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Cocatalytic Enzyme System for the Michael Addition Reaction of in-situ-Generated *ortho*-Quinones

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Keywords: Enzyme catalysis / Laccase / Lipase / Michael addition / Cascade reaction / Quinones

A laccase-lipase cocatalytic system was used to catalyze the domino reaction between catechols and nucleophilic reagents including 1,3-dicarbonyl compounds and aromatic amines in aqueous medium at room temperature. Lipase acted as a catalyst for Michael addition step, and also enhanced the overall yield of the final products.

Introduction

In recent years, the advances in genomics and biotechnology have dramatically broadened the availability of low-cost enzymes. In turn, this has increased the potential application of enzymes for organic synthesis while also addressing the challenges of "green chemistry".[1] A growing field of interest in this field is the application of enzyme-initiated domino reactions.[2] Under optimized reaction conditions, it has been shown that several biocatalytic reactions can be carried out in a single reactor.[3] For example, Kroutil and his co-workers recently developed a one-pot, two-step, two-enzyme cascade reaction for the synthesis of an enantiopure epoxide.[4] Herein, we report on the use of two enzymes, laccase and lipase, in the domino reaction of in-situ-generated *o*-quinones followed by enzyme-catalyzed Michael addition.

Laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) is a multinuclear-copper-containing oxidase that has become an attractive biocatalyst that catalyzes the oxidation of various aromatic compounds, including phenols, *o*- and *p*-diphenols, and lignin derivatives.[5] This oxidoreductase enzyme frequently exhibits high oxidative selectivity in aqueous reactions and provides a unique green chemistry solution for organic synthesis.[6] In this study, laccase was used to catalyze the oxidation of catechols to the corresponding *o*-quinones which were reacted in-situ with nucleophilic reagents, such as 1,3-dicarbonyl compounds and aromatic amines, via a Michael addition reaction. In the presence of lipase, the Michael addition step was catalyzed by lipase. Although lipases (triacylglycerol hydrolase, EC 3.1.1.3) have been known to catalyze the hydrolysis and the synthesis of esters formed from alcohols and acids,[7] recent studies have reported the ability of lipases to catalyze Michael addition reactions.[8] For example, Torre et al. provided the initial demonstration that lipase was able to catalyze the Michael addition of secondary amines to acrylonitrile.[8a] This reaction is clearly different from the natural process this enzyme is usually associated with. Berglund et al. has reported the Michael addition of 1,3-dicarbonyl compounds to α,β-unsaturated carbonyl compounds catalyzed by a *C. antarctica* lipase B mutant.[8b] Moreover, Wang et al. recently established that lipase M from *Mucor javanicus* is able to catalyze the Michael addition reaction of pyrimidine with a disaccharide acrylate.[8c]

In a recent report, we demonstrated that laccase generated *o*-quinones could efficiently undergo Michael reactions with 1,3-dicarbonyl compounds in the presence of $Sc(OTf)_3$ as a catalyst for the synthesis of benzofurans. On average this reaction provided the adduct in 50–79% yield but also produced a hazardous waste from the transitional metal catalyst employed.[6l] From an environmental concern, the development of alternative methodologies to replace the lanthanide metal catalyst in this synthesis is a high priority to enhance the overall green chemistry aspect of this one-pot synthetic reaction. Therefore, this report presents the use of the enzyme, lipase, as an alternative catalyst for Michael addition reaction in conjugation with laccase for the reaction of catechols with 1,3-dicarbonyl compounds as well as with aromatic amines.

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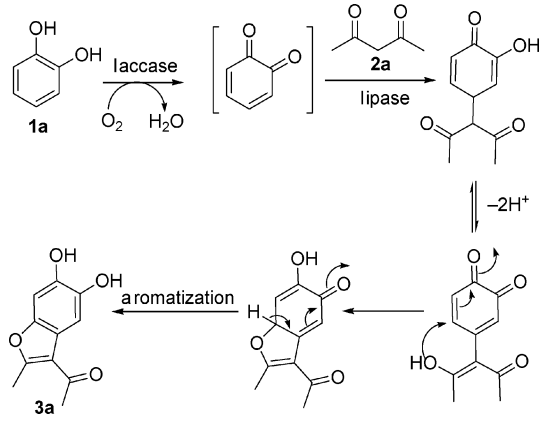
Results and Discussion

1. Laccase-Lipase Cocatalytic System for the Reaction of Catechols and 1,3-Dicarbonyl Compounds

In this study, laccase first catalyzed the oxidation of catechols to the corresponding *o*-quinones which were reacted

in-situ with 1,3-dicarbonyl compounds via a Michael addition reaction. The Michael addition step was catalyzed by lipase and the resulting addition product underwent a subsequent intramolecular cyclization to form benzofuran derivative products (see Scheme 1).

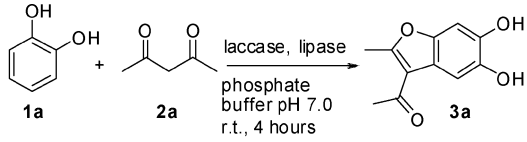


Scheme 1. Proposed reaction pathway of laccase/lipase catalytic system for the synthesis of **3a**.

In our initial studies, the reaction of catechol (**1a**) and acetylacetone (**2a**) in the presence of laccase and lipase from *Candida rugosa* (lipase CR, 60,000 U/mg) was investigated. The reaction was carried out under atmospheric conditions at room temperature (23 °C) in an aqueous buffered solution for 4 h. We found that the optimal yield of the product (**3a**) of 60% was achieved when conducting the reaction of **1a** and **2a** in 1:2 molar ratio at pH 7.0, and using 100 U of laccase and 10 mg (600,000 U) of lipase CR per 1 mmol of **1a**. Because of the high activity of in situ-generated quinone, some side products (e.g. from the polymerization of the quinone) were also observed but in this study we did not separate and identify these byproducts. For the control reaction when no laccase and lipase was added, no product **3a** was formed. When this reaction was preformed using only lipase no product was formed, and in the presence of laccase only, the product **3a** was formed in only 33% yield.

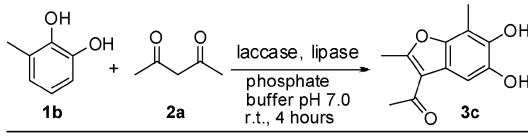
After this preliminary study, the next phase was to examine a variety of lipases for this reaction system. These lipases included lipase CR (60,000 U/mg), lipase from *Pseudomonas cepacia* (lipase PS, 46.2 U/mg), and lipase B *Candida antarctica* (CALB, 10.8 U/mg). The activity of these lipases was measured by Aldrich methods which are different for each lipase. The catalytic properties of these lipases were investigated by reacting **2a** with catechol (**1a**) or 3-methylcatechol (**1b**), in the presence of laccase, as summarized in Table 1. This study established that the optimal amount of each lipase to provide the highest yield of the product was different. The optimal amount of lipase CR, lipase PS and CALB for the reaction conditions used was 600,000 U, 924 U, and 54 U per 1 mmol of catechol, respectively. The data in Table 1 shows that the yield of the product usually increased when lipase was added to the reaction. Lipase PS and lipase CR gave a high yield of the products for both reactions while CALB was good only for reaction 2. In addition, lipase PS activity used in the reaction was much less than of lipase CR. Therefore, lipase PS was chosen for further study. In order to verify whether the lipase reaction is indeed catalyzed by the active site of lipase PS and not by the protein, the reactions using inactivated lipase PS were conducted. The results in Table 1 show that the inactivated lipase PS showed no catalytic activity for these reactions.

Table 1. Reaction with a variety of lipases.



(1)

Lipase	Yield (%)[a]
No lipase	33
Inactivated lipase from *Pseudomonas cepacia*	31
Lipase from *Candida rugosa* (Lipase CR)	60
Lipase from *Pseudomonas cepacia* (Lipase PS)	58
Lipase B *Candida antarctica* (CALB)	41



(2)

No lipase	53
Inactivated lipase from *Pseudomonas cepacia*	50
Lipase from *Candida rugosa* (Lipase CR)	56
Lipase from *Pseudomonas cepacia* (Lipase PS)	60
Lipase B *Candida antarctica* (CALB)	62

[a] Isolated yield.

To further define the catalytic benefits of lipase PS, the reaction of **1a** and **2a** in the presence of laccase with and without lipase PS were carried out. Sample aliquots were taken every 30 min during the reaction and a quantitative analysis of product **3a** was measured by ^{1}H NMR spectroscopy using 1,3,5-trioxane as an internal standard. The calculated yield of the product **3a** is higher than the isolated yield showed in Table 1 in both cases (with and without lipase PS). This can be explained by the lose of product yield during the isolation process. However, in the end of reaction, the yield difference between the reaction with and without lipase is about the same which is approximately 25%. Figure 1 shows that in the beginning of the reaction, the rate and yield of **3a** from both reactions were almost the same. This can be explained by the predominance of laccase-catalyzed oxidation of catechol at the beginning of the reaction and the gradually oxidation of catechol by laccase which lead to a low concentration of *o*-quinone. However, after 2 h of the reaction when the concentration of laccase-generated quinone was high the reaction with lipase PS was predominant with less by-products and provided a higher rate of the reaction and higher yield of the product than the reaction without lipase PS. Therefore, lipase PS can enhance the overall yield for this reaction system.

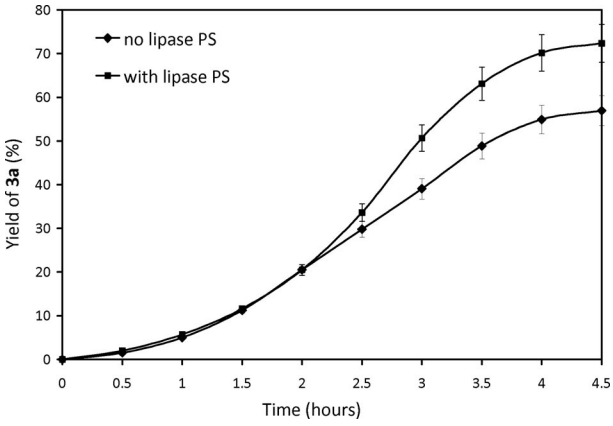
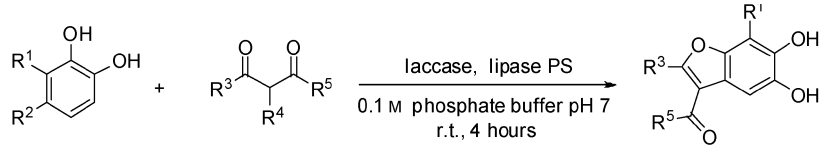


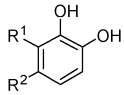
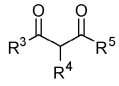
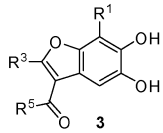
Figure 1. The formation of compound **3a** from the laccase-catalyzed reaction of **1a** and **2a** in the presence and absence of lipase PS. The percent yield of **3a** was measured by ^{1}H NMR spectroscopy.

Following these optimization studies, we evaluated the breadth of this laccase-lipase cocatalytic system for the synthesis of benzofuran derivatives using a variety of catechols and 1,3-dicarbonyl compounds. The results summarized in Table 2 clearly suggest that the inactivated lipase has no catalytic effect on the reactions. In addition, the reactivity of the 1,3-dicarbonyl compound employed also has an effect on efficiency of this two-enzyme system. When we used 1,3-dicarbonyl compounds that had an electron-withdrawing group (Cl) at the *alpha*-position, the reaction was complete in 1.5–2 h. The shorter reaction time was ascribed to the increased acidity of the *alpha*-proton of these substituted 1,3-dicarbonyl compounds. The Cl atom was eliminated during the reaction, and the proposed mechanism is illustrated in Scheme 2. Besides 3-substituted catechols, 4-substituted catechols, such as 4-chlorocatechol, can also be used for the synthesis of polyhydroxylated benzofurans (entry 11). However, the yield of the product was low. In addition, we observed that this reaction system gave only one isomer form of the possible benzofuran products.

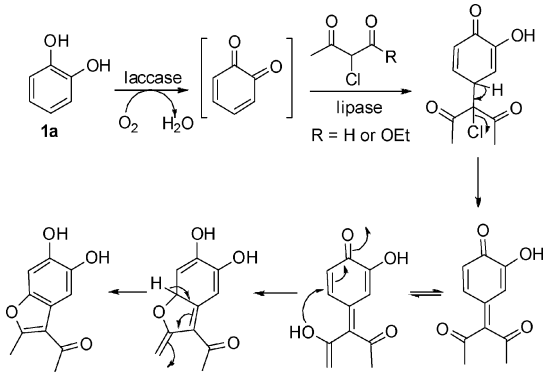
Next, we examined the recyclability of this two-enzyme catalytic system for the synthesis of benzofuran **3c**. The product **3c** is relatively insoluble in the aqueous reaction mixture and readily precipitates out of solution. Simple filtration of the product mixture facilitates reuse of the lipase/laccase reaction system. The results shown in Table 3 demonstrate that this catalytic system can be reused for a second reaction, but for the third treatment only a low yield of the product was formed.

Table 2. The reaction of catechols and 1,3-dicarbonyl compounds.



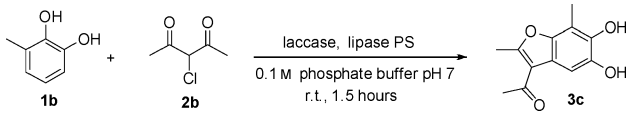
Entry	Catechol  1	1,3-Dicarbonyl compound  2	Product (% yield)[a]  3
1	**1a**: $R^1 = R^2 = H$	**2a**: $R^3 = R^5 = Me$, $R^4 = H$	**3a** (58%)
			3a (31%, used inactivated lipase PS)
2	**1a**	**2b**: $R^3 = R^5 = Me$, $R^4 = Cl$	**3a** (51%)[b]
			3a (40%, used inactivated lipase PS)[b]
3	**1a**	**2c**: $R^3 = Me$, $R^4 = H$, $R^5 = OEt$	**3b** (11%)
4	**1a**	**2d**: $R^3 = Me$, $R^4 = Cl$, $R^5 = OEt$	**3b** (53%)[b]
			3b (26%, used inactivated lipase PS)[b]
5	**1b**: $R^1 = Me$, $R^2 = H$	**2a**	**3c** (60%)
			3c (50%, used inactivated lipase PS)
6	**1b**	**2b**	**3c** (72%)[b]
			3c (52%, used inactivated lipase PS)[b]
7	**1b**	**2c**	**3d** (13%)
8	**1b**	**2d**	**3d** (66%)[b]
			3d (39%, used inactivated lipase PS)[b]
9	**1c**: $R^1 = OMe$, $R^2 = H$	**2a**	no product formed
10	**1d**: $R^1 = F$, $R^2 = H$	**2a**	no product formed
11	**1e**: $R^1 = H$, $R^2 = Cl$	**2a**	**3a** (8%)

[a] Isolated yield. [b] Reaction time is 1.5–2 h.



Scheme 2. Proposed mechanism pathway of the elimination of Cl for the laccase/lipase-catalyzed reaction of catechol and chloro-1,3-dicarbonyl compounds.

Table 3. Recycling of the catalytic system for the synthesis of 3-acetyl-5,6-dihydroxy-2,7-dimethylbenzofuran (**3c**).

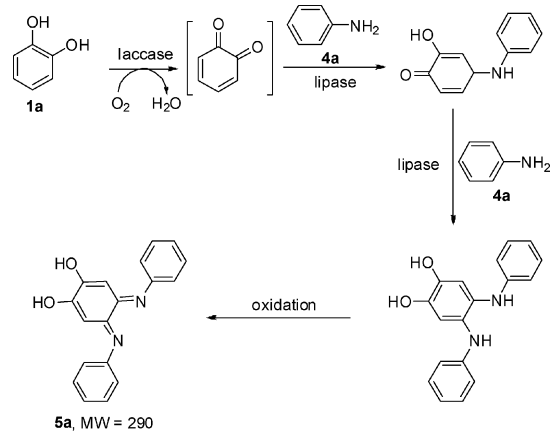


Run	Yield[a] (%) of **3c**
1	72
2	62
3	5

[a] Isolated Yield.

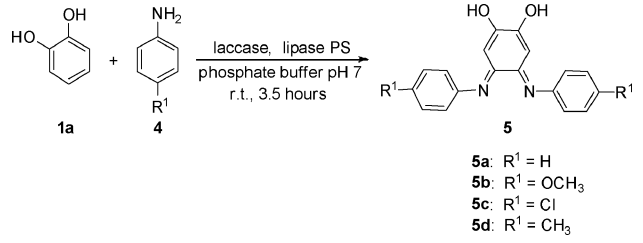
2. Laccase-Lipase Cocatalytic System for the Reaction of Catechols and Anilines

Next, we explored the feasibility of the laccase-lipase cocatalytic system for the reaction between catechol and aromatic amines, anilines. Lalk and his co-worker have demonstrated the ability to synthesize aminoquinones by laccase initiated oxidation of *p*-hydroxyquinones followed by Michael addition of primary aromatic amines in a good to excellent yields.[6a] In contrast, herein, this nuclear animation reaction with the reactive 1,2-catechols was reported to yield the corresponding products less than 35%. In the presence of lipase, we had hypothesized that thus enzyme could catalyze the Michael addition step of the reaction between the laccase-generated *o*-quinone and anilines thereby improving the overall yields. We first conducted the reaction of catechol (**1a**) (Scheme 3) and aniline (**4a**) in the presence of laccase, with or without lipase PS, in phosphate buffer pH 7.0 at room temperature for 3.5 h. The ratio of catechol and aniline was 1 to 2, and 100 U of laccase and 924 U of lipase per 1 mmol of catechol were used. An insoluble red color product precipitated out of solution during the reaction. Therefore, the product was readily collected by filtration completion of reaction. The results show that the yield of the reaction with lipase PS increased by ca. 30% when compare to the yield of the reaction without lipase PS. Next, the amount of lipase PS used in the reaction was increased from 924 U to 1848 U per 1 mmol of catechol to study the effect of lipase dose on the reaction system. This result suggests that the increase of lipase dose did not provide a significant improvement for this reaction system (Table 4). Characterization of the product by NMR and mass spectrum indicated that the product was composed of a 1:2 ratio of 1,2-benzoquinone and aniline (M^+/z = 290). Moreover, the product was not a quinone structure because the carbonyl carbon signal was not observed in ^{13}C-NMR spectrum. The proposed reaction pathway for this reaction and the structure of product (**5a**) was illustrated in Scheme 2. Product 5a is known compound. Our NMR and mass data are consistent with those in the literature.[11]



Scheme 3. Proposed reaction pathway of laccase/lipase catalytic system for the reaction between catechol (**1a**) and aniline (**4a**).

Table 4. Reactions of catechol and anilines.



Entry	R^1	Yield[a] (%) of product Without lipase	With lipase
1	H	23	30
2	H	23	28[b]
3	OCH_3	25	37
4	Cl	30	51
5	CH_3	32	50

[a] Isolated yield. [b] Used 1848U of lipase PS.

After the preliminary study, the reaction between catechol and other anilines was conducted. The results of these studies are summarized in Table 4. The reaction of catechol and anilines in the presence of laccase and lipase PS provided a higher yield than the reaction in presence of laccase only. The yield of the product in the reaction with lipase PS increased up to 70% compare to the reaction without lipase PS (Table 4, Entry 4). Therefore, the overall yield of the product of this reaction system can be enhanced by lipase PS.

Conclusion

This study demonstrates the potential of using lipase to catalyze the Michael addition reaction, and presents a new cocatalytic enzymatic system employing laccase and lipase for green chemistry synthesis. Lipase was found to catalyze the addition reaction between laccase-generated *o*-quinones and 1,3-dicarbonyl compounds in aqueous medium. In this reaction, the catalytic system of laccase and lipase PS was regioselective providing only one isomer product and is the first example of a two enzyme catalytic system for the synthesis of benzofurans. The yields of the products from reaction were shown to depend on the reactivity of the starting catechols and β-dicarbonyl compounds. Based on our experimental results, catechols with moderate reactivity yield benzofuran products in excellent yield. Moreover, lipase was also shown to catalyze the addition reaction between laccase-generated *o*-quinone and aromatic amines. In the presence of lipase and laccase, the yield of the final products increased in the range from 30 to 70% when compare to the reaction in the presence of laccase alone. Therefore, this paper illustrates a unique aqueous-based two-enzyme system for green chemistry synthesis and future applications are under study.

Experimental Section

General Information: All chemicals were used as received without further purification. Laccase (EC 1.10.3.2) from *Trametes Villosa* was donated by Novo Nordisk Biochem, North Carolina. Lipases were purchased from Aldrich. Unit definition of each lipase is different depending on the method that Aldrich used to measure lipase activity. The enzymes were kept frozen until used. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C in [D_6]DMSO or $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. All reactions were monitored by TLC. TLC was performed on aluminum sheets precoated with silica gel 60 F254 (EMD Chemicals). Column chromatography was performed on Combiflash Companion instrument (Teledyne Isco company) using RediSep normal-phase flash columns. Mass spectra were carried out in The Georgia Institute of Technology Bioanalytical Mass Spectrometry Facility. **3a**,[9] **3b**,[10] **3c**,[9] **3d**,[6] and **5a**[11] are known compounds and our ^{1}H and ^{13}C NMR spectroscopic data are consistent with those in the literature.

Enzyme Assay: Laccase used was from Trametes Villosa (EC 1.10.3.2). Laccase activity was determined by oxidation of 2,2′-azinobis(3-ethylbenzylthiozoline-6-sulfonate) (ABTS).[12] The assay mixture contained 25 μM ABTS, 0.10 M sodium acetate (pH 5.0), and a suitable amount of enzyme. The oxidation of ABTS was followed by an absorbance increase at 420 nm (ε_{420} = 3.6×104 M^{-1} cm^{-1}). Enzyme activity was expressed in units (U = μmol of ABTS oxidized per minute).

General Procedure for the Reaction Between Catechols and 1,3-Dicarbonyl Compounds: In a 250-mL round-bottom flask, 30 mL of 0.10 M phosphate buffer pH 7.0 and **1a** (0.1101 g, 1 mmol) were mixed together. Next, 100 U of laccase was added to reaction mixture and then, **2a** (0.2002 g, 205 μL, 2 mmol) and 924 U of lipase PS were added. The reaction was then stirred under air at room temperature for 4 h. After the reaction was completed, the reaction mixture was extracted by EtOAc. The organic phase was combined, dried with $MgSO_4$, and the solvents evaporated. The resulting crude products were purified by silica column chromatography, using EtOAc and petroleum ether as an eluent to obtain 3-acetyl-5,6-dihydroxy-2-methylbenzofuran (**3a**). Products were characterized by ^{1}H NMR, ^{13}C NMR and MS.

Procedure for the Study of the Reaction of 1a and 2a (with and without Lipase PS): In a 250-mL round-bottom flask, 40 mL of 0.10 M phosphate buffer pH 7.0 and **1a** (2 mmol, 0.2202 g) were mixed together. Next, 200 U of laccase was added to reaction mixture and then, **2a** (0.4004 g, 410 μL, 4 mmol) and 1848 U of lipase PS (or no lipase) were added. The reaction was then stirred at room temperature in a flask open to the atmosphere for 4.5 h. A 3 mL aliquot of the reaction mixture was taken every 30 min during the reaction and extracted with 10 mL of EtOAc. The organic phase was then dried with $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude product was submitted to quantitative ^{1}H NMR analysis to measure the formation of product **3a** using [D_6]DMSO as solvent and 1,3,5-trioxane as internal standard.

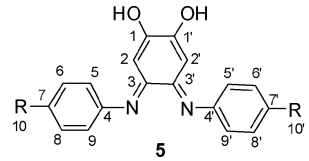
General Procedure for the Reaction Between Catechols and Anilines: In a 250-mL round-bottom flask, 30 mL of 0.10 M phosphate buffer pH 7.0 and **1a** (0.1101 g, 1 mmol) were mixed together. Next, 100 U of laccase was added to reaction mixture and then, **4a** (0.1862 g, 182 μL, 2 mmol) and 924 U of lipase PS were added. The reaction was then stirred under air at room temperature for 3.5 h. After the reaction was finished, the reaction mixture was filtered to collect the red color product **5a** as red solid (87 mg, 30%). Products were characterized by ^{1}H NMR, ^{13}C NMR and MS. ^{1}H and ^{13}C assignments and HMBC correlation for compound **5b**, **5c**, and **5d** are summarized in Table 5.

Compound 5b: Red solid, yield 129.5 mg (37%), m.p. 161–162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.50 (br. s, 1 H, OH), 7.56 (br. s, 1 H, OH), 7.08 (d, J = 7 Hz, 4 H, 4×CH arom.), 6.94 (d, J = 8 Hz, 4 H, 4×CH arom.), 6.07 (s, 2 H, 2×CH), 3.84 (s, 6 H, 2×OCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.4, 151.9, 135.3, 123.9, 114.2, 95.1, 55.1 ppm. IR (KBr): $\tilde{\nu}$ = 3293 (s), 3246 (s), 3040 (w), 2834 (w), 1739 (w), 1654 (w), 1606 (s), 1580 (s), 1525 (s), 1511 (s), 1411 (s), 1330 (m), 1286 (m), 1244 (s), 1217 (s), 1199 (s), 1173 (m), 1033 (m), 840 (m) cm^{-1}. MS (EI): m/z = 350 (86) [M^+], 319 (100), 291 (12), 174 (15), 146 (12), 92 (7), 77 (9). HRMS (EI): calcd. for $C_{20}H_{18}N_2O_4$ 350.1266; found 350.1247.

Compound 5c: Red solid, yield 182.6 mg (51%), m.p. 219–221 °C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.24 (br. s, 2 H, 2×OH), 7.44 (d, J = 7 Hz, 4 H, 4×CH arom.), 7.19 (br. s, 4 H, 4×CH arom.), 5.81 (s, 2 H, 2×CH) ppm. ^{13}C NMR (100 MHz, [D_6]-DMSO): δ = 152.0, 142.6, 129.1, 128.9, 123.7, 97.5z ppm. IR (KBr): $\tilde{\nu}$ = 3298 (s), 3031 (w), 1736 (w), 1660 (w), 1606 (m), 1573 (s), 1536 (s), 1493 (s), 1480 (s), 1415 (s), 1334 (s), 1221 (s), 1188 (s), 1087 (m), 1007 (m), 830 (m) cm^{-1}. MS (EI): m/z = 358 (M^+, 42%), 323 (80), 288 (8), 178 (18), 144 (15), 127 (100), 84 (57), 65 (18), 49 (75). HRMS (EI): calcd. for $C_{18}H_{12}N_2O_2Cl_2$ 358.0275; found 358.0266.

Compound 5d: Red solid, yield 159 mg (50%), m.p. 194–196 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.55 (br. s, 1 H, OH), 7.55 (br. s, 1 H, OH), 7.20 (d, J = 7 Hz, 4 H, 4×CH arom.), 7.02 (br. s, 4 H, 4×CH arom.), 6.09 (s, 2 H, 2×CH), 2.37 (s, 6 H, 2×CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.2, 135.5, 129.9, 122.0, 95.7, 20.9 ppm. IR (KBr): $\tilde{\nu}$ = 3297 (s), 3031 (w), 2917 (w), 1739 (m), 1663 (w), 1600 (s), 1572 (s), 1533 (s), 1511 (s), 1488 (s), 1413 (s), 1337 (s), 1219 (s), 1189 (s), 1153 (s), 897 (m), 814 (m), 732 (m)

Table 5. ^1H and ^{13}C assignments and HMBC correlation for compound **5b**, **5c**, and **5d**.[a]



5

5b: R = OCH_3
5c: R = Cl
5d: R = CH_3

Compound **5b** C atom[b]	^{13}C (δ)	^1H (δ)	^1H-^{13}C correlation
2, 2′	95.1	6.07, s, 2 H	C3, C3′
5, 5′, 9, 9′	123.9	7.08, d, 4 H (7)	C4, C4′, C6, C6′, C7, C7′, C8, C8′
6, 6′, 8, 8′	114.2	6.94, d, 4 H (8)	C4, C4′, C5, C5′, C7, C7′, C9, C9′
10, 10′	55.1	3.84, s, 6 H	C7, C7′
OH (1, 1′)		7.56, br. s, 1 H	
		8.50, br. s, 1 H	
Compound **5c** C atom[c]	^{13}C (δ)	^1H (δ)	^1H-^{13}C correlation
2, 2′	97.5	5.81, s, 2 H	C3, C3′
5, 5′, 9, 9′	129.1	7.44, d, 4 H (7)	C4, C4′, C6, C6′, C8, C8′
6, 6′, 8, 8′	123.7	7.19, br. s, 4 H	C5, C5′, C7, C7′, C9, C9′
OH (1, 1′)		9.24, br. s, 2 H	
Compound **5d** C atom[d]	^{13}C (δ)	^1H (δ)	^1H-^{13}C correlation
2, 2′	95.7	6.09, s, 2 H	C3, C3′
5, 5′, 9, 9′	122.0	7.02, br. s, 4 H	C6, C6′, C7, C7′, C8, C8′
6, 6′, 8, 8′	129.9	7.20, d, 4 H (7)	C5, C5′, C9, C9′, C10, C10′
10, 10′	20.9	2.37, s, 6 H	C6, C6′, C7, C7′, C8, C8′
OH (1, 1′)		7.55, br. s, 1 H	
		8.55, br. s, 1 H	

[a] Measure in $CDCl_3$ or [D_6]DMSO at 100 MHz (^{13}C) or 400 MHz [^1H, *J* (Hz) values in parentheses]. Chemical shifts are express in δ (ppm). [b] Compound **5b**: ^{13}C (δ) of C-3/3′, C-4/4′ and C7/7′ = 151.9, 135.3, and 157.4 ppm. [c] Compound **5c**: ^{13}C (δ) of C-3/3′, C-4/4′ and C7/7′ = 152.0, 142.6, and 128.9 ppm. [d] Compound **5d**: ^{13}C (δ) of C-3/3′ and C7/7′ = 152.2 and 135.5 ppm.

cm^{-1}. MS (EI): *m*/*z* = 318 (M^+, 42%), 303 (100), 275 (15), 158 (13), 130 (8), 91 (14), 65 (8), 49 (11). HRMS (EI): calcd. for $C_{20}H_{18}N_2O_2$ 318.1368; found 318.1348.

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