NJC

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: P. Gentili , R. Bernini, F. Crisante, E. Ussia and F. D'acunzo, *New J. Chem.*, 2016, DOI: 10.1039/C5NJ03133H.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

View Article Online
DOI: 10.1039/C5NJ03133H

Journal Name

ARTICLE

Oxidative cleavage of 1-aryl-isochroman derivatives by the *Trametes villosa* laccase/1-hydroxybenzotriazole system

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

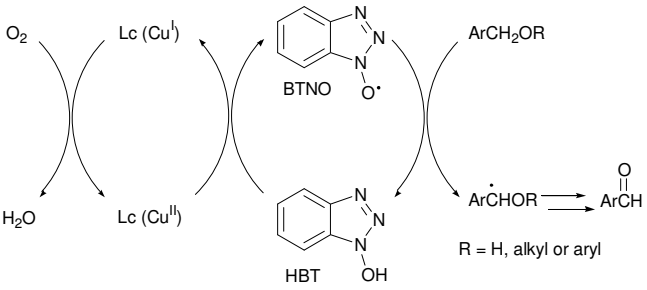
www.rsc.org/

Roberta Bernini,[a*] Fernanda Crisante,[a] Francesca D'Acunzo,[b] Patrizia Gentili,[c*] Emanuele Ussia[c]

The oxidative cleavage of the dihydropyran ring of 1-aryl-isochroman derivatives was carried out for the first time under green chemistry conditions in the presence of the *Trametes villosa* laccase/1-hydroxybenzotriazole system in buffered water/1,4-dioxane and buffered water/dimethyl carbonate as reaction media. The corresponding oxidation products [2-(2-hydroxyethyl)benzophenone derivatives] were obtained in different yields depending on the substituents on phenyl and isochroman rings. These compounds are useful intermediates for the synthesis of anticancer agents and neuroprotective drugs.

Introduction

The laccase enzymes are multicopper oxidases characterized by the presence of at least four cupric ions.[1] They are widely distributed in nature, being produced by several fungi, bacteria, insects and trees. Depending on the producing organism, their biological role can range from processes such as melanization of pathogens, lignification in plants and sclerotization in insects to wood delignification.[1a] Laccases catalyse the monoelectronic oxidation of substrates such as phenols and amines with the concomitant reduction of molecular dioxygen to water.[2] Moreover, the use of mediators can extend their oxidative capabilities for synthetic purposes.[3] Among them, 1-hydroxybenzotriazole (HBT) is particularly efficient towards the oxidation of benzylic alcohol and ether derivatives. Laccases oxidize HBT to the benzotriazole-*N*-oxyl (BTNO) radical that is responsible for the abstraction of a hydrogen atom from the benzylic position of the substrate according to the hydrogen atom transfer (HAT) mechanism (Scheme 1).[3c,3d] Our research group recently investigated the efficiency of the *Trametes villosa* laccase/HBT system in the oxidation of naturally occurring phenolic compounds under mild experimental conditions.[4] Specifically, methylated catechins and epicatechins were converted into the corresponding flavan-3,4-diols and C-4 ketones, bioactive compounds and useful intermediates for proanthocyanidins, plant polyphenols showing beneficial health properties for humans.[4a] Methylated planar catechins and bent catechins were selectively oxidized at the C-2 or C-4 benzylic position.[4c]

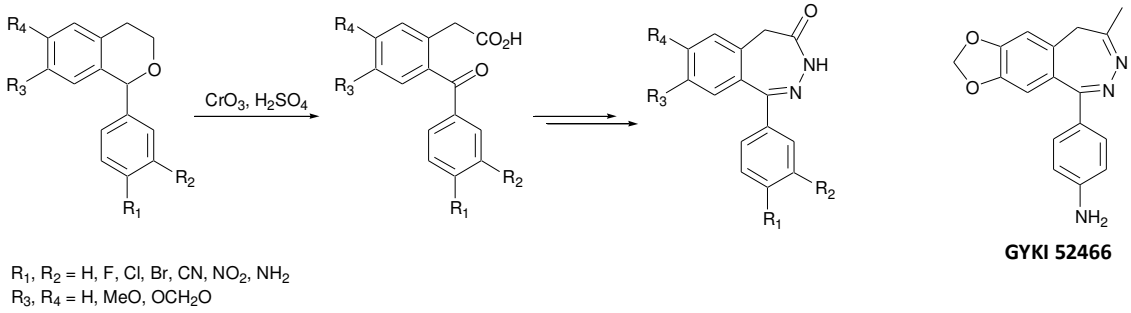


Scheme 1. Catalytic cycle of the laccase (Lc)/HBT system in the oxidation of benzylic alcohol and ether derivatives.

Following on from these interesting results, as part of our research devoted to the selective oxidation of natural compounds to obtain fine chemicals and bioactive compounds, we turned our attention to isochromans. Isochromans, or 3,4-dihydro-1*H*-benzo*[c]*pyran derivatives, are a class of natural compounds produced by only a few species of fungi, bacteria and trees.[5] Despite their sparsity in nature, they are attractive compounds exhibiting hypotensive,[6] antitumoural,[7] antibacterial [8] and antioxidant [9] activities. In addition, they are useful synthetic intermediates to obtain several classes of bioactive compounds including isochromanones,[10] benzodiazepines [11] and 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones.[12] To the best of our knowledge, there are only a few reports describing the oxidation of isochromans. Generally, the reactions were performed using stoichiometric reagents such as dimethyldioxirane [13] and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone [14] or hazardous reagents such as chromium(VI)/sulphuric acid (the Jones reagent) and antimony(V) pentachloride.[11,12,15] For example, Jones reagent was used for the oxidative cleavage of the dihydropyran ring of 1-aryl-isochromans to give the corresponding 2-benzoylphenylacetic acids, precursors of several noncompetitive

[a] Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, Via S. Camillo De Lellis, 01100 Viterbo, Italy. E-mail: berninir@unitus.it
[b] Institute of Chemical Methodologies, CNR, Via Salaria Km. 29.300, 00015 Monterotondo, Rome, Italy
[c] Department of Chemistry, IMC-CNR Section Mechanisms of Reaction, University of Rome La Sapienza, P. le A. Moro 5, 00185 Roma, Italy. E-mail: patrizia.gentili@uniroma1.it

View Article Online
DOI: 10.1039/C5NJ03133H



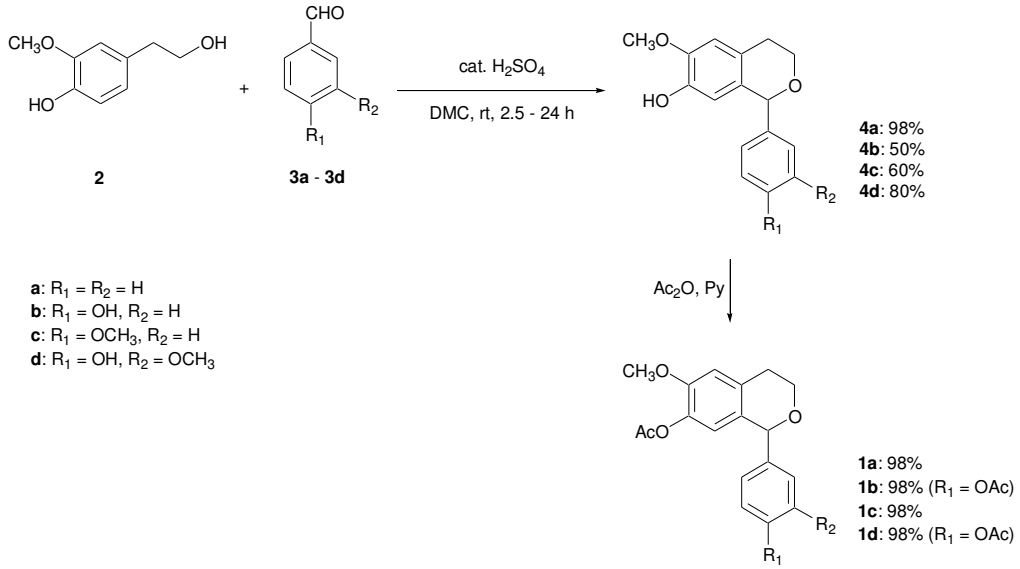
Scheme 2. Synthesis of GYKI 52466 analogues by Jones oxidation of 1-aryl-isochromans.[11c, 12]

AMPA receptor antagonists, analogues of the neuroprotective agent GYKI 52466 [1-(4-aminophenyl)-4-methyl-7,8-(methylenedioxy)-5*H*-2,3-benzodiazepine] (Scheme 2).[11c, 12]
Using data from these reports and our experience in the oxidation of natural compounds under green chemistry conditions, we describe here the oxidation of 1-aryl-isochroman derivatives by the *Trametes villosa* laccase/HBT system. The reactions were performed under aerobic conditions in reaction media [sodium acetate buffer/1,4-dioxane and sodium acetate buffer/dimethyl carbonate (DMC)] able to both solubilize isochromans and retain the activity of laccase. As reported in the literature, DMC is a friendly chemical [16] widely used in laboratories as both solvent and reagent.[17] However, until now, it has never been used as a co-solvent in enzymatic reactions. Under our experimental conditions, 1-aryl-isochromans were selectively oxidized in both reaction media at the benzyl ethereal position. The subsequent ring opening generated the corresponding 2-(2-hydroxyethyl)benzophenone derivatives, novel compounds and useful intermediates in drug synthesis.

Results and discussion

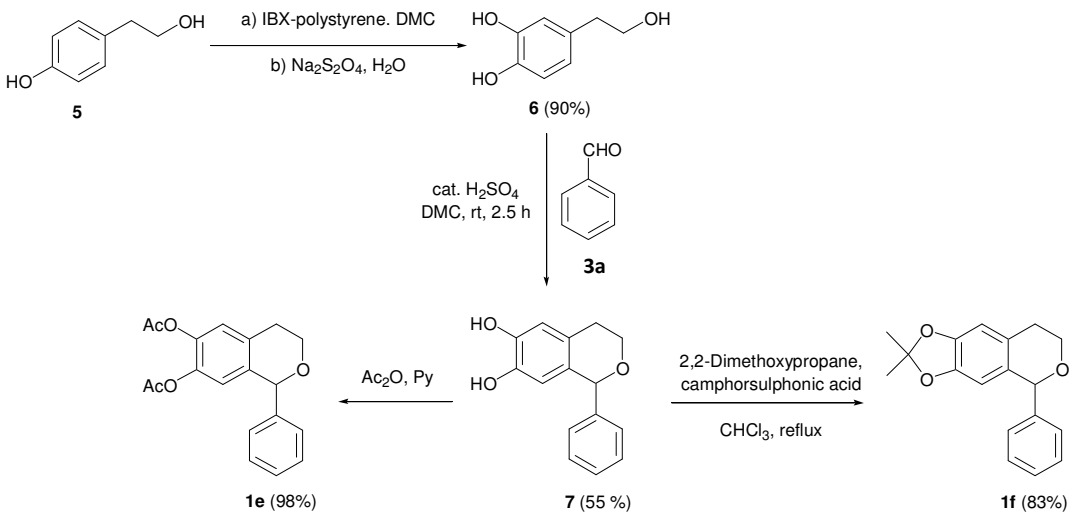
Synthesis of isochromans

1-Aryl-isochroman derivatives **1a - 1d** were synthesized by a two-step procedure depicted in Scheme 3. The first step was the oxa-Pictet-Spengler reaction from 2-(4-hydroxy-3-methoxyphenyl)ethanol (homovanillyl alcohol) **2** and benzaldehyde **3a**, 4-hydroxy-benzaldehyde **3b**, 4-methoxybenzaldehyde **3c** and 4-hydroxy-3-methoxybenzaldehyde **3d** to give the corresponding 1-aryl-7-hydroxy-6-methoxy-isochromans **4a** – **4d** (yields: 50 - 98%). The reactions were performed at room temperature in acidic medium using DMC as solvent,[17] according to the procedure recently optimized in our laboratories.[18] The following step was the acetylation of isochromans **4a** – **4d** carried out with acetic anhydride in pyridine to afford the corresponding acetylated derivatives **1a** – **1d** in quantitative yields. Protection of the phenolic groups was necessary to avoid laccase-polymerization reactions, well documented in the literature.[19]



Scheme 3. Synthesis of 1-aryl-isochromans **1a** -**1d**.[18]

View Article Online
DOI: 10.1039/C5NJ03133H



Scheme 4. Synthesis of isochromans **1e** and **1f**.

Finally, isochromans **1e** and **1f** were synthesized. As illustrated in Scheme 4, 2-(3,4-dihydroxyphenyl)ethanol **6** was first obtained by direct oxidation of tyrosol **5** with IBX-polystyrene and *in situ* reduction with sodium dithionite.[17d] Following the oxa-Pictet-Spengler reaction of **6** with benzaldehyde **3a**, isochroman **7** was isolated (yield: 55%). Subsequent treatment of **7** with acetic anhydride in pyridine afforded isochroman **1e** (quantitative yield), while with 2,2-dimethoxypropane/camphorsulphonic acid gave the corresponding acetonide **1f** (yield: 83 %).[20]

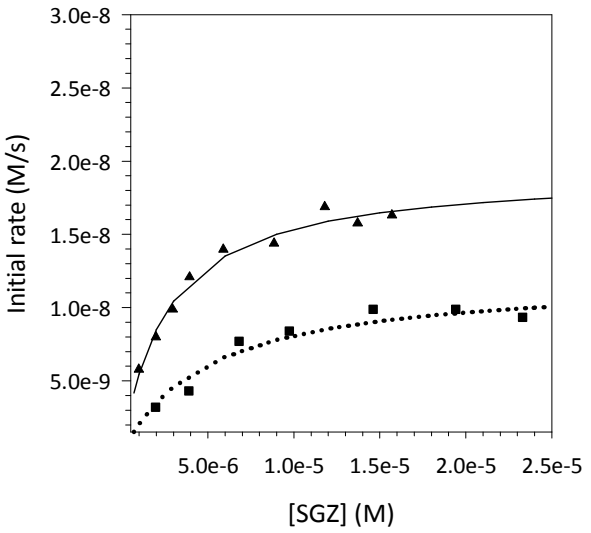


Figure 1. Michaelis-Menten plots for oxidation of SGZ by *Trametes villosa* laccase in sodium acetate buffer (▲, experimental points, — Michaelis-Menten fit) and sodium acetate buffer/DMC 10/1 (v/v, ■, experimental points, ····· Michaelis-Menten fit).

***Trametes villosa* laccase stability in the presence of DMC as co-solvent**

To the best of our knowledge, DMC is a new solvent for enzymatic reactions. First, we investigated the activity of *Trametes villosa* laccase in the presence of DMC spectrophotometrically using syringaldazine (SGZ) as a benchmark substrate.[21] Michaelis K_M and catalytic k_{cat} constants were calculated in the homogeneous mixture sodium acetate buffer/DMC = 10/1 (v/v) (a ratio chosen according to the reported solubility of DMC in water)[16] and compared to those obtained in sodium acetate buffer alone. As shown in Figure 1, laccase followed a Michaelis-Menten kinetic in both reaction media. The kinetic parameters (K_M = 4.5 µM and k_{cat} = 2483 min^{-1} in sodium acetate buffer/DMC; K_M = 2.5 µM and k_{cat} = 3793 min^{-1} in sodium acetate buffer[21]) indicated that laccase retained about 60% of its activity in the presence of DMC.

Table 1. Oxidation of 2,4,6-trichlorophenol **8** with *Trametes villosa* laccase in a mixed-solvent[a]

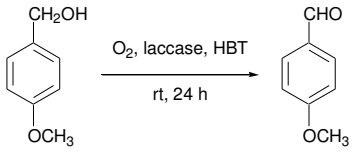
Entry	Reaction medium	Recovered 8 (%)[b]
1	Sodium acetate buffer/DMC 10/1	15
2	Sodium acetate buffer /DMC 5/1	23
3	Sodium citrate buffer/1,4-dioxane 1/1[c]	< 1

[a] [**8**] = 20 mM, [Laccase] = 3.2 U/mL, 24 h, room temperature.
[b] Determined by GC with the internal standard method; error ± 3%.
[c] From reference 22

Unfortunately, investigating the solubility of isochromans **1a** - **1f**, we found that they were almost soluble in sodium acetate buffer/DMC = 5/1 (v/v). As it was not possible to determine laccase activity spectrophotometrically in this heterogeneous medium, we verified the enzymatic efficiency directly by performing the oxidation of 2,4,6-trichlorophenol **8**, a typical substrate of laccase,

View Article Online
DOI: 10.1039/C5NJ03133H

in both sodium acetate buffer/DMC = 10/1 (v/v) and sodium acetate buffer/DMC = 5/1 (v/v) mixtures (Table 1) and compared the efficiency of its oxidation with that previously reported for the aqueous buffer/1,4-dioxane 1/1 (v/v) system.[22] Our findings indicated that laccase retained a similar activity in both mixtures (Table 1). Moreover, unlike sodium acetate buffer/1,4-dioxane medium, DMC as co-solvent is particularly advantageous, in that no further laccase addition during the oxidation is necessary to compensate for enzymatic activity loss (see Experimental section).[4] Finally, to evaluate the efficiency of the mediator HBT in the laccase-catalysed oxidation, we carried out the oxidation of 4-methoxybenzyl alcohol **9** as a model compound in both sodium acetate buffer and sodium acetate buffer/DMC = 5/1 (v/v) (Scheme 5). The satisfactory and comparable yields of the aldehyde **10** obtained in both reaction media confirmed that the activity of the laccase/HBT system was essentially unchanged in the presence of DMC (Scheme 5).



9 **10**

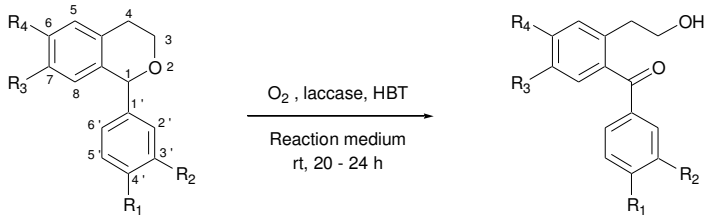
Reaction medium	*Yields (%)*
NaOAc buffer	75
NaOAc buffer/DMC 5/1	68

Scheme 5. Oxidation of 4-methoxybenzyl alcohol **9** with the *Trametes villosa* laccase/HBT system

Oxidation of isochromans 1a - 1f with the *Trametes villosa* laccase/HBT system

Based on these preliminary findings, we performed the oxidation of 1-aryl-isochroman derivatives **1a** - **1f** with the laccase/HBT system in sodium acetate buffer using 1,4-dioxane or DMC as co-solvent (Scheme 6, Table 2).

As reported, the laccase/HBT system appeared to be a selective oxidant exclusively generating products **11a – 11f** derived from the cleavage of the dihydropyran ring, even although the yields were very low when using compound **1b** as substrate (Table 2, entries 3, 4). These results could be related to its poor solubility in the reaction media. On the contrary, substrates **1a**, **1c**, **1d**, **1e** and **1f** showed a good solubility and the laccase/HBT-catalysed oxidation appeared affected by electronic effects of the substituents on the benzopyran and/or phenyl rings. In particular, compound **1a**, without substituents on the phenyl ring, was oxidized in satisfactory yields in sodium acetate buffer/1,4-dioxane and sodium acetate buffer/DMC (yield 68 and 65%; Table 2, entries 1 and 2). Of note, isochroman **1c**, having an electron-donating methoxy group on C4', i.e. in *para*-position with respect to the benzylic carbon C1, showed the highest reactivity, producing oxidation product **11c** in quantitative yield in sodium acetate buffer/1,4-dioxane = 1/1 (Table 2, entry 5). Compound **1d**, having both an acetoxy substituent on the C4' position and a methoxy substituent on the C3' position, showed a lower reactivity than **1a** and **1c**, even although product yields were satisfactory in sodium acetate buffer/1,4-dioxane and sodium acetate buffer/DMC (Table 2, entries 7 and 8). Instead, the role of the acetoxy group was clearly demonstrated when it was on the C6 position, i.e. in *para*-position with respect to the benzylic C1 carbon of the isochroman moiety. Indeed, compound **1e** was partially oxidized by the laccase/HBT system in both reaction media (Table 2, entries 9 and 10). As discussed above, the poor solubility of 1-aryl-isochroman **1b** in both reaction media prevented us from drawing any conclusions about the role of an electron-drawing group such as AcO on the C4' position of the phenyl ring (Table 2, entries 3 and 4). Interestingly, the oxidation of 1-aryl-isochroman **1f**, having a methylenedioxy group on the C6 and C7 positions of the isochroman moiety, gave product **11f** in very good yield in sodium acetate buffer/1,4-dioxane solvent (83%; Table 2, entry 11). This result is very promising for synthetic applications due to the fact that the 1,3-benzodioxole ring is a structural feature of several bioactive natural anticancer agents.[23] In particular, it is present in a series of 1,3-benzodioxole derivatives showing cytotoxic activity against several human tumour cell lines including colon carcinoma cells [24] and multidrug-resistant nasopharyngeal carcinoma cells.[25]



1a - **1f**

a: $R_1 = R_2 = H$, $R_3 = OAc$, $R_4 = OCH_3$
b: $R_1 = OAc$, $R_2 = H$, $R_3 = OAc$, $R_4 = OCH_3$
c: $R_1 = OCH_3$, $R_2 = H$, $R_3 = OAc$, $R_4 = OCH_3$
d: $R_1 = OAc$, $R_2 = OCH_3$, $R_3 = OAc$, $R_4 = OCH_3$
e: $R_1 = R_2 = H$, $R_3 = R_4 = OAc$
f: $R_1 = R_2 = H$, R_3, $R_4 = OC(CH_3)_2O$

Yields (%)	*NaOAc buffer/ 1,4 dioxane*	*NaOAc buffer/ DMC*
11a:	68	65
11b:	7	-
11c:	95	19
11d:	46	60
11e:	17	30
11f:	83	8

Scheme 6. Oxidation of isochroman derivatives **1a** - **1f** with the *Trametes villosa* laccase/HBT system in NaOAc buffer/1,4-dioxane and NaOAc buffer/DMC.

View Article Online
DOI: 10.1039/C5NJ03133H

Table 2. Oxidation of compounds **1a – 1f** with the *Trametes villosa* laccase/HBT system[a]

Entry	1-Aryl-isochroman	Experimental conditions	Conversion (%)[b]	Product (Yield, %)[b]
1	**1a**	NaOAc buffer/1,4-dioxane = 1/1, 20 h	69	**11a** (68)
2	**1a**	NaOAc buffer/DMC = 5/1, 20 h	66	**11a** (65)
3	**1b**	NaOAc buffer/1,4-dioxane = 1/1,[c] 24 h	8	**11b** (7)
4	**1b**	NaOAc buffer/DMC = 5/1,[c] 24 h	7	**11b** (7)
5	**1c**	NaOAc buffer/1,4-dioxane = 1/1, 20 h	98	**11c** (95)
6	**1c**	NaOAc buffer/DMC = 5/1,[c] 20 h	20	**11c** (19)
7	**1d**	NaOAc buffer/1,4-dioxane = 1/1, 24 h	47	**11d** (46)
8	**1d**	NaOAc buffer/DMC = 5/1, 24 h	60	**11d** (60)
9	**1e**	NaOAc buffer/1,4-dioxane = 1/1, 24 h	18	**11e** (17)
10	**1e**	NaOAc buffer/DMC = 5/1, 24 h	30	**11e** (30)
11	**1f**	NaOAc buffer/1,4-dioxane = 1/1, 20 h	85	**11f** (83)
12	**1f**	NaOAc buffer/DMC = 5/1,[c] 20 h	10	**11f** (8)

[a] [1-Aryl-isochroman]=0.05 M; [HBT]=0.016 M; [Laccase]=7.5 U/mL, room temperature. [b] Determined by HPLC with the internal standard method; error ± 3%. [c] 1-Aryl-isochroman not totally soluble.

In contrast, the low conversion of compound **1f** in acetate buffer/DMC (Table 2, entry 12) could be due to its poor solubility in this medium

Mechanistic speculations

According to the literature,[3c,3d,26] the key step in the oxidation of benzyl alcohols and ethers with the laccase/HBT system is the abstraction of a hydrogen atom from the benzylic carbon by the oxidized form of the mediator, the BTNO radical (Scheme 1). Therefore, to explain the cleavage of the isochroman ring, we assumed that once formed, the BTNO radical could abstract a hydrogen atom from the C1 position of the isochroman ring to give the corresponding benzyl radical. This radical intermediate might be further oxidized (probably by BTNO or laccase) to the corresponding carbocation and after addition of water, it evolved to a transitional hemiacetal; the subsequent opening of the hemiacetalic ring afforded derivatives **11a – 11f** (Scheme 7).[26] The observed effects of the substituents on 1-aryl-isochroman derivatives were consistent with our previous results obtained from the laccase/HBT oxidation of *para*-substituted benzyl alcohols.[3d] Electron-donor substituents heightened the rate of hydrogen abstraction (k_H) by the BTNO radical, while electron-withdrawing substituents reduced the reactivity of the HAT step. We explained the different results on the basis of both the electrophilic nature of BTNO and the polar effects of substituents on stabilization of the benzyl radical in the transition state of HAT.[3d] These previous observations shed light on the different behaviour of 1-aryl-isochromans **1a**, **1c**, **1d**, **1e** and **1f** in the oxidative cleavage with the laccase/HBT system. The presence of electron-donor substituents on the isochroman moiety and/or phenyl ring, such as in compounds **1a** and **1c**, promoted hydrogen abstraction from the benzylic C1 position by the BTNO radical. In particular, a cooperative effect could be appreciated with 1-aryl-isochromans **1c**, having two methoxy groups in *para*-position with respect to the benzylic carbon C1, and **1f**, where a methylenedioxy moiety was present on the C6 and C7 positions. When electron-withdrawing substituents were on either the isochroman or phenyl ring, the reactivity of 1-aryl-isochromans was reduced (compounds **1d** and **1e**) or almost entirely suppressed (compound **1b**).

Conclusions

Oxidative cleavage of 1-aryl-isochroman derivatives was performed for the first time with the *Trametes villosa* laccase/HBT system in aqueous media in different yields depending on the nature of the substituents on both the isochroman and phenyl moieties. The green character of this procedure was enhanced by the use of DMC as co-solvent. Even when the solubility of some compounds was partial, we demonstrated the potentiality of using DMC in laccase-catalysed oxidations by kinetic investigations. Among the oxidation products, compound **11c**, a useful intermediate for the synthesis of 2,3-benzodiazepine derivatives, was obtained in quantitative yields, while compound **11f** is remarkable due to its 1,3-benzodioxole ring, a leitmotif present in bioactive natural anticancer agents and neuroprotective drugs.

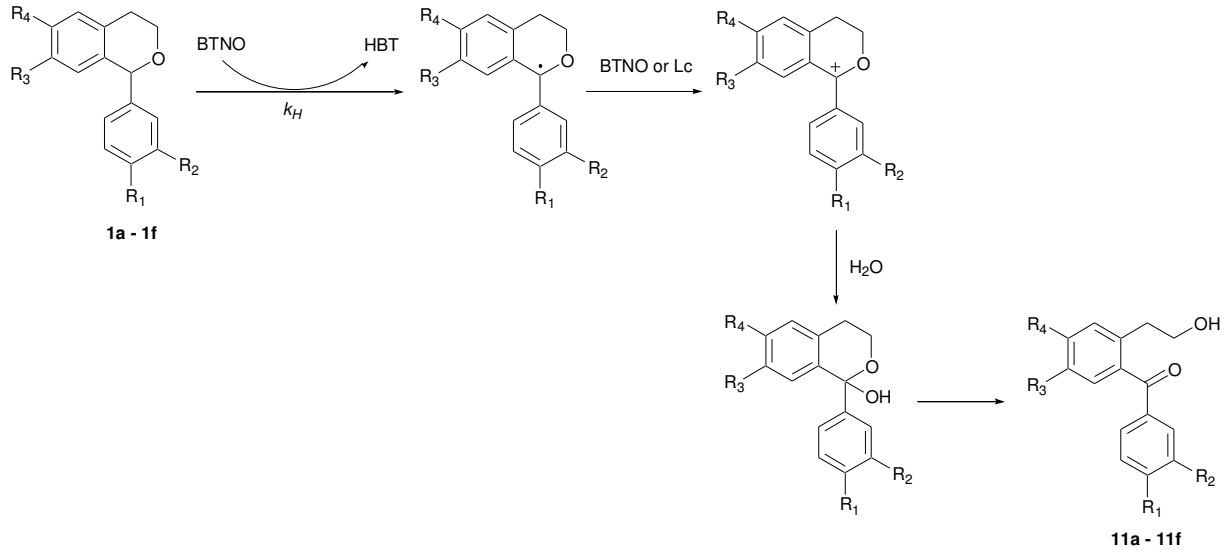
This new mild oxidative approach could lead to new synthetic methodologies for 1,3-benzodioxole derivatives with important bioactive properties, thereby providing an alternative to current procedures using harsh experimental conditions and/or harmful oxidants.

Experimental

Materials and instruments

Reagents and solvents were supplied by Sigma-Aldrich (Milan, Italy) and used without further purification. Polymer-supported IBX was purchased from Novabiochem (loading factor = 1.1 mmol/g). Crude laccase from *Trametes villosa* (Novozym 51002) was a kind gift from Novo Nordisk Co. It was free of lignin or manganese peroxidases and purified as above described by us.[4a] Silica gel (200 - 300 mesh) and silica gel F254 plates were furnished from Merck (Milan, Italy). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a spectrometer 200 and 400 MHz Bruker using $CDCl_3$ as solvent. All chemical shifts are expressed in parts per million (δ scale) and coupling constants are reported in Hertz (Hz). GC-MS analyses were performed on a

View Article Online
DOI: 10.1039/C5NJ03133H



Scheme 7. Mechanism of the oxidative cleavage of 1-aryl-isochroman derivatives **1a - 1f** by the *Trametes villosa* laccase/HBT system.

Shimadzu VG 70/250S apparatus equipped with a Supelco SLB^{TM} 5 ms column (30 m x 0.25 mm x 0.25 μm). The analyses were performed using an isothermal temperature profile of 100 °C for 2 min, followed by a 10 °C min^{-1} temperature gradient until 280 °C for 15 min. The injector temperature was 280 °C. HPLC analyses were carried out with an instrument equipped with a C18 column (150 mm x 4.6 mm x 5 mm) and a UV detector selected at λ = 280 nm. The chromatographic profiles were recorded at a flow rate of 1.0 mL/min using the following method: acetonitrile/water = 10/90 (0 - 10 min); acetonitrile/water = 60/40 (10 - 25 min); acetonitrile/water = 60/40 (5 min); acetonitrile/water = 80/20 (30 – 35 min) and finally acetonitrile/water = 80/20 (5 min). ATR FT-IR spectra were recorded with a Thermo Scientific Nicolet iS10 spectrometer equipped with a triglycine sulfate detector (DTGS), and acquired with Omnic vers. 8.1.10 software. The spectra were the result of 32 scans with a spectral resolution of 4 cm^{-1}. A Smart iTR ATR accessory equipped with a diamond ATR crystal was used. An ATR correction algorithm and automatic atmospheric suppression was applied to all spectra. The enzymatic activity was determinated using a diode array spectrophometer Varian equipped with a thermostated cuvette holder.

Synthesis of hydroxytyrosol (6). Hydroxytyrosol was synthesized as already described by us.[17d] Briefly, tyrosol **5** (1.0 mmol) was dissolved in dimethyl carbonate (10 mL) at room temperature under magnetic stirring and polymer supported-IBX (954 mg, 2.1 mmol) was added. After 1h, the mixture was filtered to recover the polymer while the solution was treated with sodium dithionite (348 mg, 2.0 mmol) and water (8 mL) for 30 min. After evaporation of the solvent under reduced pressure, the final product was extracted with ethyl acetate (3 x 10 mL) from the aqueous residue. The combined organic phases were washed with a saturated solution of NaCl and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, hydroxytyrosol **6** was obtained as a colorless oil. Spectroscopic data were according to the literature.[17d]

Synthesis of 1-aryl-isochromans. Isochromans were synthesized according to a procedure optimized by us.[18] 2-(4-Hydroxy-3-methoxyphenyl)ethanol **2** (168 mg, 1.0 mmol) was dissolved in dimethyl carbonate (8 mL), then sulphuric acid (0.2 mmol) and the appropriate benzaldehyde (1.2 mmol) were added. The mixture was kept at room temperature under magnetic stirring for 2.5-12 h depending on the substrate. At the end, the solvent was removed under reduced pressure; the crude was treated with water (10 mL) and extracted with ethyl acetate (3 x 10 mL). The organic phases were reunited and washed with a saturated solution of NaCl, then dried over anhydrous Na_2SO_4. After evaporation of the solvent under reduced pressure, the 1-aryl-isochroman was recovered and purified in a silica gel chromatographic column using hexane/ethyl acetate = 3/1 or 3/2 as eluents. In a similar manner, 1-aryl isochroman **7** was synthetized from fresh hydroxytyrosol **6** and benzaldehyde **3a**.

6-Methoxy-1-phenylisochroman-7-ol (4a). Quantitative yield; colorless oil; spectroscopic data were according to the literature.[18]

1-(4'-Hydroxyphenyl)-6-methoxyisochroman-7-ol (4b). Yield: 50%; colorless oil; spectroscopic data were according to the literature.[18]

6-Methoxy-1-(4'-methoxyphenyl)isochroman-7-ol (4c). Yield: 60%; colorless oil. Found: C, 72.14; H, 6.20 %; M$^+$, 286. $C_{17}H_{18}O_4$ requires C, 71.31; H, 6.34 %; M, 286. ν_{max}/cm^{-1}: 3304 (br, OH), 3011, 2937, 2882, 1609, 1511, 1455, 1259, 1170, 1075. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 7.20 (d, *J* = 8.7 Hz, 2H, Ph-H), 6.84 (d, *J* = 8.7 Hz, 2H, Ph-H), 6.60 (s, 1H, Ph-H), 6.30 (s, 1H, Ph-H), 5.59 (s, 1H, CH), 4.18-4.10 (m, 1H, CH), 3.92-3.81 (m, 1H, CH), 3.83 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.09-2.94 (m, 1H, CH), 2.74-2.62 (m, 1H, CH). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ: 159.4, 145.6, 143.8, 134.6, 130.3, 130.0, 125.3, 113.8, 112.8, 110.6, 78.8, 63.7, 55.9, 55.2, 28.5. GC/MS (m/z, %): 286 (M$^+$, 80), 258 (42), 179 (52), 135 (100).

1-(4'-Hydroxy-3'-methoxyphenyl)-6-methoxyisochroman-7-ol (4d). Yield: 80%; colorless oil; spectroscopic data were according to the literature.[18]

1-Phenylisochroman-6,7-diol (7). Yield: 55 %; colorless oil; spectroscopic data were according to the literature.[18]

View Article Online
DOI: 10.1039/C5NJ03133H

Acetylation of 1-aryl-isochromans. To a solution of isochroman (reaction scale: 100 mg) in dry pyridine (1.5 mL) was added acetic anhydride (1.5 mL). The mixture was kept under stirring at room temperature overnight. At the end, the mixture was poured into ice-water (5 mL), treated with 1 N HCl and extracted with ethyl acetate (2 x 10mL). Finally, the combined organic phases were washed with a saturated solution of NaCl and dried over anhydrous Na_2SO_4. After evaporation of the solvent under reduced pressure, pure sample of isochroman derivative was obtained after purification by silica gel chromatographic column using hexane/ethyl acetate = 3/1 as eluent.

6-Methoxy-1-phenylisochroman-7-yl acetate (1a). Quantitative yield; colorless oil. Found: C, 73.05; H, 5.95 %; M^+, 298. $C_{18}H_{18}O_4$ requires C, 72.47; H, 6.08 %; M, 298. ν_{max}/cm^{-1}: 3002, 2944, 2853, 1758 (acetate CO stretch), 1616, 1509, 1452, 1256, 1182, 1091. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.36-7.27 (m, 5H, Ph-H), 6.74 (s, 1H, Ph-H), 6.40 (s, 1H, Ph-H), 5.64 (s, 1H, CH), 4.24-4.14 (m, 1H, CH), 3.94-3.82 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.12-3.02 (m, 1H, CH), 2.78-2.68 (m, 1H, CH), 2.05 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 169.1, 149.7, 148.9, 141.8, 137.8, 132.4, 129.7, 128.8, 128.5, 128.2, 121.0, 112.2, 79.2, 63.9, 55.9, 28.7, 20.6. GC/MS (m/z, %): 298 (M^+, 20), 256 (70), 228 (32), 179 (100).

1-(4'-Acetoxyphenyl)-6-methoxyisochroman-7-yl acetate (1b). Quantitative yield; colorless oil. Found: C, 68.45; H, 5.99 %, M^+, 356. $C_{20}H_{20}O_6$ requires C, 67.41; H, 5.66%, M, 356. ν_{max}/cm^{-1}: 3010, 2964, 2850, 1760 (acetate CO stretch), 1610, 1509, 1454, 1256, 1180, 1091. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.29 (d, 2H, *J* = 8.5 Hz, Ph-H), 7.05 (d, 2H, *J* = 8.5 Hz, Ph-H), 6.72 (s, 1H, Ph-H), 6.41 (s, 1H, Ph-H), 5.63 (s, 1H, CH), 4.24-4.14 (m, 1H, CH), 3.94-3.82 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.11-3.02 (m, 1H, CH), 2.76-2.68 (m, 1H, CH), 2.27 (s, 3H, CH_3), 2.20 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 169.3, 169.1, 150.5, 149.8, 139.3, 137.9, 132.5, 129.9 (2C), 129.3, 121.5 (2C), 121.1, 112.,3, 78.4, 63.8, 55.9, 28.7, 21.1, 20.6. GC/MS (m/z, %): 356 (M^+, 20), 314 (80), 244 (100), 178 (80).

6-Methoxy-1-(4'-methoxyphenyl)isochroman-7-yl acetate (1c). Quantitative yield; colorless oil. Found: C, 69.82; H, 6.04 %; M^+, 328. $C_{19}H_{20}O_5$ requires C, 69.50; H, 6.14 %; M, 328. ν_{max}/cm^{-1}: 3008, 2946, 2850, 1756 (acetate CO stretch), 1612, 1510, 1455, 1252, 1178, 1090. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.21 (d, 2H, *J* = 8.7 Hz, Ph-H), 6.85 (d, 2H, *J* = 8.7 Hz, Ph-H), 6.73 (s, 1H, Ph-H), 6.41 (s, 1H, Ph-H), 5.59 (s, 1H, CH), 4.19-4.10 (m, 1H, CH), 3.94-3.81 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.14-3.01 (m, 1H, CH), 2.78-2.67 (m, 1H, CH), 2.20 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 169.0, 159.6, 149.7, 137.9, 134.2, 132.5, 130.2, 121.1, 113.9, 112.3, 78.7, 63.7, 55.9, 55.2, 28.8, 20.5. GC/MS (m/z, %): 328 (M^+, 64), 286 (74), 258 (87), 225 (33), 178 (61), 135 (100).

1-(4'-Acetoxy-3'-methoxyphenyl)-6-methoxyisochroman-7-yl-acetate (1d). Quantitative yield; colorless oil. Found: C, 66.32; H, 5.52 %; M^+, 386. $C_{21}H_{22}O_7$ requires C, 65.28; H, 5.74 %; M, 386. ν_{max}/cm^{-1}: 3010, 2974, 2843, 1766 (acetate CO stretch), 1608, 1506, 1455, 1259, 1197, 1093. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.24-7.03 (m, 3H, Ph-H), 6.78 (s, 1H, Ph-H), 6.44 (s, 1H, Ph-H), 5.59 (s, 1H, CH), 4.24-4.09 (m, 1H, CH), 3.94-3.82 (m, 1H, CH), 3.81 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.12-2.99 (m, 1H, CH), 2.79-2.67 (m, 1H, CH), 2.24 (s, 3H, CH_3), 2.14 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 169.1, 168.9, 151.8, 149.9, 140.5, 139.7, 132.4, 129.1, 122.5, 121.3, 121.1, 112.7, 112.3, 78.8, 63.8, 55.9, 28.6, 20.6, 20.5. GC/MS (m/z, %): 386 (M^+, 22), 344 (30), 302 (100).

1-Phenylisochroman-6,7-diyl diacetate (1e). Quantitative yield; colorless oil. Found C, 70.12; H, 5.22 %; M^+, 326. $C_{19}H_{18}O_5$ requires C, 69.93; H, 5.56 %; M, 326. ν_{max}/cm^{-1}: 3031, 2933, 2855, 1770 (acetate CO stretch), 1503, 1371, 1211, 1171, 1083. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.31-7.28 (m, 5H, Ph-H), 7.00 (s, 1H, Ph-H), 6.54 (s, 1H, Ph-H), 5.65 (s, 1H, CH), 4.21-4.08 (m, 1H, CH), 3.95-3.84 (m, 1H, CH), 3.14-3.01 (m, 1H, CH), 2.77-2.69 (m, 1H, CH), 2.24 (s, 3H, CH_3), 2.15 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 168.4, 168.3, 141,3, 140.7, 140.0, 136.0, 132.6, 128.9, 128.6, 128.4, 123.3, 121.6, 79.4, 63.9, 28.3, 20.6, 20.5. GC/MS (m/z, %): 326 (M^+, 22), 284 (15), 242 (100), 214 (30), 165 (90), 152 (20).

Synthesis of 2,2-dimethyl-5-phenyl-7,8-dihydro-5H-[1,3]dioxolo[4,5-g]isochromene (1f).[20] Isochroman **7** was dissolved in $CHCl_3$ (6.5 mL), then 2,2-dimethoxypropane (544 µl, 4.5 mmol) and camphorsulphonic acid (19.36 mg, 0.09 mmol) were added. The mixture was kept under stirring at reflux temperature for 6 h. At the end, the reaction was neutralized by shaking with $NaHCO_3$ (saturated solution) and the separated aqueous phases were extracted with diethyl ether (3 x 10 mL). The collected organic phases were dried over anhydrous Na_2SO_4 and evaporated in vacuo obtaining a crude mixture. After the purification on chromatographic column, isochroman **1f** was isolated. Yield: 83%; colorless oil. Found: C, 77.62; H, 6.25 %, M^+, 282. $C_{18}H_{18}O_3$ requires C, 76.57; H, 6.43; %; M, 282. ν_{max}/cm^{-1}: 3083, 3026, 2958, 2859, 1490, 1386, 1240, 1095, 981, 856. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.35-7.28 (m, 5H, Ph-H), 6.52 (s, 1H, s, Ph-H), 6.11 (s, 1H, Ph-H), 5.60 (s, 1H, CH), 4.17- 4.09 (m, 1H, CH), 3.94-3.80 (m, 1H, CH), 3.12-3.00 (m, 1H, CH), 2.70-2.42 (m, 1H, CH), 1.62 (s, 6H, 2xCH_3). ^{13}C-NMR (200 MHz, $CDCl_3$) δ: 146.3, 145.8, 142.3, 129.7, 128.8, 128.4, 128.0, 126.3, 117.6, 108.1, 106.6, 79.7, 63.9, 28.8, 25.8. GC/MS (m/z, %): 282 (M^+, 100), 267 (52), 252 (42).

Oxidation of 2,4,6-trichlorophenol (8) and 4-methoxybenzyl alcohol (9) with the *T. villosa* laccase and laccase/HBT system. The oxidation reactions were performed in air at room temperature in magnetically stirred sodium acetate buffer (0.1 M, pH 4.7) or sodium acetate buffer (0.1 M, pH 4.7)/DMC 10/1 (v/v) or sodium acetate buffer (0.1 M, pH 4.7)/DMC 5/1 (v/v). Compounds **8** (90 µmol) and **9** (60 µmol) were dissolved in 3.0 mL of the appropriate reaction medium followed by addition of laccase (10 U); HBT (22 µmol) was added only in the presence of **9**. After 24 h, the internal standard was introduced in the reaction mixtures, respectively 1-bromonaphthalene (29 µmol) for compound **8** and 4'-methoxy-acetophenone (53 µmol) for compound **9**. The crudes were extracted with ethyl acetate; finally, the organic phases were washed with a saturated solution of NaCl and dried over Na_2SO_4. The yield of the oxidation reactions were determined by GC analysis with the internal standard method; suitable response factors were determined from authentic products.

Oxidation of 1-aryl-isochromans with the *T. villosa* laccase/HBT system. The reaction was performed in air at room temperature under stirring for 24 h. The suitable substrate (0.1 mmol) was dissolved in 2.0 mL of reaction medium [sodium acetate buffer (0.05 M, pH 4.7)/1,4-dioxane 1:1 (v/v) or sodium acetate buffer/DMC 5:1

View Article Online
DOI: 10.1039/C5NJ03133H

(v/v)] followed by the addition of HBT (0.033 mmol) and laccase (15 U). Additional aliquots of laccase were added every 8 h only when buffered water/1,4-dioxane was used as solvent. The reactions were monitored by TLC analysis. At the end, the internal standard (1-methyl-6-methoxy-3,4-dihydro-1H-isochromen-7-ol, 0.1 mmol)[4a] was added; the mixture was extracted with ethyl acetate and the organic phases were washed with a saturated solution of NaCl and dried over anhydrous Na_2SO_4. The oxidation yields were determined by HPLC analysis with the internal standard method; suitable response factors were determined from authentic products. Authentic products were synthesized by the same procedure. After the work-up and extraction, the organic solution was concentrated in vacuum. The residue was purified on silica gel column chromatography using petroleum ether/ethyl acetate = 3/1 as eluent. All products were characterized by spectroscopic analysis.

5-Benzoyl-4-(2-hydroxyethyl)-2-methoxyphenyl acetate (11a). Colorless oil. Found: C, 69.98; H, 5.45 %; (M^+-H_2O), 296. $C_{18}H_{18}O_5$ requires C, 68.78; H, 5.77 %; M, 314. ν_{max}/cm^{-1}: 3420 (br, OH), 3062, 2922, 2849, 1763 (acetate CO stretch), 1659 (ketone CO stretch), 1607, 1511, 1448, 1324, 1277, 1205, 1093. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 7.80-7.40 (m, 5H, Ph-H), 7.02 (s, 1H, Ph-H), 6.97 (s, 1H, Ph-H), 3.96-3.87 (m, 5H, OCH_3 + OCH_2), 2.99 (t, 2H, *J* = 6.1 Hz, CH_2), 2.24 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ: 197.1, 168.8, 153.5, 140.3, 137.9, 133.1, 130.5, 130.3, 128.4, 128.2, 125.4, 114.8, 64.0, 56.0, 36.4, 20.6. GC/MS (m/z, %): 296 (M^+-H_2O, 30), 254 (100).

4-(5-Acetoxy-2-(2-hydroxyethyl)-4-methoxybenzoyl)phenyl acetate (11b). Colorless oil. Found: C, 65.02; H, 5.29 %; (M^+-H_2O), 354. $C_{20}H_{20}O_7$ requires C, 64.51; H, 5.41 %; M, 372. ν_{max}/cm^{-1}: 3390 (br, OH), 3075, 3033, 2971, 2847, 1759 (acetate CO stretch), 1659 (ketone CO stretch), 1601, 1510, 1371, 1203, 1085. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 7.82 (d, 2H, *J* = 8.8 Hz, Ph-H), 7.17 (d, 2H, *J* = 8.8 Hz Ph-H), 7.08 (s, 1H, Ph-H), 6.96 (s, 1H, Ph-H), 3.95-3.89 (m, 5H, OCH_3 + OCH_2), 2.98 (t, 2H, *J* = 6.0 Hz, CH_2), 2.31 (s, 3H, CH_3), 2.26 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ: 197.1, 168.1, 154.4, 153.5, 140.3, 140.2, 138.2, 137.0, 135.4, 125.2, 121.7, 114.8, 64.0, 56.0, 36.4, 21.1, 20.5. GC/MS (m/z, %): 354 (M^+-H_2O, 32), 312 (38), 270 (100).

4-(2-Hydroxyethyl)-2-methoxy-5-(4-methoxybenzoyl)phenyl acetate (11c). Colorless oil. Found: C, 67.42; H, 5.48 %; (M^+-H_2O), 326. $C_{19}H_{20}O_6$ requires C, 66.27; H, 5.85 %; M, 344. ν_{max}/cm^{-1}: 3412 (br, OH), 3076, 3009, 2937, 2852, 1765 (Acetate CO stretch), 1651 (ketone CO stretch), 1599, 1510, 1321, 1256, 1218, 1164, 1094, 1028. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 7.77 (d, 1H, *J* = 9.0 Hz, Ph-H), 7.04 (s, 1H, Ph-H), 6.95 (s, 1H, CH), 6.91 (d, 2H, *J* = 8.9 Hz, Ph-H), 3.92-3.85 (m, 8H, 2xOCH_3 + OCH_2), 2.93 (t, 2H, *J* = 6.1 Hz, CH_2), 2.25 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 200 MHz) δ: 195.7, 168.8, 163.8, 153.0, 139.5, 136.9, 133.0, 130.9, 130.5, 124.7, 114.7, 113.7, 64.0, 56.0, 55.5, 36.4, 20.5; GC/MS (m/z, %): 326 (M^+-H_2O, 42), 284 (40), 270 (100).

4-(5-Acetoxy-2-(2-hydroxyethyl)-4-methoxybenzoyl)-2-methoxyphenyl acetate (11d). Colorless oil. Found: C, 63.78; H, 5.25 %; (M^+-H_2O), 384. $C_{21}H_{22}O_8$ requires C, 62.68; H, 5.51 %; M, 402. ν_{max}/cm^{-1}: 3551 (br, OH), 3053, 3014, 2924, 2851, 1765 (acetate CO stretch), 1652 (ketone CO stretch), 1601, 1513, 1412, 1328, 1260, 1207, 1032. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 7.48-7.06 (m, 5H, Ph-H); 6.96 (s, 1H, Ph-H), 3.96-3.80 (m, 8H, 2XOCH_3+OCH_2), 2.98 (t, 2H, *J* = 6.1 Hz, CH_2), 2.32 (s, 3H, CH_3), 2.27 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ: 195.7, 168.9, 168.5, 153.5, 151.3, 141.8, 140.2, 136.8, 136.5, 130.0, 125.5, 124.3, 122.7, 114.8, 113.8, 64.0, 56.1, 56.0, 20.6, 20.5. GC/MS (m/z, %): 384 (M^+-H_2O, 40), 318 (100).

4-Benzoyl-5-(2-hydroxyethyl)-1,2-phenylene diacetate (11e). Colorless oil. Found: C, 67.72; H, 5.42 %; (M^+-H_2O), 324. $C_{19}H_{18}O_6$ requires C, 66.66; H, 5.30 %; M, 342. ν_{max}/cm^{-1}: 3360 (br, OH), 3040, 2938, 2860, 1760 (acetate CO stretch), 1656 (ketone CO stretch), 1505, 1366, 1209, 1165, 1080. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 7.84-7.18 (m, 5H, Ph-H); 7.07 (s, 1H, Ph-H), 6.96 (s, 1H, Ph-H), 3.96-3.80 (m, 2H, OCH_2), 2.92 (t, 2H, *J* = 6.0 Hz, CH_2), 2.32 (s, 3H, CH_3), 2.27 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ: 195.8, 167.9, 167.8, 143.8, 139.5, 138.4, 138.0, 132.4, 130.3, 128.6, 128.4, 122.7, 119.6, 63.5, 35.9, 20.6, 20.5. GC/MS (m/z, %): 324 (M^+-H_2O, 35), 282 (20), 240 (100).

6-(2-Hydroxyethyl)-2,2-dimethylbenzo[*d*][1,3]dioxol-5-yl)(phenyl)methanone (11f). Colorless oil. Found C, 73.51; H, 6.21 %; (M^+-H_2O), 280. $C_{18}H_1O_4$ requires C, 72.47; H, 6.08 %; M, 298. ν_{max}/cm^{-1}: 3320 (br, OH), 3061, 2979, 2931, 2865, 1658 (ketone CO stretch), 1509, 1496, 1377, 1272, 1244, 1219, 1094. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 7.81-7.40 (m, 5H, Ph-H), 6.75 (s, 1H, s, Ph-H), 6.69 (s, 1H, Ph-H), 4.12 (t, 2H, *J* = 6.0 Hz, OCH_2), 2.87 (t, 2H, *J* = 6.0 Hz, CH_2), 1.74 (s, 6H, 2xCH_3). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ: 197.9, 150.0, 148.6, 138.0, 135.1, 133.1, 131.1, 130.6, 128.1, 119.2, 110.7, 109.9, 64.1, 36.2, 25.9, 25.8. GC/MS (m/z, %): 280 (M^+-H_2O, 32), 265 (50), 250 (100).

Determination of the *T. villosa* laccase activity and kinetic parameters. *T. villosa* laccase activity was determined spectrophotometrically using syringaldazine (SGZ) as benchmark substrate.[21] The enzyme (4 µL of a solution composed of 7.1 U of *T. villosa* laccase in 1 mL of sodium acetate buffer 0.05 M, pH 4.7) was added into a cuvette containing SGZ at different concentrations (9.8 10^{-7} M ÷ 2.5 10^{-5} M) in 2.8 mL of sodium acetate buffer or sodium acetate buffer/DMC = 10/1 (v/v). The final molar concentration of laccase was 2.9 10^{-10} M. After mixing, the increase in absorbance at λ = 532 nm was monitored each 5 s for 30 minutes at 25°C. All experiments were carried out in triplicate. Initial rates of reaction were calculated from the linear range of absorbance *vs* time plots with ε = 72331 M^{-1} cm^{-1} (at λ = 532 nm)[27] for the oxidation product. Plotting initial rates *versus* SGZ concentrations gave a Michaelis-Menten curve. Maximum rate V_{max}, Michaelis K_M and catalytic k_{cat} constants were determined by fitting experimental points with the following equation: $V = V_{max}\,[S]/(K_M + [S])$

Acknowledgements

Thanks are due to the Ministero dell'Istruzione, dell'Università della Ricerca (MIUR) for financial support, PRIN 2010-2011 (2010PFLRJR) project (PROxi project).
Thanks to the ''Complex Equipment Center'' (University of Tuscia, Viterbo, Italy) for the availability of NMR Bruker spectrometer.

References

1 (a) P. Giardina, V. Faraco, C. Pezzella, A. Piscitelli, S. Vanhulle and G. Sannia, *Cell. Mol. Life Sci.,* 2010, **67**, 369; (b) S.

View Article Online
DOI: 10.1039/C5NJ03133H

Rodriguez-Couto, *Pan Stanford Series on Biocatalysis* (2015), **1** (Industrial Biocatalysis), 697.
2 E. I. Solomon, P. Chem, M. Metz, S.-K. Lee and A. E. Palmer, *Angew. Chem. Int. Ed*., 2001, **40**, 4570
3 (a) P. Astolfi, P. Brandi, C. Galli, P. Gentili, M. F. Gerini, L. Greci and O. Lanzalunga, *New J. Chem.,* 2005, **29,** 1 and references cited therein; (b) O. V. Morozova; G. P. Shumakovich; S. V. Shleev and Y. I. Yaropolov, *Appl. Biochem. Micro*., 2007, **43**, 523; (c) C. Galli and P. Gentili, *J. Phys. Org. Chem.*, 2004, **17**, 973 and references cited therein; (d) F. d'Acunzo, P. Baiocco, M. Fabbrini, C. Galli and P. Gentili, *New J. Chem.*, 2002, **26**, 1791
4 (a) R. Bernini, F. Crisante, P. Gentili, F. Morana, M. Pierini and M. Piras, *J. Org. Chem.,* 2011, **76**, 820; (b) *Ibidem,* 2011, **76**, 4220; (c) R. Bernini, F. Crisante, P. Gentili, S. Menta, F. Morana and M. Pierini, *RSC Adv.,* 2014, **4**, 8183; (d) F. D'Acunzo, A. M. Barreca and C. Galli, *J. Mol. Catal. B: Enzym.* 2004*,* **31***, 25*
5 (a) F. I. Hsu and J. Y. Chen, *Phytochemistry*, 1993, **34**, 1625; (b) J. Ralph, J. Peng, and F. Lua, *Tetrahedron Lett.,* 1998, **39**, 4963; (c) J. P. Peng, F. Lu, and J. Ralph, *Phytochemistry,* 1999, **50**, 659
6 J. M. McCall, R. B. McCall, R. E. Tenbrink, B. V. Kamdsar, S. J. Humphrey, V. H. Sethy, D. W. Harri and C. Daenzer, *J. Med. Chem.,* 1982, **25**, 75
7 M. Tobe, T. Tashiro, M. Sasaki and H. Takikawa, *Tetrahedron,* 2007, **63**, 9333
8 K. Trisuwan, V. Rukachaisirikul, Y. Sukpondma, S. Phongpaichit, S. Preedanon and J. Sakayaroj, *Tetrahedron,* 2010, **66**, 4484
9 (a) G. I. Togna, A. R. Togna, M. Franconi, C. Marra and M. Guiso, *J. Nutr.,* 2003, **133**, 2532; (b) A. Bendini, L. Cerretani, A. Carrasco-Pancorbo, A. M. Gómez-Caravaca, A. Segura-Carretero, A. Fernández Gutiérrez and G. Lercker, *Molecules,* 2007, **12**, 1679; (c) M. Guiso, C. Marra and R. R. Arcos, *Nat. Prod. Res.,* 2008, 1403
10 (a) P. P. Pradhan, J. M. Bobbit and W. F. Bailey, *J. Org. Chem.,* 2009, **74**, 9524; (b) J. Wegner, S. Ceyla, C. Friese and A. Kirschnuing, *Eur. J. Org. Chem.,* 2010, 4372; (c) D. Garcia, F. Foubelo and M. Yus, *Eur. J. Org. Chem.,* 2010, 2893
11 (a) R. Gitto, M. Zappalà, G. De Sarro and A. Chimirri, *Il Farmaco,* 2002, **57**, 129; (b) F. Gatta, D. Piazza, M. R. Del Giudice and M. Massotti, *Farmaco, Edizione Scientifica*, 1985, **40**, 942; (c) B. Elger, A. Huth, R. Neuhaus, E. Ottow, H. Schneider, B. Seilheimer and L. Turski, *J. Med. Chem.,* 2005, **48***,* 4618
12 A. Chimirri, G. De Sarro, A. De Sarro, R. Gitto, S. Grasso, S. Quartatone, M. Zappalà, P. Giusti, V. Libri, A. Constantini and A. G. Chapman, *J. Med. Chem.,* 1997, **40**, 1258
13 (a) P. Bovicelli, A. Sanetti, R. Bernini and P. Lupattelli, *Tetrahedron* 1997, **53**, 9755; (b) P. Bovicelli, P. Lupattelli, B. Crescenzi, A. Sanetti and R. Bernini, *Tetrahedron,* 1999*,* **55***,* 14719
14 (a) M. Guiso, C. Marra and F. Piccioni, *Nat. Prod. Res.,* 2010, **24**, 331; (b) S. Solyom, I. Pallagi, G. Abraham, M. Kertesz, G. Horvath and P Berzsenyi, *Med. Chem.,* 2005, **1**, 481
15 (a) G. Ábrahám, S. Sólyom, E. Csuzdi, P. Berzsenyi, I. Ling, I. Tarnawa, T. Hámori, I. Pallagi, K. Horváth, F. Andrási, G. Kapus, L. G. Hársing Jr., I. Király, M. Patthy and G. Horváth, *Bioorgan. Med. Chem.,* 2000*,* **8***,* 2127; (b) M. Miura, M. Nojima and S. Kusabayashi, *J. Am. Chem. Soc.,* 1981, **103**, 1789
16 (a) P. Tundo, *Pure Appl. Chem.,* 2001, **73**, 1117; (b) P. Tundo and M. Selva, *Acc. Chem. Res.*, 2002**, 35**, 706
17 (a) R. Bernini, E. Mincione, M. Barontini, F. Crisante, A. and A. Gambacorta, *Tetrahedron,* 2007, **63***,* 6895; (b) R. Bernini, E. Mincione, F. Crisante, M. Barontini, G. Fabrizi and P. Gentili, *Tetrahedron Lett.,* 2007, **48**, 7000; (c) R. Bernini, E. Mincione, M. Barontini and F. Crisante, *J. Agr. Food Chem.,* 2008, **56**, 8897; (d) R. Bernini, E. Mincione, F. Crisante, M. Barontini, and G. Fabrizi, *Tetrahedron Lett.,* 2009, **50***,* 1307; (e) R. Bernini, F. Crisante and M. C. Ginnasi , *Molecules,* 2011, **16**, 1418; (f) R. Bernini, F. Crisante, M. Barontini, D. Tofani, V. Balducci and A. Gambacorta, *J. Agric. Food Chem.,* 2012, **60**, 7408
18 R. Bernini, F. Crisante, G. Fabrizi and P. Gentili, *Curr. Org. Chem.,* 2012*,* **16**, 1051
19 (a) J. E. Chung, M. Kurisawa, Y. Tachibana, H. Uyama, and S. Kobayashi, *Chem. Lett.* 2003, **32**, 620; (b) M. Kurisawa, J. C. Chung, and S. Kobayashi, *Macromol. Biosci.,* 2003, **3**, 758; (c) A. M. Osman, K. K. Y. Wong, A. S. Fernyhough, and N. Z. Rotorua, *Enzyme Microb. Technol.,* 2007, **40***,* 1272; (d) N. Itoh, Y. Katsube, K. Yamamoto, N. Nakajima, and K. Yoshida, *Tetrahedron,* 2007, **63**, 9488; (e) H.-L. Ma, S. Kermasha, J.-M. Gao, R. M. Borges and X.-Z. Yu, *J. Mol. Catal. B: Enzym.* 2009, **57**, 89
20 (a) A. Gambacorta, D. Tofani, R. Bernini and A. Migliorini, *J. Agric. Food Chem.,* 2007, **55***,* 3386; (b) R. Bernini, S. Cacchi, G. Fabrizi and E. Filisti, *Org. Lett.,* 2008, **10**, 3457
21 F. Xu, W. Shin, S. H. Brown, J, A. Wahleithner, U. M. Sundaram and E. I. Solomon, *Biochim. Biophys. Acta,* 1996, **1292**, 303
22 F. d'Acunzo, C. Galli, P. Gentili and F. Sergi, *New J. Chem.,* 2006, **30**, 583
23 (a) D. R. de Magalhães Moreira, A. C. Lima Leite, P. M. Pinheiro Ferreira, P. Marçal da Costa, L. V. Costa Lotufo, M. O. de Moraes, D. J. Brondani and C. do Ó Pessoa, *Eur. J. Med. Chem.,* 2007, **42***,* 351; (b) M. Gordaliza, M. A. Castro, J. M. M. del Corral and A. San Feliciano, *Curr. Pharm. Des*., 2000, **6***,* 1811; (c) M. Gordaliza, P.A. García, J. M. M. del Corral, M. A. Castro and M. A. Gómez-Zurita, *Toxicon,* 2004, **44**, 441
24 (a) G. A. Potter and P. C. Butler, Patent US7598294 B2 , 2009; (b) S. Yokota, M. Kitahara and K. Nagata, *Cancer Res.,* 2000, **60***,* 2942
25 Y. Xia, Z. Yang, P. Xia, K.F. Bastow, Y. Nakanishi and K. Lee, *Bioorg. Med. Chem. Lett.,* 2000, **10***,* 699
26 F. d'Acunzo, P. Baiocco and C. Galli, *New J. Chem.*, 2003, **27**, 329

View Article Online
DOI: 10.1039/C5NJ03133H

Oxidative cleavage of 1-aryl-isochroman derivatives by the *Trametes villosa* laccase/1-hydroxybenzotriazole system

Roberta Bernini,* Fernanda Crisante, Francesca D'Acunzo, Patrizia Gentili,* Emanuele Ussia

Dimethyl carbonate was firstly used as co-solvent in a green oxidative cleavage 1-aryl-isochroman derivatives yielding useful synthetic intermediates of drugs.