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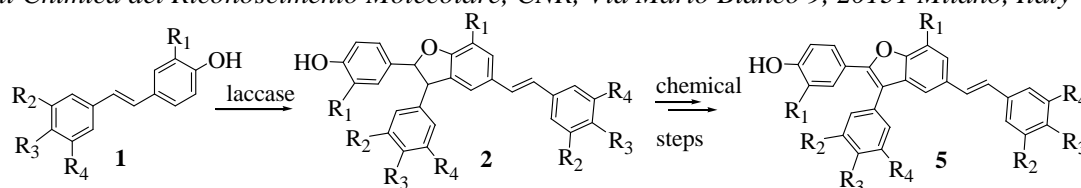
Chemo-enzymatic synthesis of new resveratrol-related dimers containing the benzo[b]furan framework and evaluation of their radical scavenger activities

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Chemo-enzymatic synthesis of new resveratrol-related dimers containing the benzo[b]furan framework and evaluation of their radical scavenger activities

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ABSTRACT

The chemo-enzymatic synthesis and the evaluation of radical scavenger performance of resveratrol-related dimers with antioxidative power have been investigated. The dimeric compounds, containing the benzo[b]furan framework, were prepared via an oxidative dimerization catalyzed by a laccase from *Trametes versicolor*, followed by a treatment with the organic oxidant DDQ. This methodology can be useful for the synthesis of various 2,3-diarylbenzo[b]furans derivates, a class of compound that exhibits a wide range of biological activities.

1. Introduction

Plant derived polyphenols have attracted a great deal of interest in recent years, because of their possible benefits on human health.[1]

Stilbenoids are an important class of phenolic phytoalexins found in a number of plant families, including *Vitaceae*. Their structure is characterized by the presence of the 1,2-diphenylethylene (stilbene) skeleton. The most famous example of which is *trans*-Resveratrol (3,5,4'-trihydroxystilbene, **1a**), a phytoalexin produced by plants, particularly in grapevines, pines and legumes, via a metabolic sequence induced in response to biotic or abiotic stress factors.[2] It has been proposed to be one of the components of red wines with beneficial effects to human cardiovascular health (French paradox).[3] In addition to resveratrol, its oligomers, in particular the so-called "viniferins", have also been found in plants as a results of infection or stress. Both monomeric and oligomeric stilbenes (oligostilbenoids) are reported to be potentially important cancer chemoprotective agents, being able to inhibit cellular events associated with carcinogenesis.[4,5] Thus, a number of hydroxylated stilbenes derived from natural sources and possessing a range of interesting biological activities have been described in the literature.[6] As a consequence, it is not surprising that several papers have been published on the synthesis and the evaluation of the antitumor activity of **1a** and of its analogues.[7] Additionally, both monomeric and oligomeric stilbenes have been described as potentially important antimicrobial, anti-HIV and anti-inflammatory agents,[8] in accordance to the enhanced biological properties exhibited by many high molecular weight polyphenols.[9]

Due to the structural diversity and attractiveness of oligostilbenoids, different studies were undertaken to fulfill their synthesis. Of particular significance are the works on biomimetic dimerization approaches by Sako[10], Luo[11], and Weber[12] and on rational synthetic approaches by Sarpong[13], Nicolaou[14] and, most notably, Snyder[15] groups.

Another interesting chemical framework is the benzo[b]furan nucleus, which is prevalent in a wide variety of biologically active natural and unnatural compounds.[16] Many benzo[b]furans, including the 2,3-disubstituted derivatives, are of interest because they exhibit a wide range of biological activities,[17] including, for example, anticancer effect.[18] A number of synthetic approaches to this class of compounds have been introduced in recent years.[19] Thus we reasoned that the enzyme-catalyzed oxidative dimerization and/or oligomerization of phenolic derivatives might also be a useful approach. In this context, a few years ago we have described the synthesis of a resveratrol dimer and of some new dimeric hydroxystilbenes by an approach that combines the application of chemical C-C bond coupling reactions and oxidoreductases,[20] that is the ligation of phenols and aromatic carboxylic acids to give hydroxystilbenes followed

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by oxidative dimerisation reactions catalyzed by laccases, a group of copper-containing oxidases.[21]

Laccases are oxidoreductases (the so-called "blue oxidases"), widely distributed in fungi and in some bacteria and higher plants, having high stability, and multiple industrial applications.[22] These enzymes are able to catalyse the oxidation of a wide range of substrates by a radical catalyzed reaction mechanism.[23]

With the aim of finding new dimeric hydroxystilbene derivatives possessing antioxidative power, we report here the chemo-enzymatic synthesis and the evaluation of the radical scavenger performances of a series of resveratrol-related dimers containing the benzo[b]furan framework.

2. Results and discussion

A few years ago we reported an investigation on the laccase-catalyzed oxidative dimerization of a series of hydroxystilbene derivatives.[20] affording 4-O-α-ß-5 (dihydrofuran-like) dimers as main products.

Looking for an improved reaction protocol, it was found that the best conditions for the synthesis of these compounds were based on the use of the commercially available laccase from *Trametes versicolor* in a mildly shaken biphasic system made of an organic phase containing the substrate and a water phase containing the enzyme. Accordingly, resveratrol (1a) and the other hydroxystilbenes (1b-e) were dissolved in AcOEt, while the laccase was dissolved in an equal volume of 20 mM acetate buffer, pH 4.5. The biphasic systems were shaken at room temperature and monitored by TLC. When the products were prevalent with respect to the initial substrates, the reactions were quenched by phase separation followed by AcOEt extraction of the water solution. The organic solvent was evaporated and the products were isolated by flash chromatography and identified as the (E)-dehydrodimers (2a-e) by mono- and bi-dimensional ^{1}H- and ^{13}C-NMR spectra.[20]
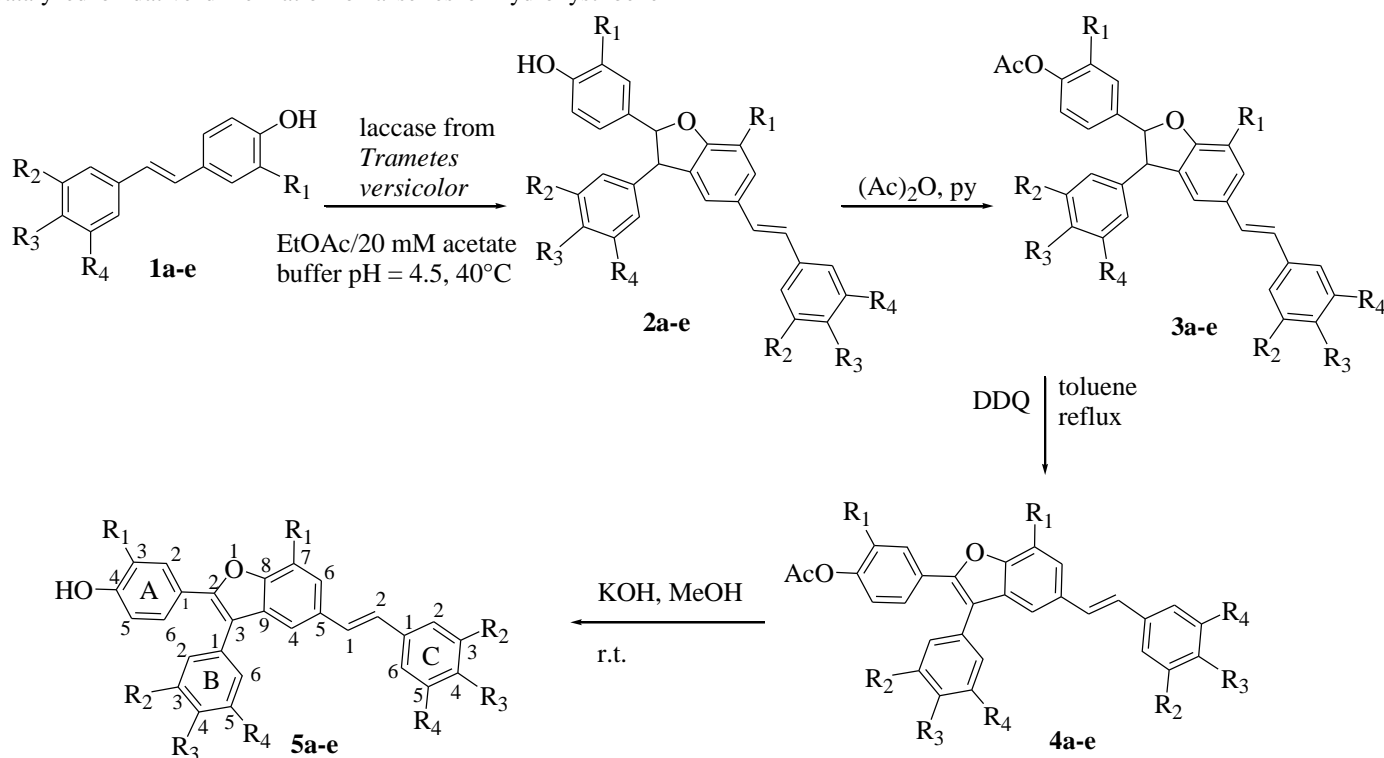
	R$_1$	R$_2$	R$_3$	R$_4$
1a, 2a, 5a	H	OH	H	OH
1b, 2b, 5b	H	H	OCH$_3$	H
1c, 2c, 5c	H	H	OCH$_3$	OCH$_3$
1d, 2d, 5d	OCH$_3$	H	OCH$_3$	H
1e, 2e, 5e	OCH$_3$	H	OCH$_3$	OCH$_3$
3a, 4a	H	OAc	H	OAc
3b, 4b	H	H	OCH$_3$	H
3c, 4c	H	H	OCH$_3$	OCH$_3$
3d, 4d	OCH$_3$	H	OCH$_3$	H
3e, 4e	OCH$_3$	H	OCH$_3$	OCH$_3$

Scheme 1. Chemo-enzymatic synthesis of compounds 5a-e

As shown in **Scheme 1**, the acetylated (E)-dehydrodimers (**3a-e**) have been then refluxed for 9 h in dry toluene containing an excess of the organic oxidant DDQ, and the resulting crude mixtures were deacetylated and purified by flash chromatography to give the new dimeric derivatives **5a-e** in 74-99 % isolated yields. These new dimers were characterized by mass spectrometry and NMR (mono- and bi-dimensional ^{1}H- and ^{13}C-NMR) analysis and identified as 2,3-diarylbenzo[b]furan derivatives: a common feature of all this dimeric product is the absence of the characteristic signals due to the dihydrobenzofuran ring protons (H-2 and H-3), indicating the removal of these adjacent protons as a consequence of the oxidation of the derivatives **2a-e**; furthermore, the signal of the C-2 and C-3 benzofuran carbons in the ^{13}C-NMR spectra of **5a-e** are in the typical range of the double-bond carbons.

As an example, the characterization of the oxidized dimer obtained from resveratrol is reported. The mass analysis (HRESI-MS) of the isolated product showed a molecular ion peak [M+Na]$^+$ at m/z 475.11469±1.1 Da (calculated 475.11521) and a molecular ion peak [M-H]$^-$ at 451.11862±0.2 Da (calculated 451.11871), consistent with a fully conjugated dimeric compound. The proposed structure of the oxidized dimer **5a** was unambiguously confirmed by NMR analysis.

Initial inspection of the ^{1}H-NMR spectrum of **5a** in DMSO-d_6 (Figure 1) showed the absence of the characteristic signals due to the dihydrobenzofuran ring protons (resonating in the precursor **2a** at δ 4.47, d, J = 7.96 Hz and 5.45 ppm, d, J = 7.96 Hz),[20a] indicating the removal of these adjacent protons as a consequence of the oxidation of **2a**. Furthermore, the signals of three different types of aromatic OH's were present at δ 9.85, 9.40 and 9.21 ppm (integrating for 1H, 2H and 2H, respectively), confirming the penta-phenolic structure. Additionally, the ^{1}H-NMR and ^{1}H-^{1}H correlation spectroscopy (COSY) spectra (Figure 2) exhibited the presence of two ortho-coupled aromatic signals assignable to a p-hydroxyphenyl group in an AA'BB' type of arrangement (multiplet at 6.79 ppm and 7.47 ppm,), protons related to two 3,5-dihydroxyphenyl moieties in an A$_2$B type of arrangement (triplet at 6.28 ppm, 1H; doublet at 6.32 ppm, 2H; triplet at 6.16 ppm, 1 H; doublet at 6.45 ppm, 2 H), a set of protons coupled in an ABX system on a 1,2,4-trisubstituted benzene ring at 7.54 and 7.60 ppm (integrating for 1H and 2H respectively) and two *trans* olefinic protons (doublets at 6.98 and 7.16 ppm). A complete assignments of the signals could be made on the basis of ^{1}H,^{13}C-inverse detected single-quantum (HSQC)[24] and multiple-bond (HMBC)[25] correlation experiments, and the data are reported in Table 1.
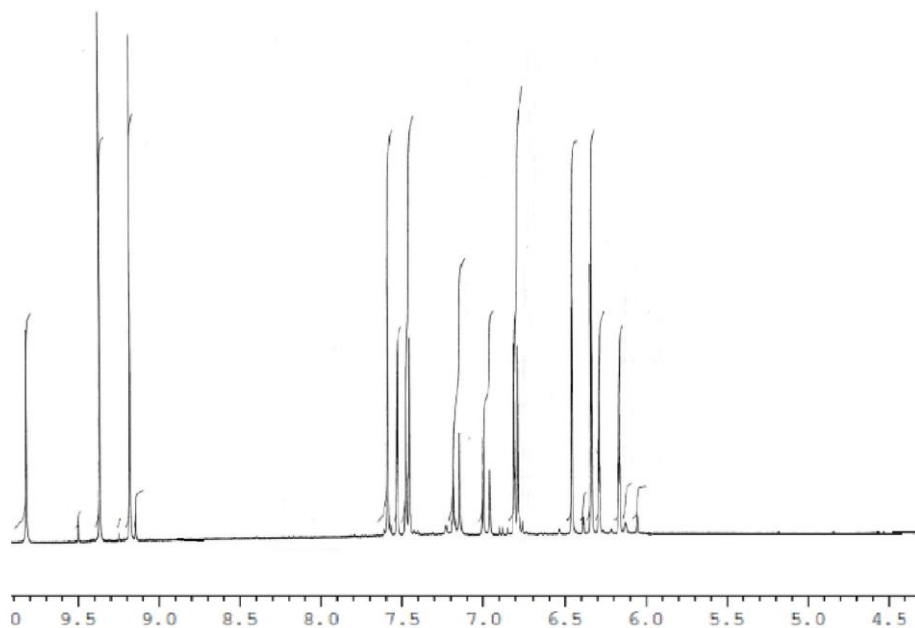
Figure 1. ^{1}H-NMR spectrum of conpound **5a**
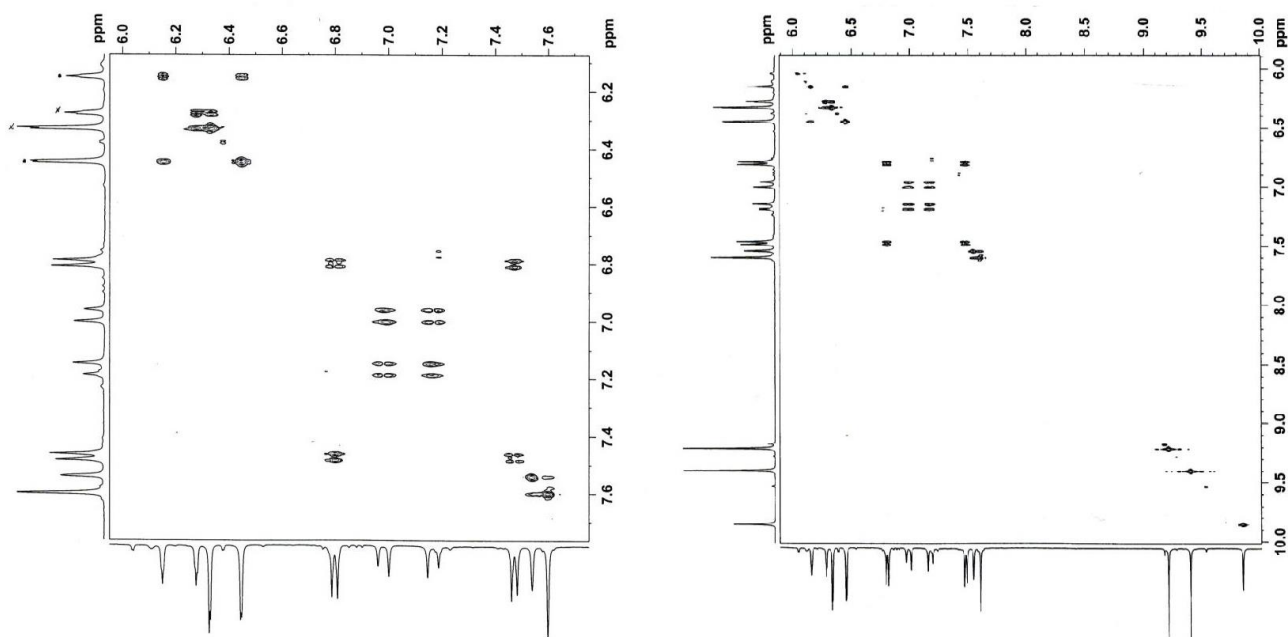
Figure 2. ^{1}H-^{1}H correlation (COSY) spectrum of conpound **5a**

Table 1: ^{1}H and ^{13}C NMR data for compounds **2a** and **5a**

framework	δ$_H$ (mult., J Hz)			δ$_C$	
	2a [a]	**5a**		**2a** [a]	**5a**
4-hydroxyphenyl					
H-2,6	7.24 (BB' multiplet)	7.47 (BB' multiplet)	C-1	133.28	nd
H-3,5	6.85 (AA' multiplet)	6.79 (AA' multiplet)	C-2,6	129.23	128.77
OH		9.85 (bs)	C-3,5	116.85	116.02
			C-4	159.10	158.58
benzofuran					
H-2	5.45 (d, 7.96)	-	C-2	94.72	151.30
H-3	4.47 (d, 7.96)	-	C-3	58.52	115.75
H-4	7.26 (broad s)	7.54 (broad s)	C-4	124.62	117.92
H-6	7.43 (dd, 8.26, 1.63)	7.60 (broad s)	C-5	132.43	133.0
H-7	6.87 (d, 8.26)	7.60 (broad s)	C-6	129.29	123.64
			C-7	110.81	111.63
			C-8	161.30	153.1
			C-9	132.81	nd
3,5-dihydroxyphenyl					
H-2,6	6.20 (d, 2.16)	6.32 (d, 2.18)	C-3,5	160.44	159.46
H-4	6.29 (t, 2.16)	6.28 (t, 2.18)	C-4	103.08	102.60
OH		9.40 (bs)	C-2,6	108.11	107.80
			C-5	145.91	nd
(3,5-dihydroxyphenyl)vinyl					

vinyl protons					
H-1	7.06 (d, 16.33)	7.16 (d, 16.36)	C-1	129.80	128.67
H-2	6.90 (d, 16.33)	6.98 (d, 16.36)	C-2	127.92	128.34
3,5-dihydroxyphenyl					
H-2,6	7.21 (d, 2.10)	6.45 (d, 2.09)	C-3,5	160.22	158.95
H-4	6.82 (t, 2.10)	6.16 (t, 2.09)	C-4	103.43	102.60
OH		9.21 (bs)	C-2,6	106.42	105.09
			C-5	141.46	nd

[a]ref. 20a

Antioxidant activity

The antioxidant activities of the hydroxystilbenes **1a-e** and of their dimeric derivatives **2a-e** and **5a-e** were evaluated by using the well-known DPPH reduction method, that is by plotting the remaining percentage of DPPH as a function of the molar ratios of the compound over DPPH.[26] The results are reported in Table 2 and expressed as IC_{50} as a results of a mean of three determinations. As a general trend, it was found that the monomeric hydroxystilbenes **1a-e** and the benzofuran derivatives **5a-e** show comparable antiradical activities, which are one order of magnitude higher than those of the dehydrodimers **2a-e**.

As an example, the resveratrol dehydrodimer **2a** reacted with DPPH and reached a steady state after about 3 h, whereas the dimer **5a** reached the steady state after approximately 1.5 h. An EC_{50} value of $(9.5 \pm 0.4) \times 10^{-4}$ mmol/ml was determined for the dehydrodimer **2a**, a higher value with respect to the one obtained with resveratrol $[(6.1 \pm 0.3) \times 10^{-5}$ mmol/ml]. On the contrary, the new dimer **5a** showed an EC_{50} of $(1.1 \pm 0.4) \times 10^{-4}$ mmol/ml, comparable to that of resveratrol.

These experimental results might be explained by analyzing the structural features of the two derivatives **2a** and **5a**. Thus, the molecules of δ-viniferin **2a** and of 5-{5-[2-(3,5-dihydroxyphenyl)vinyl]-2-(4-hydroxyphenyl)-benzofuran-3-yl}benzene-1,3-diol **5a** were modeled using HyperChem[TM] and then optimized using the Polak-Ribière conjugate gradient minimisation to a final potential energy gradient of 0.05 kcal mole[-1] Å^{-1} (Figure 3). Both these compounds possess a planar moiety defined by their stilbenic framework. However, in **5a** the presence of a double bond between the C2-C3 carbon of the benzofuran ring makes the whole molecule almost planar. Specifically, this additional double bond leads to an extended resonance delocalization for the 4'-phenoxyl radical, as the spin density can flow to the adjacent ring. On the contrary, the absence of this olefinic double bond in **2a** causes a decrease in the possible radical resonance delocalization and, consequently, a significant decrease in the antioxidant activity of this molecule.

Table 2: antioxidant activities of the hydroxystilbenes **1a-e** and of their dimeric derivatives **2a-e** and **5a-e**

derivatives	IC_{50} (mmol/ml)
1a	$6.1 \cdot 10^{-5}$
2a	$9.5 \cdot 10^{-4}$
5a	$1.1 \cdot 10^{-4}$
1b	$1.6 \cdot 10^{-4}$
2b	$1.8 \cdot 10^{-3}$
5b	$1.6 \cdot 10^{-4}$
1c	$3.1 \cdot 10^{-5}$
2c	$8.4 \cdot 10^{-4}$
5c	$2.4 \cdot 10^{-4}$
1d	$2.0 \cdot 10^{-5}$
2d	$3.5 \cdot 10^{-4}$
5d	$2.4 \cdot 10^{-5}$
1e	$2.1 \cdot 10^{-5}$
2e	$3.8 \cdot 10^{-4}$
5e	$1.4 \cdot 10^{-4}$

Furthermore, as the C2-C3 double bond in **5a** is in the *cis* conformation, the two aromatic rings linked at C-2 and C-3 are sterically hindered. As observed in a previous paper,[3c] the spin delocalization of the 4-phenoxyl radical via the double bond is partially hampered by the lack of full co-planarity of the π system. This effect might explain the observed slightly lower antioxidant activity of the derivative **5a** with respect to resveratrol.
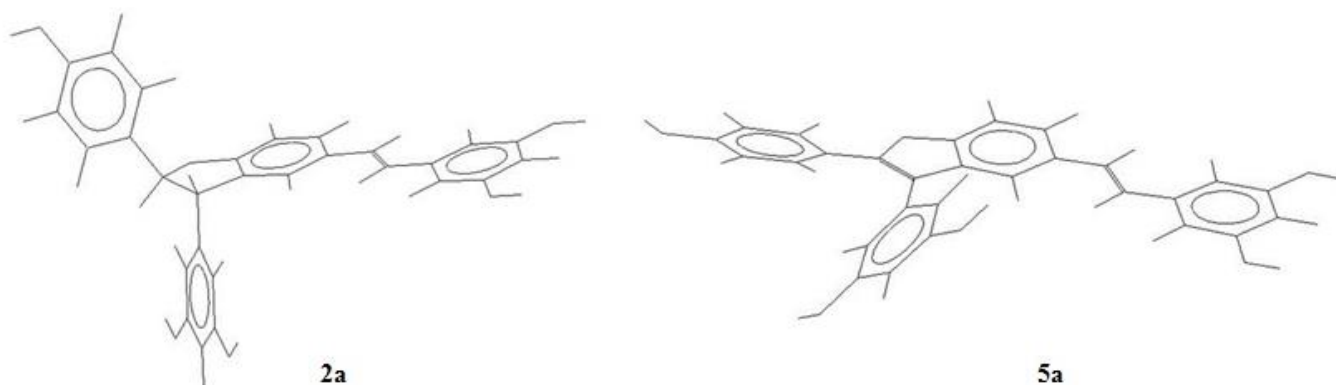
Figure 3. 3D structure visualization of molecules **2a** and **5a**.

3. Conclusion

We have reported here a convenient chemo-enzymatic synthesis of a new hydroxystilbene dimers containing the benzo[b]furans moiety and possessing an antioxidant activity comparable to that of the parent monomeric hydroxystilbenes. The proposed methodology can be useful for the synthesis of various 2,3-diaryl benzo[b]furan derivatives, a class of compounds that exhibits a wide range of biological activities, starting from suitable hydroxystilbenic precursors.

4. Experimental

4.1 General

Laccase from *Trametes versicolor* was from Fluka and its activity was evaluated by monitoring the oxidation of ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] at 436 nm. An enzymatic solution (10 mL) was added to a 1 mL cuvette containing 20 mM acetate buffer pH 3.5 (890 µL) and ABTS (100 µL of a 10 mM solution of ABTS in H_2O). One enzyme unit is defined as the amount of laccase that oxidizes 1 µmol of ABTS per minute under these conditions (ε_{ABTS} 29.3 mM^{-1}cm^{-1}).

trans-Resveratrol were obtained from Sigma. Compounds **1b-e** were synthesized by Perkins condensation starting from the suitable substituted hydroxy-benzaldehydes and phenylacetic acids in the presence of acetic anhydride and triethylamine, as described elsewhere.[20]

TLC were performed on precoated silica gel 60 F254 plates. Flashchromatography were performed using silica gel 60 (70–230 mesh).

Mass spectra were recorded with ion-trap coupled with a gas chromatograph operating under EI conditions (electron energy 70 eV). High resolution electrospray mass spectra (HRESI-MS) were acquired with an FT-ICR (Fourier Transfer Ion Cyclotron Resonance) instrument equipped with a 4.7 Tesla cryo-magnet. Samples were dissolved in MeOH and injected into the instrument equipped with its own ESI source. Spectra were recorded in the HR mode.

NMR spectra were obtained at 400 MHz using a FT-NMR instrument. All spectra were run on solutions in DMSO with tetramethylsilane as external reference. Chemical shifts are reported in parts per million to high frequency of the reference and coupling constants *J* are in Hertz.

Energy minimized stereostructures of **2a** and **5a** were obtained by MM+ calculation using HyperChem version 7.0 molecular modeling program(Hypercube, Inc., Gainesville, FL).

Antiradical activity was determined by DPPH method:[26] spectrophotometric data were acquired using a UV/VIS spectrometer.

4.2 General procedure for the oxidation of hydroxystilbenes catalyzed by T. versicolor laccase

Hydroxystilbenes (**1a-e**) (0.70 mmol) were dissolved in AcOEt (10 mL), while the laccase (40 U) was dissolved in 20 mM acetate buffer, pH 4.5 (10 mL). The biphasic systems were shaken at 40ºC and monitored by TLC. When the TLC spots indicated that the products were prevalent with respect to the initial substrates (6-72h), the reaction was quenched by phase separation followed by AcOEt extraction of the water solution. The organic solvent was evaporated and the crude residue was purified by flash chromatography to give the dehydrodimer (**2a-e**). The compounds were identified by comparison with the previously reported UV, mass, and ¹H-NMR spectral data.[20, 27]

2a. Yield = 31%. TLC (PetEt:EtOAc:MeOH, 3:7:1): R_f = 0.5. EI⁺-MS (m/z): 454 (M⁺).
2b. Yield = 29%. TLC (PetEt:AcOEt, 4:6): R_f 0.6. EI⁺-MS (m/z): 450 (M⁺).
2c. Yield = 19%. TLC (PetEt:AcOEt, 5:5): R_f 0.26. EI⁺-MS (m/z): 510 (M⁺).
2d. Yield = 22%. TLC (PetEt:AcOEt, 7:3): R_f 0.15. EI⁺-MS (m/z): 510 (M⁺).
2e. Yield = 17%. TLC (PetEt:AcOEt, 6:4): R_f 0.14. EI⁺-MS (m/z): 570 (M⁺).

4.3 General procedure for the acetylation of dehydrodimers

A solution of dehydrodimer (**2a-e**) (0.15mmol) in acetic anhydride (1 ml) and pyridine (1 ml) was stirred overnight at room temperature. The reaction was monitored by TLC (PetEt-AcOEt, 6:4).The organic solvent was evaporated and the crude residue containing the dimer acetate (**3a-e**) was used as such for the following oxidative step.

3a.Yield = 88%. TLC (PetEt:EtOAc, 6:4): R_f = 0.6. EI⁺-MS (m/z): 664 (M⁺).

3b. Yield = 71%. TLC (PetEt:AcOEt, 8:2): R_f 0.3. EI$^+$-MS (m/z): 492 (M$^+$).

3c. Yield = 81%. TLC (PetEt:AcOEt, 6:4): R_f 0.4. EI$^+$-MS (m/z): 552 (M$^+$).

3d. Yield = 50%. TLC (CHCl$_3$:CH$_3$OH, 10:0.01): R_f 0.4. EI$^+$-MS (m/z): 552 (M$^+$).

3e. Yield = 91%. TLC (CHCl$_3$:CH$_3$OH, 10:0.05): R_f 0.75. EI$^+$-MS (m/z): 612 (M$^+$).

4.4 General methodology for the oxidation of acetylated dehydrodimers

A crude sample (0.15 mmol) of acetylated dehydrodimer (**3a-e**) and DDQ (2,3-dichloro-5,6-dicyano-*p*-benzoquinone, 1.9 eq) in toluene (25 ml) was stirred under reflux. DDQ was added into the reaction mixture every 15 h (totally 9.5 eq). The course of the reaction was monitored by TLC (toluene-acetone, 20:1). After 90 hr, the organic solvent was evaporated and the crude residue containing the acetylated benzofuran derivative (**4a-e**) was purified by flash chromatography (PetEt-AcOEt-MeOH, 6:4) to remove the excess of DDQ.

4a. Yield = 100%. TLC (Toluene:Acetone, 20:1): R_f 0.6. EI$^+$-MS (m/z): 662 (M$^+$)

4b.Yield = 79%. TLC (Toluene:Acetone, 20:1): R_f 0.39. EI$^+$-MS (m/z): 490 (M$^+$)

4c. Yield = 30%. TLC (Toluene:Acetone, 20:1): R_f 0.3. EI$^+$-MS (m/z): 550 (M$^+$)

4d. Yield = 24%. TLC (Toluene:Acetone, 20:1): R_f 0.4. EI$^+$-MS (m/z): 550 (M$^+$)

4e. Yield = 54%. TLC (Toluene:Acetone, 20:1): R_f 0.16. EI$^+$-MS (m/z): 610 (M$^+$)

4.5 General methodology for the deacetylation of the acetylated benzofuran derivativs

A solution of the acetylated benzofuran derivative (**4a-e**) (0.06 mmol) and potassium hydroxide (150 mg) in methanol (20 ml) was stirred at room temperature. After 1 hr, the solution was diluted with cold water (15 ml) and then neutralized with 1% hydrochloric acid. The solution was extracted with ethyl acetate (each 10 ml, 3 times). The extract was washed with brine and then dried over sodium sulfate. After evaporation of the solvent, the residue was subjected to flash chromatography to give the products (**5a-e**) .

5a. Yield 99%. EI$^+$-MS (m/z): 452 (M$^+$). ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ = 6.16 (1H, t, 2.09 Hz, H-4 C); 6.28 (1H, t, 2.18 Hz, H-4 B); 6.32 (2H, d, 2.18 Hz, H-2,6 B); 6.45 (2H, d, 2.09 Hz, H-2,6 C); 6.79 (2H, AA' multiplet, H-3,5 A); 6.98 (1H, d, 16.36 Hz, H-2 vinyl); 7.16 (1H, d, 16.36 Hz, H-1 vinyl); 7.47 (2H, BB' multiplet, H-2,6 A); 7.54 (1H, broad s, H-4); 7.60 (1H, broad s, H-6); 7.60 (1H broad s, H-7); 9.21 (2H, bs, OH C); 9.40 (2H, bs, OH B); 9.85 (1H, bs, OH A). ^{13}C-NMR (400 MHz, DMSO-d$_6$): δ = 102.6 (C-4 B); 102.6 (C-4 C); 105.1 (C-2,6); 107.8 (C-2,6 B); 111.6 (C-7); 115.8 (C-3); 116.0 (C-3,5 A); 117.9 (C-4); 123.6 (C-6); 128.3 (C-2 vinyl); 128.8 (C-2,6 A); 128.7 (C-1 vinyl); 133.0 (C-5); 151.3 (C-2); 153.1 (C-8); 158.6 (C-4 A); 158.9 (C-3,5 C); 159.5 (C-3,5 B).

5b. Yield: 80%. EI$^+$-MS (m/z): 448 (M$^+$) ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ = 3.76 (3H, s, OC$\underline{H}_3$ C); 3.84 (3H, s, OC$\underline{H}_3$ B); 6.19 (1H, d, 16.41 Hz, H-2 vinyl); 6.79 (2H, AA' multiplet, H-3,5 A); 6.92 (2H, AA' multiplet, H-3,5 C); 7.09 (2H, AA' multiplet, H-3,5 B); 7.11 (1H, d, 16.41, H-1 vinyl); 7.41 (2H, BB' multiplet, H-2,6 B); 7.43 (2H, BB' multiplet, H-2,6 A); 7.52 (2H, BB' multiplet, H-2,6 C); 7.52 (1H, s, H-4); 7.58 (2H, s, H-6, H-7); 9.86 (1H, bs, OH). ^{13}C-NMR (400 MHz, DMSO-d$_6$): δ = 55.5 (2 x OCH_3);111.6 (2C, C-3, C-7); 114.6 (2C, C-3,5 C); 115.1 (2C, C-3,5 B); 116.0 (2C, C-3,5 A); 117.4 (C-4); 121.3 (C-1 A); 123.1 (C-6); 124.6 (C-1 B); 126.9 (C-2 vinyl); 127.3 (C-1 vinyl); 128.1 (2C, C-2,6 C); 128.6 (2C, C-2,6 A); 130.4 (C-1 C); 130.9 (C-9); 131.1 (2C, C-2,6 B); 133.4 (C-5); 151.3 (C-2); 153.0 (C-8); 158.5 (C-4 A); 159.2 (C-4 C); 159.2 (C-4 B).

5c. Yield: 79%.EI$^+$-MS (m/z): 508 (M$^+$) ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ = 3.72 (3H, s, C3-OC$\underline{H}_3$ C); 3.77 (3H, s, C3-OC$\underline{H}_3$ B); 3.81 (3H, s, C4-OC$\underline{H}_3$ B); 3.85 (3H, s, C4-OC$\underline{H}_3$ C); 6.80 (2H, AA' multiplet, H-3,5 A); 6.94 (1H, d, 6.79 Hz, H-5 C); 7.03 (1H, s, H-2 B); 7.05 (1H, d, 8.42 Hz, H-5 B); 7.10 (1H, d, 6.79 Hz, H-6 C); 7.12 (1H, d, 8.42 Hz, H-6 B); 7.12 (1H, d, 16.1 Hz, H-2 vinyl);7.26 (2H, BB' multiplet, H-2,6 A); 7.26 (1H, bs, H-2 C); 7.27 (1H, d, 16.1 Hz, H-1 vinyl); 7.56 (1H, bs, H-4);7.61 (2H, bs, H-6, H-7); 9.87 (1H, bs, OH). ^{13}C-NMR (400 MHz, DMSO-d$_6$): δ = 56.0 (4 x OCH_3); 109.8 (C-2 C), 111.6 (C-7), 112.4 (C-5 C), 112.9 (C-6 B), 113.7 (C-2 B), 116.0 (C-3), 116.1 (C-3,5 A), 117.8 (C-4), 120.2 (C-6 C), 121.3 (C-1 A), 122.3 (C-5 B), 123.1 (C-6), 125.0 (C-1 B), 127.3 (C-1 vinyl), 127.6 (C-2 vinyl), 128.6 (C-2,6 A), 130.8 (C-1 C), 133.4 (C-5), 134.4 (C-9), 148.8 (C-4 C), 148.9 (C-3 B), 149.3 (C-4 B), 149.5 (C-3 C), 151.5 (C-2), 153.0 (C-8), 158.6 (C-4 A).

5d. Yield: 99%.EI$^+$-MS (m/z): 508 (M$^+$) ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ = 3.61 (3H, s, OCH_3 A); 3.78 (3H, s, OCH_3 C); 3.84 (3H, s, OCH_3 B); 4.06 (3H, s, OCH_3); 6.79 (1H, d, 8.74 Hz, H-5 A); 6.94 (2H, AA' multiplet, H-3,5 C); 7.04-7.07 (2H, multiplet, H-2,6 A); 7.10 (1H, s, H-4); 7.11 (2H, AA' multiplet, H-3,5 B); 7.17 (1H, s, H-1,2 vinyl); 7.25 (1H, s, H-6); 7.42 (2H, BB' multiplet, H-2,6 B); 7.54 (2H, BB' multiplet, H-2,6 C); 9.44 (1H, bs, OH). ^{13}C-NMR (400 MHz, DMSO-d$_6$): δ = 55.6 (OCH_3 C); 55.7 (OCH_3 B); 55.8 (OCH_3 A); 56.5 (OCH_3); 105.3 (C-6); 110.9 (C-2 A); 114.8 (2C, C-3,5 C); 115.1 (C-3); 115.8 (2C, C-3,5 B); 116.2 (C-5 A); 120.3 (C-4); 120.3 (C-6 A); 121.5 (C-1 A); 127.3 (C-1 vinyl); 127.5 (C-2 vinyl); 128.0 (2C, C-2,6 C); 130.4 (C-1 C); 131.3 (C-1 B); 131.3 (2C, C-2,6 B); 132.2 (C-9); 134.4 (C-5); 142.2 (C-8); 145.4 (C-7); 147.9 (C-3 A, C-4 A); 151.2 (C-2); 159.2 (C-4 B); 159.3 (C-4 C).

5e. Yield: 82%.EI$^+$-MS (m/z): 568 (M$^+$) ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ = 3.62 (3H, s, OCH_3 A); 3.74 (3H, s, C3-OC$\underline{H}_3$ C); 3.77 (3H, s, C3-OC$\underline{H}_3$ B); 3.82 (3H, s, C4-OC$\underline{H}_3$ B); 3.84 (3H, s, C4-OC$\underline{H}_3$ C); 4.07 (3H, s, OC$\underline{H}_3$); 6.81 (1H, d, 8.0 Hz, H-5 A); 7.03 (1H, d, 6.79 Hz, H-5 C); 7.03 (1H, d, H-5 B); 7.04 (1H, d, H-2 B); 7.09 (1H, d, 8.0 Hz, H-6 A); 7.13 (1H, d, 6.79 Hz, H-6 C); 7.13 (1H, d, H-6 B); 7.14 (1H, bs, H-4); 7.16 (1H, d, 16.0 Hz, H-2 vinyl); 7.24 (1H, d, 16.0 Hz, H-1 vinyl); 7.26 (1H, bs, H-2 C); 7.26 (1H, bs, H-2 A); 7.26 (1H, bs, H-6); 9.45 (1H, bs, OH). ^{13}C-NMR (400 MHz, DMSO-d$_6$): δ = 55.9 (2 x OCH_3 B); 56.0 (2 x OCH_3 C); 55.7 (OCH_3 A); 56.4 (OCH_3); 105.0 (C-6); 109.5 (C-2 A); 109.5 (C-2 C); 110.8 (C-4); 112.3 (C-5 C); 112.9 (C-6 B); 113.7 (C-2 B); 116.0 (C-3); 116.1 (C-5 A); 120.1 (C-6 C); 120.3 (C-6 A); 121.5 (C-1 A); 122.5(C-5 B); 125.0 (C-1 B); 127.4 (C-1 vinyl); 127.8 (C-2 vinyl); 130.8 (C-1 C); 134.2 (C-5); 134.4 (C-9); 142.1 (C-8); 145.4 (C-7); 147.9 (C-3 A); 149.0 (C-4 C); 149.0 (C-4 A); 149.0 (C-3 B); 149.4 (C-4 B); 149.6 (C-3 C); 151.2 (C-2);

4.6 Antiradical activity

The antioxidant activities of the compounds **1a-e**, **2a-e** and **5a-e** were determined using DPPH as a free radical, following the method described by Berset,[26] using different concentrations (0.03-1 mM) of the compound to be tested. A methanolic

solution of compound **1a-e**, **2a-e** or **5a-e** (100 µL) was added to 3.9 mL of a 0.06 mM DPPH methanolic solution. The decrease in absorbance at 515 nm was evaluated until the reaction reached a plateau (about 3 h). The percentage of residual DPPH at the steady state was reported onto a graph as a function of the molar ratio of antioxidant to DPPH. Antiradical activity was defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (efficient concentration).

Acknowledgements

Supplementary materials

NMR spectra of the compounds are available.

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