by one of us [42]. For comparison, hydroquinone (known as strong, but toxic tyrosinase inhibitor), caffeic acid and protocatechuic acid were used as positive controls. The results obtained are presented in Fig. 2.

Compared to standards, the inhibitory activities increase in the following order: CA ≤ **4d** ≤ **5** ≈ **4e** < **4c** ≤ HQ ≤ PCA ≈ **4b** ≤ **4a**, and thus the compounds tested can be considered as weak (**4d**, **4e** and **5**) or strong (**4a**, **4b** and **4c**) mushroom tyrosinase inhibitors. These results suggest that **4a**, **4b** and **4c** are superior to the rest in the inhibition of tyrosinase and that the number and the position of substituents into the cinnamic acid moiety (ring B) seem to play an important role in triggering the inhibitory effects. Considering the former compounds, it can be seen that the introduction of an additional hydroxyl group slightly decreases the enzyme inhibition, and thus to be concluded that the presence of up to two hydroxyl groups into ring B is essential for potent inhibitory activity. Furthermore, the insertion of third hydroxyl group into position 5 in ring B diminished dramatically the tyrosinase inhibitory effect, and thus **4d** is the least active compound in the series. It is worthy to note, that the same effect was observed for the two butylated *cis*-stilbenes **4e** and **5**, suggesting that the steric effects of the substituents play an important role, when the solute is approaching to the active site of the enzyme. Based on the above arguments, the compounds studied can be considered as competitive tyrosinase inhibitors due to their structure similarity to the natural substrates *L*-tyrosine and *L*-DOPA (Fig. 3). However, considering the results obtained for CA and PCA, it could be deduced that the protocatechuic

acid fragment contributes in a great value to the inhibitory effect since it ensures a catechol group capable to chelate copper in the enzyme. On the other hand, it may not be illogical to be assumed that the homophthalic acid fragment may be also in charge of the apparent activity, and thus the presence of the two carboxylic groups to be taken into account. This assumption can be supported by recent studies [46-48], reporting that homophthalic acid may play a role as a linker in the formation of metal complexes (including Cu(II)) with rigid nitrogen containing ligands. Based on the above, compounds **4** can be also considered as non-competitive or mixed tyrosinase inhibitors, which binds equally well to the enzyme whether or not it has already bound the substrate. However, in order the role of each fragment incorporated into the compounds structure to be clarified and the above speculations to be converted to a conclusive interpretation, additional work is needed.

## 3. Conclusions

A series of polyhydroxy *cis*-stilbenes, structurally based on two independent pharmacophoric fragments such as protocatechuic acid and cinnamic acid, were synthesized by means of a novel one-pot procedure employing commercially available starting materials. The latter allows the room temperature synthesis of stilbenes gathering two well-known pharmacophoric fragments, in good yields and for short reaction times. The incorporated *trans*-cinnamic acid fragment into the target compounds structure was shown to ensure *cis*-orientation of the stilbene backbone and to prevent further isomerization along the carbon–carbon double bond, the configuration of which was unequivocally elucidated by means of gated decopuling $^{13}C$ NMR spectra and 2D NOESY experiments.

The conducted preliminary biological screening showed that the tested compounds possess a triple biological action as potent radical scavengers, antifungal agents and tyrosinase inhibitors in micromolar concentrations. The most promising bioactive compound amongst the others was **4a**, acting as excellent radical scavenger against $DPPH^{\bullet}$ radical ($IC_{50} \leq 10$ μM), antifungal agent suppressing the growth of *F. graminearum* (89% at 0.17 μmol/mL) and better than hydroquinone tyrosinase inhibitor at 23 μM. Moreover, it was shown that the combination of two different and independently acting fragments of well-known pharmacological profiles into one covalently bonded hybrid molecule can evoke synergistic effect resulting in higher than the expected activity.

The biological potential of the synthesized compounds **4a-e** and **5** found in this preliminary study suggest that further work is worth performing. In this regard, in order the role of each fragment incorporated into the compounds structure to be clarified, experiments on the synthesis of more complete series of structural analogues are in progress in our laboratory and the results will be published in due course.

## 4. Experimental section

### *4.1. General remarks*

Most chemicals and mushroom tyrosinase (EC 1.14.18.1) used in this study were purchased from Sigma–Aldrich (FOT, Bulgaria). Hydroquinone was obtained from Ferak (Germany). The organic solvents were of analytical grade. The test microorganism strains used for the microbiological assays were taken from National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC), Bulgaria, except for *Aspergillus niger* (A3), which belongs to the Department of Biotechnology, Biological Faculty, Sofia University, Bulgaria. Melting points were determined on a Kofler microscope Boetius PHMK 0.5. All the reactions were monitored by thin layer chromatography (TLC) on pre-coated polyesters sheets POLIGRAM® SIL G/$UV_{254}$; spots were visualized with UV light. MASCHEREY-NAGEL Silica gel 60 (230-400 mesh) was used for column chromatography. The IR spectra were acquired in Nujol on a Specord 75 and are reported in reciprocal centimeters. NMR spectra were recorded on a Bruker Avance (250.13 MHz and 62.90 MHz for $^{1}H$ and $^{13}C$, respectively) using DMSO-$d_6$ as a solvent and TMS as an internal standard. The chemicals shifts (δ) are given in ppm and *J* values are reported in Hz. The exact mass of compounds **4a-e**, **5** was determinate by HRMS analyses on DFS High Resolution GC/MS (Thermo), after derivatization with $(CH_3)_3SiCl$. The $DPPH^{\bullet}$ radical scavenging activity assay was performed on Shimadzu UV/VIS CECIL Aurius 3021 spectrophotometer and the tyrosinase inhibitory activity on Agilent 8453 UV–Vis spectrophotometer.

### *4.2. Chemistry*

*General procedure for one-pot synthesis of polyhydroxy (E)-2-(1-carboxy-2-phenylvinyl)benzoic acids (**4a-e**)*

An equimolar mixture of 6,7-dimetoxihomophthalic anhydride (**1**), aldehyde **2a-e** and DMAP in dry dichloromethane (10 mL) was stirred at room temperature and the consumption of the

reagents was monitored by TLC. At the end of the reaction (10-15 min), boron tribromide (1.6 M solution in dichloromethane) was slowly added (one equivalent per heteroatom) and the reaction mixture was stirred additionally for 10-60 min. At the end of the reaction, the mixture was treated with 50% NaOH (pH = 12), stirred for 10 min and acidified with conc. HCl (pH = 1). The water layer was then saturated with NaCl and the products were extracted with ethyl acetate. The organic phase was washed with brine to a constant pH, dried over anhydrous $Na_2SO_4$ and the solvent was evaporated under reduced pressure. The residue was further purified by column chromatography (Mobile phase: acetone/petroleum ether/formic acid – 1/1/0.01) and the products **4a-e** were isolated after crystallization from appropriate mixture of solvents.

*4.2.1. (E)-2-(1-carboxy-2-phenylvinyl)-4,5-dihydroxybenzoic acid (**4a**)*

0.986 g oil (99%) from reaction of 6,7-dimetoxihomophthalic anhydride (**1**) (0.74 g, 3.33 mmol), benzaldehyde (**2a**) (0.34 mL, 3.33 mmol), 4-dimethylaminopyridine (0.40 g, 3.33 mmol) and boron tribromide (18.7 mL, 29.97 mmol) in 10 mL dichloromethane. White crystals (0.51 g, 51%) after column chromatography and crystallization form methanol/dichloromethane – 1/5. M.p. = 229-231 $^{o}$C; IR (Nujol) $\nu$ (cm$^{-1}$): 1680 (C=O, COOH); $^{1}$H NMR (DMSO-$d_6$, 250.13 MHz) $\delta$ 6.36 (s, 1H), 6.97-7.09 (m, 2H, =CH–), 7.15-7.26 (m, 3H, =CH–), 7.46 (s, 1H, =CH–), 7.49 (s, 1H, =CH–), 9.38 (bs, 1H, OH), 9.63 (bs, 1H, OH), 12.23 (bs, 2H, 2 x COOH); $^{13}$C NMR (DMSO-$d_6$, 62.5 MHz) $\delta$ 117.6 (=CH–), 118.2 (=CH–), 121.5 (C), 128.4 (2 x =CH–), 128.5 (=CH–), 130.0 (2 x =CH–), 130.8 (C), 135.1 (=CH–), 135.2 (C), 144.5 (C), 149.4 (C), 167.2 (COOH), 168.3 (COOH); HRMS: *m/z* calculate: 588.2215, found: 588.2199 for $C_{28}H_{44}O_6Si_4$.

*4.2.2. (E)-2-(1-carboxy-2-(4-hydroxyphenyl)vinyl)-4,5-dihydroxybenzoic acid (**4b**)*

0.95 g oil (95%) from reaction of 6,7-dimetoxihomophthalic anhydride (**1**) (0.70 g, 3.16 mmol), 4-methoxybenzaldehyde (**2b**) (0.38 mL, 3.162 mmol), 4-dimethylaminopyridine (0.39 g, 3.16 mmol) and boron tribromide (19.8 mL, 31.62 mmol) in 10 mL dichloromethane. Yellow crystals (0.47 g, 47%) after column chromatography and crystallization from methanol/ethyl acetate – 1/5. M.p. = 186-188 $^{o}$C; IR (Nujol) $\nu$ (cm$^{-1}$): 1660 (C=O, COOH); $^{1}$H NMR (DMSO-$d_6$, 250.13 MHz) $\delta$ 6.38 (s, 1H), 6.58 (d, 2H, =CH–, $J$ = 8.5), 6.85 (d, 2H, =CH–, $J$ = 8.5), 7.41 (s, 1H, =CH–), 7.45 (s, 1H, =CH–), 9.31 (bs, 1H, OH), 9.60 (bs, 1H, OH), 9.74 (bs, 1H, OH), 12.01 (bs, 2H, 2 x COOH); $^{13}$C NMR (DMSO-$d_6$, 62.5 MHz) $\delta$ 115.2 (2 x =CH–), 117.7 (=CH–), 118.2

(=CH–), 121.4 (C), 126.0 (C), 131.2 (C), 131.4 (C), 131.9 (2 x =CH–), 135.6 (=CH–), 144.3 (C), 149.4 (C), 158.0 (C), 167.2 (COOH), 168.6 (COOH); HRMS: *m/z* calculate: 676.25594, found: 676.25572 for $C_{31}H_{52}O_7Si_5$.

*4.2.3. (E)-2-(1-carboxy-2-(3,4-dihydroxyphenyl)vinyl)-4,5-dihydroxybenzoic acid (**4c**)*

0.92 g oil (92%) from reaction of 6,7-dimetoxihomophthalic anhydride (**1**) (0.70 g, 3.01 mmol), 3,4-dimethoxybenzaldehyde (**2c**) (0.50 g, 3.01 mmol), 4-dimethylaminopyridine (0.37g, 3.01 mmol) and boron tribromide (20.7 mL, 33.11 mmol) in 10 mL dichloromethane. Solid foam (0.43 g, 43%) after column chromatography. IR (Nujol) $\nu$ ($cm^{-1}$): 1675 (C=O, COOH); $^1$H NMR (DMSO-$d_6$, 250.13 MHz) $\delta$ 6.29-6.45 (m, 3H, =CH–), 6.56 (d, 1H, =CH–, $J$ = 8.1), 7.32 (s, 1H, =CH–), 7.45 (s, 1H, =CH–), 8.86 (bs, 1H, OH), 9.28 (bs, 2H, 2 x OH), 9.56 (bs, 1H, OH), 12.00 (bs, 2H, 2 x COOH); $^{13}$C NMR (DMSO-$d_6$, 62.5 MHz) $\delta$ 115.3 (=CH–), 117.5 (=CH–), 117.8 (=CH–), 118.2 (=CH–), 121.3 (C), 122.8 (=CH–), 126.5 (C), 131.2 (C), 131.3 (C), 136.0 (=CH–), 144.2 (C), 144.8 (C), 146.5 (C), 149.3 (C), 167.2 (COOH), 168.7 (COOH); HRMS: *m/z* calculate: 764.29038, found: 764.28984 for $C_{34}H_{60}O_8Si_6$.

*4.2.4. (E)-2-(1-carboxy-2-(3,4,5-trihydroxyphenyl)vinyl)-4,5-dihydroxybenzoic acid (**4d**)*

0.95 g (95%) oil from reaction of 6,7-dimetoxihomophthalic anhydride (**1**) (0.64 g, 2.87 mmol), 3,4,5-trimethoxybenzaldehyde (**2d**) (0.56 g, 2.87 mmol), 4-dimethylaminopyridine (0.35 g, 2.87 mmol) and boron tribromide (21.5 mL, 34.46 mmol) in 10 mL dichloromethane. Yellow crystals (0.42 g, 42%) after column chromatography and crystallization form dichloromethane/ethyl acetate – 1/5. M.p. = 178-179 $^o$C; IR (Nujol) $\nu$ ($cm^{-1}$): 1680 (C=O, COOH); $^1$H NMR (DMSO-$d_6$, 250.13 MHz) $\delta$ 6.02 (s, 2H, =CH–), 6.36 (s, 1H, =CH–), 7.22 (s, 1H, =CH–), 7.44 (s, 1H, =CH–), 8.77 (bs, 3H, 3 x OH), 9.23 (bs, 1H, OH), 9.56 (bs, 1H, OH), 12.01 (bs, 2H, 2 x COOH); $^{13}$C NMR (DMSO-$d_6$, 62.5 MHz) $\delta$ 110.0 (2 x =CH–), 117.8 (=CH–), 118.2 (=CH–), 121.3 (C), 125.4 (C), 131.2 (C), 131.5 (C), 134.6 (C), 136.3 (=CH–), 144.2 (C), 145.4 (2 x C), 149.2 (C), 167.3 (COOH), 168.8 (COOH) HRMS: *m/z* calculate: 852.32482, found: 852.32428 for $C_{37}H_{68}O_9Si_7$.

*4.2.5. (E)-2-(1-carboxy-2-(3,5-di-tert-butyl-4-hydroxyphenyl)vinyl)-4,5-dihydroxybenzoic acid (**4e**)*

0.96 g (96%) oil from reaction of 6,7-dimetoxihomophthalic anhydride (**1**) (0.52 g, 2.33 mmol), 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (**2e**) (0.58 g, 2.33 mmol), 4-dimethylaminopyridine (0.29 g, 2.33 mmol) and boron tribromide (14.6 mL, 23.34 mmol) in 10 mL dichloromethane. Yellow crystals (0.50 g, 50%) after column chromatography and crystallization form dichloromethane/ethyl acetate – 1/5. M.p. = 236-238 $^{o}$C; IR (Nujol) $\nu$ (cm$^{-1}$): 1725 (COOH), 1680 (COOR); $^{1}$H NMR (DMSO-$d_6$, 250.13 MHz) $\delta$ 1.20 (s, 18H 2 x *t*-Bu), 6.41 (s, 1H, =CH–), 6.89 (s, 2H, =CH–), 7.18 (s, 1H), 7.43 (s, 1H), 7.46 (s, 1H), 9.23 (bs, 1H, OH), 9.58 (bs, 1H, OH), 12.01 (bs, 2H, 2 x COOH); $^{13}$C NMR (DMSO-$d_6$, 62.5 MHz) $\delta$ 30.1 (6 x $CH_3$), 34.3 (2 x C), 117.6 (=CH–), 118.1 (=CH–), 121.2 (C), 126.1 (C), 127.6 (2 x =CH–), 131.4 (C), 131.5 (C), 136.6 (=CH–), 138.3 (2 x C), 144.3 (C), 149.6 (C), 154.7 (C), 167.1 (COOH), 168.6 (COOH); HRMS: *m/z* calculate: 716.34161, found: 716.34107 for $C_{36}H_{60}O_7Si_4$.

*4.2.6. Synthesis of (E)-2-(1-carboxy-2-(3,5-di-tert-butyl-4-hydroxyphenyl)vinyl)-4,5-dimethoxybenzoic acid (**5**)*

To a mixture 6,7-dimethoxyhomophthalic anhydride (**1**) (0.49 g, 2.19 mmol) and 1.1 equiv. of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (**2e**) (0.57 g, 2.41 mmol) in 5 mL dry dichloromethane, 1 equiv. DMAP (0.27 g, 2.19 mmol) was added. The reaction mixture was stirred for 15 min at room temperature. At the end of the reaction (TLC), the reaction mixture was acidified (pH = 3) with 15% HCl, diluted with ethyl acetate and the obtained carboxylic acids were extracted with 10% $NaHCO_3$. The aqueous layer was acidified (pH = 3) with 15% HCl and extracted with ethyl acetate. The organic layer was dried with $Na_2SO_4$, filtered and the solvent was evaporated under reduced pressure. The residual oil crystallized from ethyl acetate/petroleum ether – 1/1. Yellow crystals (0.53 g, 53%). M.p. = 214-216 $^{o}$C; IR (Nujol) $\nu$ (cm$^{-1}$): 1680 (C=O, COOH); $^{1}$H NMR (DMSO-$d_6$, 250.13 MHz) $\delta$ 1.18 (s, 18H, 2 x *t*-Bu), 3.65 (s, 3H, O–$CH_3$), 3.82 (s, 3H, O–$CH_3$), 6.63 (s, 1H), 6.85 (s, 2H), 7.23 (s, 1H), 7.51 (s, 1H), 7.56 (s, 1H), 12.33 (bs, 2 x COOH); $^{13}$C NMR (DMSO-$d_6$, 62.5 MHz) $\delta$ 30.0 (6 x $CH_3$), 34.7 (2 x C), 55.8 (2 x O–$CH_3$), 113.9 (2 x =CH–), 122.7 (C), 126.0 (C), 127.6 (2 x =CH–), 130.8 (C), 133.1 (C), 137.2 (=CH–), 138.4 (2 x C), 147.7 (C), 152.3 (C), 154.9 (C), 167.0 (COOH), 168.4 (COOH); HRMS: *m/z* calculate: 600.29386, found: 600.29346 for $C_{32}H_{48}O_7Si_2$.

*4.3. Biological activity*

*4.3.1. DPPH$^{\bullet}$ radical assay*

For estimation of the DPPH$^{\bullet}$ radical scavenging activity, the analyses were performed at 3.3 μM of compounds **4a-e, 5**. In general, 0.5 mL methanol solution with exact concentration of the tested compounds was added to 0.5 mL of DPPH$^{\bullet}$ stock solution (100 μM) and the absorbance of the DPPH$^{\bullet}$ at 518 nm was recorded at every 10 min until the reaction reached equilibrium. The experiments were performed in an excess of DPPH$^{\bullet}$ radical (molar ratio DPPH$^{\bullet}$/substrate of 15) in order to exhaust the H-donating capacity of polyphenols. Methanol was used as blank sample and caffeic acid (**CA**) was used as a reference compound. The scavenging activity was calculated by means of Eq. 1.

$$\text{DPPH reduction, \%} = (A_0\text{-}A_f)/A_0 \times 100 \qquad (1),$$

where $A_0$ and $A_f$ correspond to the absorbencies of DPPH$^{\bullet}$ radical at the beginning and at the end of the reaction, respectively.

*4.3.2. Antibacterial and Anti-yeast Activity (In Vitro)*

The synthesized compounds were screened for antibacterial activity against four bacterial strains. i.e., *Bacillus subtilis* (ATCC 6633), *Staphilococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeroginosa* (ATCC 10145) and one yeast strain *Candida albicans* (ATCC 10231) using agar well diffusion method. Overlays of each test strain (106 CFU/mL) were prepared on agar plates and allowed to dry. Wells (7 mm) were made in the agar plates and 100 μL of the test sample (0.5 mg/mL in DMSO) was introduced into respective wells. Other wells were supplemented with DMSO and reference antimicrobial drugs (amikacin 30 μg dick for bacterial test strains and 100 μL nystatine 10000 IU/mL for *C. albicans*) serving as negative and positive controls, respectively. The plates were allowed to diffuse through the agar for 60 min at 4 $^{o}$C prior to incubation for 24 h at 30 or 37 $^{o}$C, after which inhibitory zones were measured (in mm). All tests were done in triplicate. Growth inhibition was calculated with reference to positive control.

*4.3.3. Antifungal Activity (In Vitro)*

The synthesized compounds were tested for antifungal activity against three fungal strains *Aspergillus niger* (A3), *Aspergillus flavus* (IMI 052104) and *Fusarium graminearum* (ATCC 24373). Stock solutions of pure compounds (1 mg/mL) were prepared in sterile DMSO. Test compounds (0.5 mL) were mixed with 20 mL temperate PDA (Oxoid) agar at 45 $^{o}$C into plates. After drying of the plates, 3 µL of each molds spore suspension (105 spores/mL) were dropped on the agar, and the plates were incubated for 7 days at 30 $^{o}$C. The diameter of the mould colonies was measured and compared with the controls on media with 0.5 mL DMSO and pure PDA. All tests were done in triplicate. The inhibition effect was calculated as:

Ihibition effect, % = 100-(d[colony]/d[control colony]*100)

*4.3.4. Mushroom Tyrosinase Assay*

Tyrosinase activity was determined spectrophotometrically by the modified dopachrome method, using L-tyrosine as a substrate. The reaction media (3 mL) contained: phosphate buffer (1.0 mL, 0.1 M, pH 6.8); tyrosine (1.0 mL, 1.5 mM) dissolved in deionized water; inhibitor (0.35 mL, 0.2 mM) dissolved in DMSO; deionized water (0.35 mL); mushroom tyrosinase aqueous solution (0.30 ml, 192 U/mL). The reference solution was prepared with 0.35 mL of DMSO instead of inhibitor. After adding of mushroom tyrosinase solution, the reaction mixture was incubated at 37 $^{o}$C for 30 min. The UV absorbance of the reaction mixture was measured at 475 nm. The percentage of mushroom tyrosinase inhibitory activity was calculated by following equation:

Mushroom tyrosinase activity, % = B/A x 100

where A is the absorbance of the reference solution, and B is the absorbance of the test sample solution. Each experiment was performed in triplicate and averaged. Hydroquinone was used as positive controls.

**Acknowledgements**

The financial support of the National Science Fund of Bulgaria at the Ministry of Education, Youth and Science (projects DMU-03-10/2011 and DRNF-02-13/2009) and Sofia University Fund (project 029/2013) is greatly acknowledged by the authors.

**References**

[1] G. Likhtenshtein, (2009) Bioactive Stilbenes, in Stilbenes: Applications in Chemistry, Life Sciences and Materials Science, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

[2] J. Chong, A. Poutaraud, P. Hugueney, Metabolism and roles of stilbenes in plants, Plant Sci. 177 (2009) 143–155.

[3] F. Mazué, D. Colin, J. Gobbo, M. Wegner, A. Rescifina, C. Spatafora, D. Fasseur, D. Delmas, P. Meunier, C. Tringali, N. Latruffe, Structural determinants of resveratrol for cell proliferation inhibition potency: Experimental and docking studies of new analogs, Eur. J. Med. Chem. 45 (2010) 2972–2980.

[4] H. Li, W.K.K. Wu, Z. Zheng, C.T. Che, L. Yu, Z.J. Li, Y.C. Wu, K.-W. Cheng, J. Yu, C.H. Cho, M. Wang, 2,3',4,4',5'-Pentamethoxy-*trans*-stilbene, a resveratrol derivative, is a potent inducer of apoptosis in colon cancer cells via targeting microtubules, Biochem. Pharmacol. 78 (2009) 1224–1232.

[5] A. Gosslau, S. Pabbaraja, S. Knapp, K.Y. Chen, *trans*- and *cis*-Stilbene polyphenols induced rapid perinuclear mitochondrial clustering and p53-independent apoptosis in cancer cells but not normal cells, Eur. J. Pharmacol. 587 (2008) 25–34.

[6] S. Quideau, D. Deffieux, L. Pouysegu, Resveratrol Still Has Something To Say about Aging!, Angew. Chem. Int. Ed. 51 (2012) 6824–6826.

[7] C. Lu, Y. Guo, J. Li, M. Yao, Q. Liao, Z. Xie, X. Li, Design, synthesis, and evaluation of resveratrol derivatives as $A\beta_{1-42}$ aggregation inhibitors, antioxidants, and neuroprotective agents, Bioorg. Med. Chem. Lett. 22 (2012) 7683–7687.

[8] J.-C. Jung, E. Lim, Y. Lee, J.-M. Kang, H. Kim, S. Jang, S. Oh, M. Jung, Synthesis of novel *trans*-stilbene derivatives and evaluation of their potent antioxidant and neuroprotective effects, Eur. J. Med. Chem. 44 (2009) 3166–3174.

[9] D.-J. Ding, X.-Y. Cao, F. Dai, X.-Z. Li, G.-Y. Liu, D. Lin, X. Fu, X.-L. Jin, B. Zhou, Synthesis and antioxidant activity of hydroxylated phenanthrenes as *cis*-restricted resveratrol analogues, Food Chem.135 (2012) 1011–1019.

[10] S. Sangjun, E. de Jong, S. Nijmeijer, T. Mutarapat, S. Ruchirawat, M. van den Berg, M.B.M. van Duursen, Induction of cell cycle arrest in human MCF-7 breast cancer cells by *cis*-stilbene derivatives related to VIOXX®, Toxicol. Lett. 186 (2009) 115–122.

[11] J. Liu, C.-H. Zheng, X.-H. Ren, F. Zhou, W. Li, J. Zhu, J.-G. Lv, Y.-J. Zhou, Synthesis and Biological Evaluation of 1-Benzylidene-3,4-dihydronaphthalen-2-one as a New Class of Microtubule-Targeting Agents, J. Med. Chem. 55 (2012) 5720–5733.

[12] K.V. Balan, Y. Wang, S.W. Chen, J.-C. Chen, L.-F. Zheng, L. Yang, Z.-L. Liu, P. Pantazis, J.H. Wyche, Z. Han, Proteasome-independent down-regulation of estrogen receptor-α (ERα) in breast cancer cells treated with 4,4'-dihydroxy-*trans*-stilbene, Biochem. Pharmacol. 72 (2006) 573–581.

[13] O.I. Petrov, M. Gerova, Ch. Chanev, K.V. Petrova, New efficient synthesis of Combretastatin A-4 via Colvin rearrangement, Synthesis (2011) 3711–3715.

[14] R. Cella, H.A. Stefani, Ultrasound-assisted synthesis of *Z* and *E* stilbenes by Suzuki cross-coupling reactions of organotellurides with potassium organotrifluoroborate salts, Tetrahedron 62 (2006) 5656–5662.

[15] K. Gaukroger, J.A. Hadfield, L.A. Hepworth, N.J. Lawrence, A.T. McGown, Novel syntheses of *cis* and *trans* isomers of Combretastatin A-4, J. Org. Chem. 66 (2001) 8135–8138.

[16] A.G. Myers, D. Tanaka, M.R. Mannion, Development of a Decarboxylative Palladation Reaction and Its Use in a Heck-type Olefination of Arene Carboxylates, J. Am. Chem. Soc. 124 (2002) 11250–11251.

[17] G. Bellucci, C. Chiappe, G. Lo Moro, Crown ether catalyzed stereospecific synthesis of *Z*- and *E*-stilbenes by Wittig reaction in a solid-liquid two-phases system, Tetrahedron Lett. 37 (1996) 4225–4228.

[18] M. Gerova, S. Georgieva, C. Chanev, O.I. Petrov, An improved protocol for the synthesis of natural combretastatin A-4 via modified Wittig reaction, C. R. Acad. Bulg. Sci. 64 (2011) 1415–1420.

[19] C. Borrel, S. Thoret, X. Cachet, D. Guernard, F. Tillequin, M. Koch, S. Michel, New antitubulin derivatives in the combretastatin A-4 series: synthesis and biological evaluation, Bioorg. Med. Chem. 13 (2005) 3853–3864.

[20] H.-Y. Sun, C.-F. Xiao, Y.-C. Cai, Y. Chen, W. Wei, X.-K. Liu, Z.-L. Lv, Y. Zou, Efficient Synthesis of Natural Polyphenolic Stilbenes: Resveratrol, Piceatannol and Oxyresveratrol, Chem. Pharm. Bull. 58 (2010) 1492–1496.

[21] C.-F. Xiao, Y. Zou, J.-L. Du, H.-Y. Sun, X.-K. Liu, Hydroxyl Substitutional Effect on Selective Synthesis of cis, trans Stilbenes and 3-Arylcoumarins Through Perkin Condensation, Synth. Commun. 42 (2012) 1243–1258.

[22] A.K. Sinha, V. Kumar, A. Sharma, A. Sharma, R. Kumar, An unusual, mild and convenient one-pot two-step access to (*E*)-stilbenes from hydroxy-substituted benzaldehydes and phenylacetic acids under microwave activation: a new facet of the classical Perkin reaction, Tetrahedron 63 (2007) 11070–11077.

[23] C.S. Letizia, J. Cocchiara, A. Lapczynski, J. Lalko, A.M. Api, Fragrance material review on cinnamic acid, Food Chem. Toxicol. 43 (2005) 925–943.

[24] X. Li, X. Wang, D. Chen, S. Chen, Antioxidant activity and mechanism of protocatechuic acid in vitro, Funct. Foods Health Dis. 7 (2011) 232–244.

[25] T. Tanaka, T. Tanaka, M. Tanaka, Potential cancer chemopreventive activity of protocatechuic acid, J. Exp. Clin. Med. 3 (2011) 27–33.

[26] I. Medina, I. Undeland, K. Larsson, I. Storrø, T. Rustad, C. Jacobsen, V. Kristinová, J.M. Gallardo, Activity of caffeic acid in different fish lipid matrices: A review, Food Chem. 131 (2012) 730–740.

[27] M. Touaibia, J. Jean-Francois, J. Doiron, Caffeic Acid, A versatile pharmacophore: An overview, Mini Rev. Med. Chem. 11 (2011) 695–713.

[28] L.D. Rocha, M.C. Monteiro, A.J. Teodoro, Anticancer properties of hydroxycinnamic acids: A review, Cancer Clin. Oncol. 1 (2012) 1927–4866.

[29] Y. Kadoma, S. Fujisawa, A comparative study of the radical-scavenging activity of the phenolcarboxylic acids: caffeic acid, *p*-coumaric acid, chlorogenic acid and ferulic acid, with or without 2-mercaptoethanol, a thiol, using the induction period method, Molecules 13 (2008) 2488–2499.

[30] M.G. Bogdanov, M. Palamareva, *cis*/*trans*-Isochromanones. DMAP induced cycloaddition of homophthalic anhydride and aldehydes, Tetrahedron 60 (2004) 2525–2530.

[31] M.G. Bogdanov, B. Gocheva, D. Dimitrova, M. Palamareva, New isochromans. 1. Synthesis and antimicrobial activity of 4-substituted (±)-1H-spiro[benzo[c]pyran-3(4H),1′-cyclohexane]-1-ones, J. Heterocycl. Chem. 44 (2007) 673–677.

[32] M.G. Bogdanov, Y. Mitrev, I. Tiritiris, New highly diastereoselective Perkin/Michael addition domino reaction between homophthalic anhydride and aromatic aldehydes: A facile approach to blue-fluorescent dibenzo[c,h]chromenones. Eur. J. Org. Chem. (2011) 377–384.

[33] U. Vogeli, W. von Philipsborn, Vicinal C,H spin coupling in substituted alkenes. Stereochemical significance and structural effects, Org. Magn. Reson. 7 (1975) 617–627.

[34] R.L. Prior, X. Wu, K. Schaich, Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements, J. Agric. Food Chem. 53 (2005) 4290–4302.

[35] L.M. Reid, R.W. Nicol, T. Ouellet, M. Savard, J.D. Miller, J.C. Young, D.W. Stewart, and A.W. Schaafsma, Interaction of *F. graminearum* and *F. moniliforme* in Maize Ears: Disease Progress, Fungal Biomass, and Mycotoxin Accumulation, Phytopathology 89 (1999) 1028–1037.

[36] A. Zinedine, J.M. Soriano, J.C. Molto, J. Manes, Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin, Food Chem. Toxicol. 45 (2007) 1–18.

[37] J.J. Pestka, A.T. Smolinski, Deoxynivalenol: Toxicology and Potential Effects on Humans, J. Toxicol. Environ. Health. Part B: Critical Reviews 8 (2005) 39–69.

[38] S.-Y. Kwak, J.-K. Yang, H.-R. Choi, K.-C. Park, Y.-B. Kim, Y.-S. Lee, Synthesis and dual biological effects of hydroxycinnamoyl phenylalanyl/prolyl hydroxamic acid derivatives as tyrosinase inhibitor and antioxidant, Bioorg. Med. Chem. Lett. 23 (2013) 1136–1142.

[39] S. Okombi, D. Rival, S. Bonnet, A.-M. Mariotte, E. Perrier, A. Boumendjel, Analogues of N-hydroxycinnamoylphenalkylamides as inhibitors of human melanocyte-tyrosinase, Bioorg. Med. Chem. Lett. 16 (2006) 2252–2255.

[40] J.-M. Noh, Y.-S. Lee, Inhibitory activities of hydroxyphenolic acid–amino acid conjugates on tyrosinase, Food Chem. 125 (2011) 953–957.

[41] S. Kermasha, M. Goetghebeur, A. Monfette, M. Metche, B. Rovel, Inhibitory effects of cysteine and aromatic acids on tyrosinase activity, Phytochemistry 34 (1993) 349–353.

[42] L. Georgiev, M. Chochkova, I. Totseva, K. Seizova, E. Marinova, G. Ivanova, M. Ninova, H. Najdenski, T. Milkova, Anti-tyrosinase, antioxidant and antimicrobial activities of hydroxycinnamoylamides, Med. Chem. Res. (2012) doi:10.1007/s00044-012-0419-x.

[43] Q. Fan, H. Jiang, E.-d. Yuan, J.-x. Zhang, Z.-x. Ning, S.-j. Qi, Q.-y. Wei, Tyrosinase inhibitory effects and antioxidative activities of novel cinnamoyl amides with amino acid ester moiety, Food Chem. 134 (2012) 1081–1087.

[44] K. Iwai, N. Kishimoto, Y. Kakino, K. Mochida, and T. Fujita, In Vitro Antioxidative Effects and Tyrosinase Inhibitory Activities of Seven Hydroxycinnamoyl Derivatives in Green Coffee Beans, J. Agric. Food Chem. 52 (2004) 4893–4898.

[45] I. Kubo, I. Kinst-Hori, Tyrosinase Inhibitory Activity of the Olive Oil Flavor Compounds, J. Agric. Food Chem. 47 (1999) 4574-4578.

[46] G.-Z. Liu, J. Zhang, L.-Y. Wang, A comparison of the coordination preference of Cd, Zn, Cu(II) with flexible homophthalic acid and rigid bipyridine ligands, Polyhedron 30 (2011) 1487–1493.

[47] C.M. Rogers, C.Y. Wang, G.A. Farnum, R.L. LaDuca, Divalent metal homophthalate coordination polymers with long-spanning dipyridyl ligands containing piperazine moieties, Inorg. Chim. Acta (2013), http://dx.doi.org/10.1016/j.ica.2013.01.005 .

[48] Y. Wang, S.-Q. Shen, J.-H. Zhou, T. Wang, S.-N. Wang, G.-X. Liu, Synthesis, structural diversities and properties of a series of transition metal-organic frameworks based on asymmetric dicarboxylic acid and N-donor auxiliary ligand, Inorg. Chem. Commun. 30 (2013) 5–12.

**Table, Scheme and Figure Captions**

**Table 1**. Structure and atom numbering of **4a-e.** Chemical shifts ($\delta$, ppm) and heteronuclear coupling constants (in brackets, *J*, Hz) for selected representative nuclei.

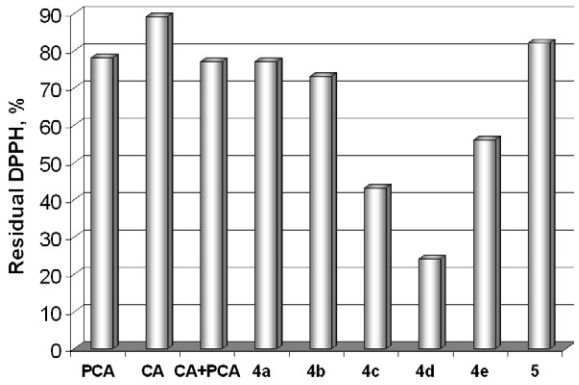
**Table 2**. *In vitro* anti-yeast and antifungal activity of **4a-e** and **5**.

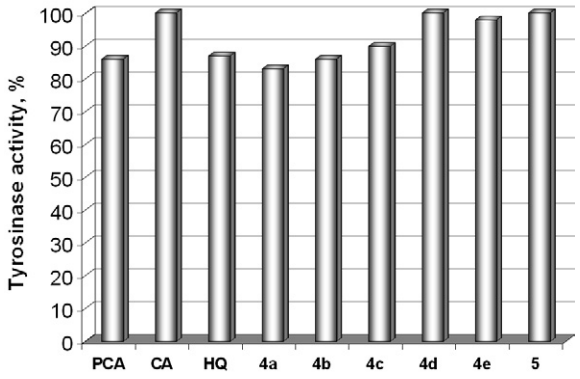
**Scheme 1**. One-pot synthesis of *cis*-restricted polyhydroxy stilbenes **4a-e**. Reagents and conditions: (*i*) DMAP/$CH_2Cl_2$, 10-15 min, rt, then (*ii*) $BBr_3$/$CH_2Cl_2$, rt, 10-60 min.

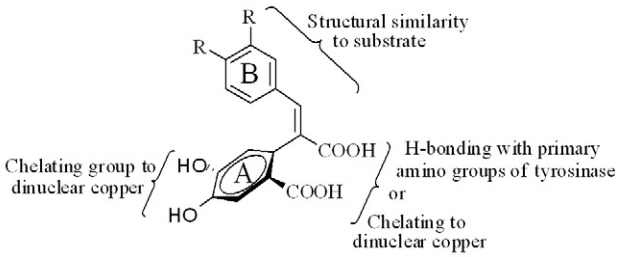
**Figure 1.** $DPPH^{\bullet}$ scavenging activity of **4a-e, 5** at 3.3 μM. The analyses were performed in a molar ratio $DPPH^{\bullet}$/substrate of 15. Caffeic acid (**CA**), protocatechuic acid (**PCA**) and their equimolar mixture were used as reference compounds.

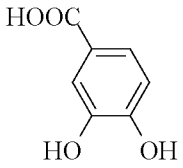
**Figure 2.** Mushroom tyrosinase inhibitory activity of **4a-e, 5** at 23 μM. The analyses were performed in a mole ratio tyrosine/substrate of 22. Hydroquinone (**HQ**), caffeic acid (**CA**) and protocatechuic acid (**PCA**) were used as reference compounds.

**Figure 3.** Possible tyrosinase inhibition mechanism. Given structural fragments suggest **4** as both competitive and non-competitive tyrosinase inhibitors.

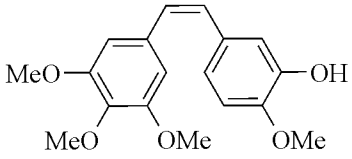




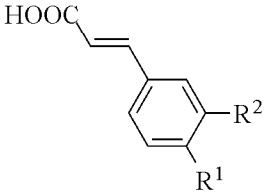




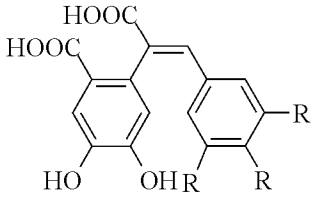

protocatechuic acid



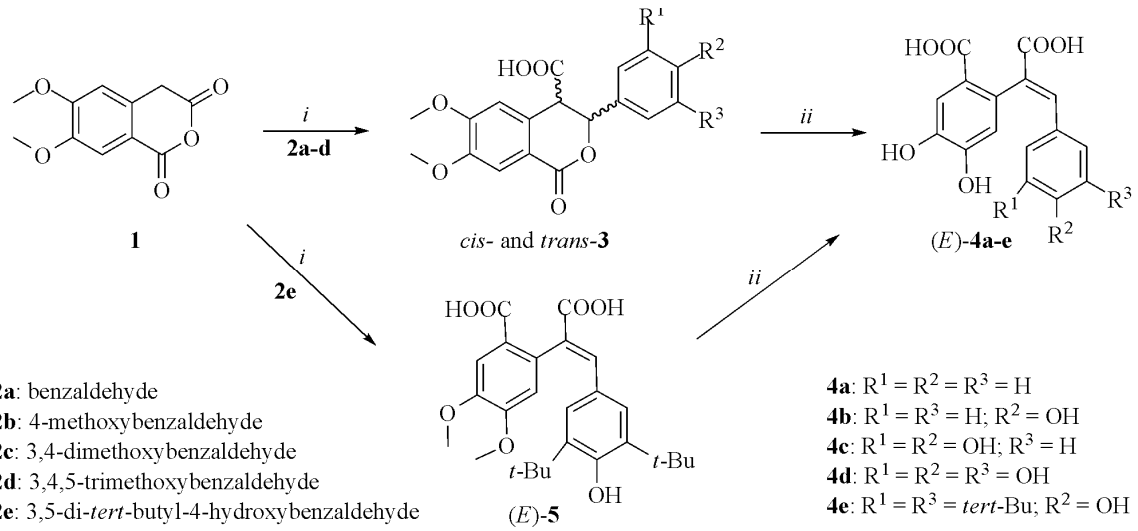

combretastatin A-4




cinnamic acid: $R^1 = R^2 = H$
*p*-coumaric acid: $R^1 = OH$; $R^2 = H$
caffeic acid: $R^1 = R^2 = OH$




Target compounds
in this study
R = H or OH or *t*-Bu




**2a**: benzaldehyde
**2b**: 4-methoxybenzaldehyde
**2c**: 3,4-dimethoxybenzaldehyde
**2d**: 3,4,5-trimethoxybenzaldehyde
**2e**: 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde

**4a**: $R^1 = R^2 = R^3 = H$
**4b**: $R^1 = R^3 = H$; $R^2 = OH$
**4c**: $R^1 = R^2 = OH$; $R^3 = H$
**4d**: $R^1 = R^2 = R^3 = OH$
**4e**: $R^1 = R^3 =$ *tert*-Bu; $R^2 = OH$

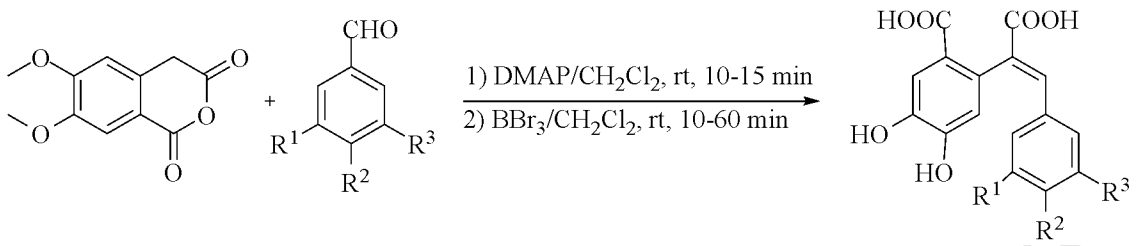
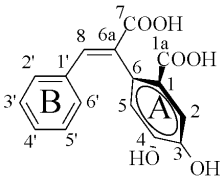


**Table 1**. Structure and atom numbering of **4a-e.** Chemical shifts ($\delta$, ppm) and heteronuclear coupling constants (in brackets, *J*, Hz) for selected representative nuclei.




| | **4a** | **4b** | **4c** | **4d** | **4e** |
|---|---|---|---|---|---|
| C-7 | 168.25 (7.0) | 168.55 (7.3) | 168.70 | 168.74 (7.3) | 168.60 |
| C-1a | 167.12 (4.0) | 167.14 (4.4) | 167.20 | 167.27(4.2) | 167.10 |
| H-2 | 7.46, s, 1H | 7.45, s, 1H | 7.45 | 7.44, s, 1H | 7.45** |
| H-5 | 6.36, s, 1H | 6.38, s, 1H | ol* | 6.36, s, 1H | 6.41 |
| H-8 | 7.50, s, 1H | 7.41, s, 1H | 7.32 | 7.22, s, 1H | 7.43** |

*Overlapping

**The opposite assignment is possible

Table 2. *In vitro* anti-yeast and antifungal activity of **4a-e**, **5**.

| Comp. | Inhibitory effect, % | | | |
|---|---|---|---|---|
| | *C. albicans*[a] | *A. niger*[b] | *A. flavus*[b] | *F. graminearum*[b] |
| **4a** | 40 | 34 | 43 | 89 |
| **4b** | 34 | 38 | 42 | 72 |
| **4c** | 45 | 37 | 42 | 72 |
| **4d** | 40 | 37 | 42 | 71 |
| **4e** | 40 | 44 | 52 | 76 |
| **5** | 48 | 44 | 48 | 70 |
| **CA**[c] | nd[d] | 35 | 16 | 63 |
| **PCA**[c] | nd[d] | 40 | 9 | 76 |

[a] Inhibitory effect according Nystatin, % (see Section 4.3.2).

[b] Inhibitory effect according control, % (see Section 4.3.3).

[c] CA and PCA refer to caffeic acid and protecatechuic acid, respectively.

[d] Not determined.