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Sustainable Synthesis of α-Glucosidase Inhibitors by Gas-Free Pd-Carbonylation of Nature-Based Hydroxytyrosol

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Abstract: This study outlines the sustainable synthesis of novel hydroxytyrosol (HT) and tyrosol (T) ester derivatives via a Pd-catalyzed alkoxycarbonylation of aromatic iodides. The high sustainability of the process is attributed to the use of (1) a solid carbon monoxide source, $Mo(CO)_6$, in place of dangerous gaseous CO; (2) a biomass-derived organic solvent, CPME (cyclopentyl methyl ether); (3) naturally occurring hydroxylated compounds, such as HT and T, which could be derived from agricultural waste rather than produced from petroleum-based sources. The method enables the regioselective preparation of various HT and T esters in a short reaction time (4–8 h), under mild temperatures (80 °C), and with moderate-to-excellent yields (62–93%). Moreover, in vitro biological tests have demonstrated that, in addition to the well-known antioxidant properties typical of natural phenolic compounds such as HT and T, some of the newly synthesized derivatives have a safe profile and are effective inhibitors of the α-glucosidase with potential application in the management of hyperglycemia. This synthetic approach offers a promising strategy for exploring biologically relevant chemical space, bridging the gap between natural products and sustainable drug synthesis.

Keywords: alhoxycarbonylation; palladium catalysis; hydroxyderivatives; a-glucosidase inhibitors; antioxidants; biomass-derived solvent; waste valorization; molybdenum hexacarbonyl; cyclopentyl methyl ether

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1. Introduction

The sustainability of a chemical process is influenced by a multitude of interrelated factors, including the use and production of non-toxic compounds, energy consumption, waste generation, and the integration of renewable feedstocks, as emphasized in the foundational framework provided by the Twelve Principles of Green Chemistry [1]. Overall, these principles advocate for the design of processes that minimize environmental impact and enhance overall efficiency.

Waste generated by human activities plays a significant role in environmental challenges, including the increasing levels of carbon dioxide in the atmosphere, which is regarded as a major driver of dramatic climate change. In 2023, global CO_2 emissions reached an unprecedented 37.8 gigatons, primarily driven by the burning of fossil fuels for heating and electricity production. The manufacture of organic chemicals accounted for 1.86 gigatons, representing 5% of the total emissions [2].

As reported by the European Environment Agency, the chemical industry experienced a 21% rise in the production of both hazardous and non-hazardous waste from 2012 to 2018 estimated at around 13.5 megatons, followed by a decline between 2018 and 2020 (11.6 megatons), likely attributed to the impact of the COVID-19 pandemic, while the share of waste classified as hazardous due to the presence of harmful chemicals remained stable at about 50% between 2012 and 2020, averaging 5.8 megatons annually [3].

The use of biomass as a source of organic compounds could be one of the strategies to achieve carbon-neutral industrial chemistry; over the last five decades, the perception of waste has changed significantly, evolving from a focus on waste management and mitigation to prioritizing prevention, utilization, and ultimately, its valorization.

Significant efforts in advancing sustainable processes have been recently made to convert bio-renewable feedstocks into biofuels and biopolymers [4]. However, comparatively less emphasis has been placed on innovating methods to convert biomass into a wide range of fine chemicals currently derived from non-renewable petrochemical sources [5].

Many studies have been made to convert lignocellulosic biomass into benzenoid feedstocks. However, the processes, which rely on depolymerization and transformation sequences, often prove to be costly, resource-intensive, and low-yielding [6].

Moreover, in the context of the sustainability of organic syntheses on an industrial scale, the challenges posed by the extensive use of organic solvents are particularly significant. Their volatile nature can lead to fugitive emissions, contributing to air pollution and posing potential health hazards to workers due to inhalation or accidental exposure. Furthermore, the difficulty in recovering and recycling many organic solvents exacerbates their environmental footprint.

To address these issues, strategies such as solvent-free reactions and the adoption of alternative solvents (e.g., water [7], Deep Eutectic Solvents [8], ionic liquids [9], biomass-derived solvents [10], or supercritical fluids [11]), are being actively developed. These approaches aim to align industrial practices with the principles of atom economy, pollution prevention, and the use of safer chemicals, thereby advancing the sustainability of organic synthesis processes.

Our recent research has been dedicated to developing sustainable synthetic methodologies through two main approaches. The first involves utilizing multi-bond formation strategies [12–14], while the second focuses on substituting toxic volatile organic solvents with ionic eutectic mixtures [15–17], preferably derived from bio-renewable resources. Driven by the desire to develop increasingly sustainable chemical processes, as well as in the recovery of organic compounds from natural matrices, we recently described a methodology for the extraction of hydroxytyrosol from the olive leaves by using non-toxic, non-flammable ionic solvents (Deep Eutectic Solvents) derived from natural sources. The one-pot, two-step method consists of the extraction of oleuropein from powdered olive leaves and subsequent hydrolysis to produce a hydroxytyrosol (HT) solution in DES/water. After a liquid–liquid extraction with ethyl acetate and solvent evaporation, the process yields a solid extract containing up to 12.3 $\pm$ 0.9 g of HT per kilogram of leaves (0.12 $\pm$ 0.03 g HT per gram of extract) [18].

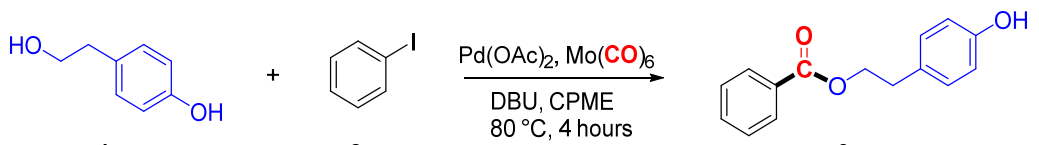
In this paper, we illustrate a gas-free carbonylative coupling of phenols HT and tyrosol (T) with (hetero)aryl iodides to obtain biologically valuable ester derivatives. The Pd-catalyzed reaction has a high degree of sustainability since it is performed in an eco-friendly solvent, for example cyclopentyl methyl ether (CPME); uses $Mo(CO)_6$ as a safe source of carbon monoxide; and employs natural compounds as substrates that can be potentially extracted from agricultural waste (olive leaves) rather than produced from fossil fuels. Moreover, biological characterization highlights that the derivatives prepared with this strategy continue to primarily show a safe profile like their precursors and an

interesting inhibitor activity on α-glucosidase, beside a well-known antioxidant activity typical of natural phenolic compounds derivatives. This synthetic approach represents a promising bridge between the natural chemical space and the sustainable development of new pharmaceuticals.

2. Results and Discussion

In recent studies, we found that the gas-free carbonylation of aryl iodides using $Mo(CO)_6$ as a safer carbon monoxide source was significantly enhanced by Deep Eutectic Solvents (DESs) [19,20], yielding the desired esters in high efficiency within just two hours. Building on this understanding, we started to study the reaction between T (**1a**), iodobenzene (**2a**), and $Mo(CO)_6$ in ChCl/urea with the aim to obtain valuable aroylated tyrosol derivatives **3**; due to its large availability, the tyrosol molecule was chosen as a model substrate to simulate the reactivity of other polyphenols such as hydroxytyrosol. The gas-free carbonylative coupling between the alcohol **1a** and the aryliodide **2a** (Table 1), in the eutectic mixture ChCl/urea (1:2 mol/mol), was executed in the presence of a catalytic system consisting of $Pd(OAc)_2$ and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The latter is thought to promote the release of CO from $Mo(CO)_6$ even at a relatively low temperature of 80 °C. Under these experimental conditions, the target ester **3aa** did not form at all, even after 4 h (Table 1, entry 1). The presence of an acidic phenolic moiety in substrate **1**, most likely existing as a phenate in the ionic medium, probably caused the formation of palladium complexes with negligible catalytic activity. In fact, only traces of benzoic acid, deriving from the carbonylation of **2a**, were revealed by GC-MS and ^{1}H-NMR analyses of the crude reaction mixture. We then decided to drastically vary the nature of the reaction medium and moved from ionic DES to less polar solvents, again with a high degree of sustainability [21].

Table 1. The optimization of the Pd-catalyzed carbonylative coupling between tyrosol **1** and iodobenzene **2a** in the presence of $Mo(CO)_6$ as a safe source of CO [a].



1a **2a** **3aa**

Entry	1a (mmol)	2a (mmol)	Solvent	3aa (Yield%) [b]
1	0.5	0.6	ChCl/Urea	0
2	0.5	0.6	2-MeTHF	36
3	0.5	0.6	DMC	traces
4	0.5	0.6	CPME	50
5	0.75	0.5	CPME	71

[a] Reaction conditions: tyrosol **1a**, iodobenzene **2a**, DBU (1.5 mmol), and $Mo(CO)_6$ (0.5 mmol) were suspended in 2 mL of solvent and then $Pd(OAc)_2$ (5.0 mol%) was added. The mixture was heated at 80 °C for 4 h under vigorous magnetic stirring. Of note, to minimize the impact of impurities on the carbonylation process and to ensure accuracy, we used highly pure T and HT (99% by HPLC analysis), obtained by reducing the corresponding carboxylic acids, rather than by extracting from olive leaves, which yielded compounds with a purity below 90%. [b] Calculated via ^{1}H NMR analysis of the crude reaction mixture using the internal standard technique (NMR internal standard: dimethyl sulfone).

In 2-methyltetrahydrofuran (2-MeTHF), as the reaction medium, we observed the formation of small amounts of target benzoate **3aa** (36% yield, Table 1, entry 2), whereas in dimethyl carbonate (DMC) only traces of **3aa** were detected by GC-MS (entry 3). Particularly, the major product isolated from the reaction mixture was the unsymmetrical 2-(4-hydroxyphenyl)ethyl methyl carbonate (98% yield) deriving from the direct nucle-

ophilic attack of tyrosol to DMC. When cyclopentyl methyl ether (CPME) was used as bio-mass-derived green medium, the formation of the target product **3aa** increased up to 50% yield (entry 4).

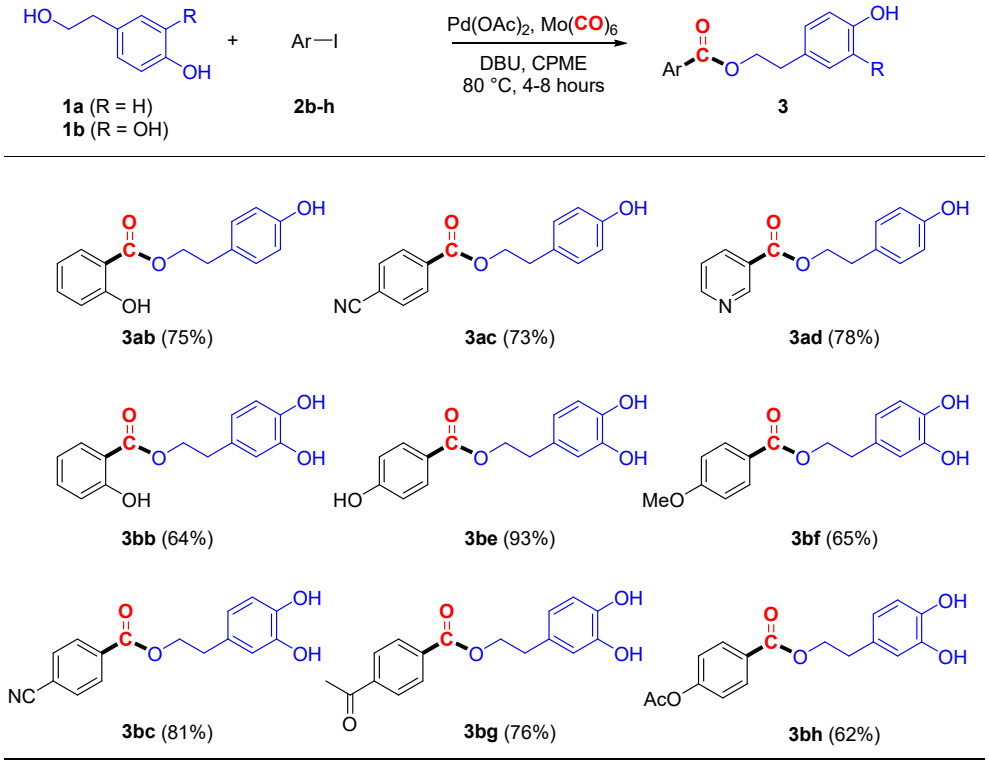
Using a slight excess of **1a** (0.75 mmol) compared to the iodide **2a** (0.5 mmol), we found that the carbonylative process was more efficient, leading to the ester **3aa** in a satisfactory 71% yield (entry 5). These experimental conditions were chosen for the synthesis of all the derivatives reported in the present study.

The use of a different source of Pd (e.g., $PdCl_2$ or Pd/C) highlighted the unique catalytic activity of $Pd(OAc)_2$ in promoting the alkoxycarbonylation reaction.

Specifically, only trace amounts of product **3aa** were detected in the crude mixture when other Pd sources were used instead of $Pd(OAc)_2$. Moreover, attempts to replace DBU with greener alternatives, such as NaOAc, KOH, or K_2CO_3, yielded unsatisfactory results, with the desired ester **3aa** being obtained in yields of only 12–36% (see ESI for further details on the optimization of model reaction).

To investigate a wider range of structural variations in tyrosol derivatives and evaluate the scope of the carbonylative process, a series of aryl iodides **2b**–**h** with various functional groups was employed in the reaction with tyrosol **1a** (Scheme 1). The presence of a hydroxy group in the 2-position (**2b**) or a cyano group in the 4-position (**2c**) positively influenced the reaction outcome, resulting in the formation of esters **3ab** and **3ac** with yields of 75% and 73%, respectively (Scheme 1). Also, 3-iodopyridine **2d** was efficiently coupled with tyrosol **1a**, leading to the product **3ad** with a 78% yield. It is worth noting that the ester **3ad** can be considered a chimeric compound [22], integrating two molecular structures of significant biological importance: tyrosol and nicotinic acid. Encouraged by the promising results obtained with these gas-free carbonylative couplings in CPME, we extended the approach to the synthesis of hydroxytyrosol derivatives using **1b** as a starting material [21]. The protocol proceeded smoothly under the same experimental conditions previously optimized for **1a** (Table 1, entry 5). Both 2- and 4-iodophenol (**2b** and **2e**) were successfully coupled with hydroxytyrosol **1b**, yielding polyhydroxylated esters **3bb** and **3be** in moderate-to-excellent yields (64–93%, Scheme 1). However, a less efficient coupling was observed when 4-iodoanisole (**2f**) was reacted with **1b**, resulting in the isolation of ester **3bf** in a yield of 65%, even after 8 h of reaction time (Scheme 1). Good ester yields (76–81%) were instead achieved with the employment of cyano- and acetyl-substituted iodides **2c** and **2g**, allowing the preparation of polyfunctionalized molecules **3bc** and **3bg**. When 4-iodophenyl acetate **2h** was used as the halogenated reagent, the carbonylative coupling produced the bis-ester derivative **3bh** in only 62% yield, probably because of unwanted transesterification reactions involving the acetoxy group of the substrate **2h** and/or the product **3bh**. Despite the moderate yield in the formation of bis-ester **3bh**, it is important to emphasize that this methodology allows for the synthesis of non-symmetric bis-esters, which would have been difficult to achieve using conventional synthetic approaches, such as those based on carboxy group activation.

Regarding the reaction mechanism, we assume that DBU first facilitates the release of CO gas from the $Mo(CO)_6$ complex, as detailed in the study by Larhed et al. [23]. The resulting increase in CO pressure within the reaction medium is then expected to initiate a Pd-catalyzed carbonylative coupling between the aryl iodide and the primary alcohol (T or HT). This process follows the traditionally accepted mechanism, which involves (1) oxidative addition of the aryl halide, (2) CO coordination and insertion, and (3) alcoholysis of the resulting acyl-palladium complex [24].



Scheme 1. The scope of Pd-Catalyzed carbonylative coupling between alcohols **1a**,**b** and (hetero)aromatic iodides **2b**–**h** in CPME as a green medium, with $Mo(CO)_6$ as a safe CO source. Reaction conditions: alcohol **1a**,**b** (0.75 mmol), aryl iodide **2b**–**h** (0.5 mmol), $Pd(OAc)_2$ (5.0 mol%), DBU (1.5 mmol), $Mo(CO)_6$ (0.5 mmol), DBU (1.5 mmol), CPME (2.0 mL), 80 °C, 4 h. Isolated yields are reported in brakets. For the preparation of esters **3be**, **3bf**, **3bc** and **3bh** the reaction time is 8 h.

After completing the synthetic investigation, we conducted a series of biological tests on the newly prepared HT derivatives. Only **3bh** was not subjected to biological tests because crystallization did not yield a sample pure enough. Considering their peculiar structures, **3bb**, **3bc**, **3be**, **3bf**, and **3bg** have been tested for their ability to inhibit α-glucosidase, investigating their potential hypoglycemic effects. α-glucosidase inhibition plays an important role in the treatment of type 2 diabetes, which represents 95% of diabetic diseases, whose prevalence in the past three decades has risen dramatically in countries of all income levels [25]. Inhibitors of these enzymes function competitively, blocking their activity and helping to prevent postprandial hyperglycemia [26]. Currently, acarbose, miglitol, and voglibose have been approved by the FDA for the treatment of diabetes as oral α-glucosidase inhibitors. While this class of inhibitors offers several advantages compared to other groups of antidiabetic drugs, mild side effects have been observed. These include the absence of systemic gastrointestinal side effects and occasional allergic reactions. Over the past decade, there has been significant interest in the discovery of natural products with α-glucosidase inhibitory activity [27].

Compared to HT and Acarbose, all the derivatives tested show an enzyme inhibition activity greater than HT, which, at the highest concentration used (100 μM), inhibits α-glucosidase of 15% (IC_{50} = 245 μM). Acarbose, in our experimental conditions, has an IC_{50} of 205 μM and a percentage of inhibition of 73. Compounds **3bc** bearing a *p*-cyano group (10 μM, 90% inhibition) and **3be** that have a *p*-hydroxyl group (14 μM, 91% inhibition) were found to be the most effective α-glucosidase inhibitors. **3bb**, with *ortho*-hydroxyl group, inhibits the enzyme by 74% with an IC_{50} of 81 μM while **3bf** and **3bg**, the least polar derivatives, show less potency with an IC_{50} of 354 and 265 μM, respectively. The inhibition assay results are summarized in Figure 1.

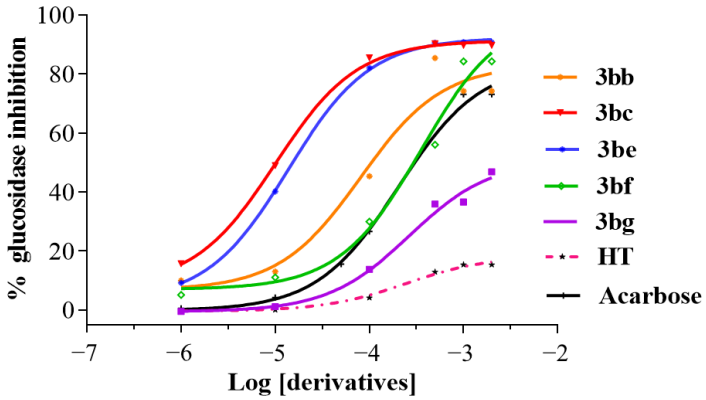


Figure 1. α-glucosidase inhibitory activity of HT-derivatives **3bb**, **3bc**, **3be**, **3bf**, and **3bg** compared to HT as reference.

Additionally, their antioxidant activity was assessed using two most common radical scavenging assays, such as 2,20-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The ABTS assay is based on the generation of a blue/green $ABTS^{\bullet+}$ that can be reduced by antioxidants, whereas the DPPH assay is based on the reduction of the purple DPPH to 1,1-diphenyl-2-picryl hydrazine. ABTS assay detects a higher antioxidant capacity with respect to DPPH assay [28], and it is more useful to detecting antioxidant capacity in a variety of foods [29]. Thus, to discriminate between two methods, **3bb**, **3bc**, **3be**, **3bf**, HT, and Gallic acid (GA), a well-known natural antioxidant compound, have been tested by using both assays [30].

By using ABTS assay, the radical scavenging activity of HT was found to be 26 μM while GA has shown an IC_{50} value of 6.9 μM. The HT-derivatives **3bf** (IC_{50} = 21 μM), **3bc** (IC_{50} = 23 μM), and **3bg** (IC_{50} = 24 μM) have been demonstrated to be slightly more potent than HT. **3be** has an IC_{50} of 29 μM, while the derivative with the lowest antioxidant property is **3bb** (IC_{50} = 41 μM) as reported in Figure 2.

By DPPH assay the newly synthesized compounds showed a good antioxidant capacity, comparable to each other. The most potent are **3bg** (IC_{50} = 12 μM), **3bc** (IC_{50} = 13 μM), and **3bf** (IC_{50} = 14 μM) followed by **3be** and **3bf** which have an IC_{50} of 20 and 21 μM, respectively (Figure 2). Also, in this case, it is confirmed a higher sensitivity of ABTS with respect to DPPH, in fact, in the first assay it is possible to have a better differentiation among the compounds.

Moreover, cytotoxic experiments have been conducted in three different cell lines to verify **3bb**, **3bc**, **3be**, and **3bf** safety profiles. MDCK-MDR1 is a transfected cell line overexpressing human P-glycoprotein (multidrug resistance 1; MDR1) [31], an efflux transporter crucial for drug absorption and usually used in drug discovery and development [32]; human hepatocellular liver carcinoma HepG2 cells, a useful model to study the action of drugs on the hepatic cells metabolism to assess the risk of their hepatotoxic activity [33]; the human colorectal adenocarcinoma Caco-2 cell line that spontaneously differentiates into a polarized cell monolayer with microvilli and many properties typical of absorptive enterocytes as found in the small intestine [34], which is widely used to assess intestinal cytotoxicity and permeability [35]. In all these three cell lines, after 24 h of exposure, viability was higher than 90% at different concentrations (1–100 μM) (see Supplementary Materials).

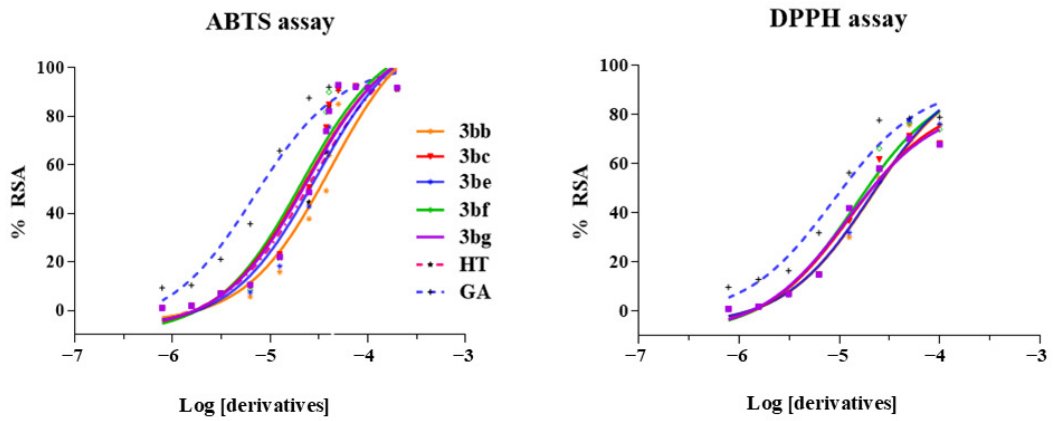


Figure 2. Antioxidant activity of HT-derivatives **3bb**, **3bc**, **3be**, **3bf**, and **3bg** measured by ABTS (**left**) and DPPH (**right**) assays. The activity is expressed as radical scavenging inhibition percentage (RSA%) against the Log of concentration and compared to HT and GA molecules, used as references.

3. Materials and Methods

3.1. General Aspects

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer and chemical shifts are reported in parts per million (δ). Dimethyl sulfone has been used as the internal standard for yield determination by ^{1}H NMR analysis of the crude reaction mixtures. FT-IR spectra were recorded on a Perkin-Elmer 681 spectrometer. Chromatography was conducted by using silica gel 60 with a particle size distribution 40–63 μm and 230–400 ASTM. GC-MS analyses were performed on HP 5995C model. High-resolution mass spectrometry (HRMS) analyses were performed using a Bruker microTOF QII mass spectrometer equipped with an electrospray ion source (ESI). Melting points were determined with an Electrothermal melting point apparatus. Reagents and solvents, unless otherwise specified, were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and TCI Europe N.V. (Zwijndrecht, Belgium) and used without any further purification. Petroleum ether refers to the 40–60 °C boiling fraction. The Deep Eutectic Solvent (DES), cholinium chloride (ChCl)/urea (1:2 mol/mol), was prepared by heating under stirring at 60–80 °C for 10–30 min the corresponding individual components until a clear eutectic mixture was obtained. Full characterization data, including copies of ^{1}H NMR and ^{13}C NMR spectra, have been reported for all the synthesized compounds. The chemical purity of compounds subjected to in vitro biological tests was assessed by HPLC analysis performed on an Agilent 1260 Infinity instrument equipped with a 1260 DAD VL + detector, and their purity is higher than 95%. The analysis was conducted in isocratic conditions by using acetonitrile H_2O = 70:30 as a mobile phase. The stationary phase was constituted by a Zorbax Eclipse Plus C18 column, 250 × 4.6 mm Agilent® (Santa Clara, CA, USA). UV detection was made at λ = 254 nm.

3.2. Experimental Procedure for the Synthesis of Tyrosol (T) and Hydroxytyrosol (HT) by Reduction of the Corresponding Carboxylic Acids

3,4-Dihydroxyphenylacetic acid or 4-hydroxyphenylacetic acid (3.3 mmol) was dissolved in anhydrous THF (25 mL), and then cooled down to 0 °C. Then, lithium aluminum hydride solution (2.0 M, 4.0 equiv., 6.5 mL) was added dropwise. The mixture, after three vacuum–nitrogen cycles, was stirred under reflux (for the reduction of 3,4-dihydroxyphenylacetic acid) or under room temperature (for the reduction of 4-hydroxyphenylacetic acid), for 90 min until the substrate was consumed (controlled by TLC). Then, the mixture was acidified with 2.0 M H_2SO_4 and extracted with AcOEt (3 × 20 mL). The combined organic phases were dried and filtered. The residue was

purified by column chromatography using eluent petroleum ether/AcOEt 80:20 (*v*/*v*) to obtain pure tyrosol (99%) or CH_2Cl_2/acetone 80:20 (*v*/*v*) to obtain hydroxytyrosol as pure compound (99%).

3.3. Experimental Procedure for the Carbonylative Coupling Between Alcohols **1a**–**b** *and Aryl Iodides* **2a**–**h** *in CPME*

In a 10 mL round bottom flask, alcohol (tyrosol 1a or hydroxytyrosol **1b**, 0.75 mmol), aryl iodide (**2a**–**h**, 0.5 mmol), $Mo(CO)_6$ (132.0 mg, 0.5 mmol), DBU (1,8-diazabicyclo[5.4.0] undec-7-ene, 228.3 mg, 224 µL, 1.5 mmol), $Pd(OAc)_2$ (trimer, FW = 673.46, 5.0 mol%, 0.025 mmol, 16.8 mg) and CPME (cyclopentyl methyl ether, 2.0 mL) were sequentially added. The reaction mixture was stirred at 80 °C for 4 h (in the case of esters **3be**, **3bf**, **3bc**, **3bh**, 8 h of reaction time was needed to ensure complete conversion of substrates). After completion of the reaction, the mixture was allowed to cool to room temperature. Subsequently, 2.5 mL of water was added, followed by the gradual addition of an aqueous HCl solution (10% *v*/*v*) until pH = 2. The mixture was then extracted with AcOEt (5 mL × 3) and the reunited organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered through a celite pad, and evaporated under reduced pressure. The crude was purified by flash column chromatography on silica gel (using as eluent petroleum ether/AcOEt 80/20 to petroleum ether/AcOEt 60/40), obtaining the desired esters as pure compounds **3ab**–**3bh**.

4. Biological Assays

4.1. α-Glucosidase Inhibition Assay

A mixture of 20 µL of different concentrations, ranging from 1 mM to 1 µM, of the compounds and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing 0.1 U/mL α-Glucosidase (Sigma Aldrich, Darmstadt, Germany, #5003) was incubated in 96-well plates at 25 °C for 10 min. After pre-incubation, 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well. The reaction mixtures were incubated at 25 °C for 10 min. Before and after incubation, absorbance was recorded at 405 nm by a TECAN-Infinite 200 Microplate Reader and compared to that of the control (100% of enzymatic activity), which had 20 µL buffer solutions in place of the compounds [36]. The α-glucosidase inhibitory activity was expressed as % inhibition and was calculated as follows:

$$\%\ \text{Inhibition} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100$$

4.2. Antioxidant Activity with ABTS Protocol

Experiments were performed on the TECAN-Infinite 200 Microplate Reader. ABTS was dissolved in acetate buffer 0.1 mmol/L to make a concentration of 7 mmol/L. $ABTS^{\bullet+}$ was produced by reacting the ABTS stock solution with 2.45 mmol/L potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the study of samples, the $ABTS^{\bullet+}$ stock solution was diluted with acetate buffer 0.1 mmol/L, pH 7.4 to an absorbance of 0.70 at 734 nm. After adding 190 µL of diluted $ABTS^{\bullet+}$ to 10 µL of the sample, with concentrations ranging from 100 µM to 1 µM, the absorbance was measured 5 min after the initial mixing. Decolorization of the assay was linear with increasing antioxidant concentrations. ABTS was purchased from Sigma Aldrich, Darmstadt, Germany, (#A9941).

4.3. Antioxidant Activity with DPPH Protocol

In a 96-well plate, each tested compound, at concentrations ranging from 100 µM to 1 µM, was dissolved in a reaction mixture of MeOH and H_2O and diluted by adding

2% DMSO. A freshly prepared solution of DPPH in reaction mixture (100 μM final concentration) was added [37]. Following vigorous stirring, each mixture was left in the dark for 10 min at room temperature, after which absorbance values for each well were read at 520 nm using a TECAN-Infinite 200 Microplate Reader.

Antioxidant activity was determined as a percentage of radical scavenging activity (%RSA). The value was calculated using the following equation:

$$\% \text{ RSA} = \left(\frac{\text{Abs}_o - \text{Abs}_i}{\text{Abs}_o} \right) \times 100$$

where Abs_o and Abs_i represent absorbance without and with antioxidants, respectively. DPPH was purchased from Sigma Aldrich, Darmstadt, Germany (#D9132).

4.4. Cell Culture and Cell Viability

MDCK-MDR1 and Caco-2 cells were grown in Dulbecco's Modified Eagle Medium and high glucose (DMEM high glucose, Euroclone S.p.A., Pero, Italy) supplemented with 10% Fetal Bovine Serum (FBS, Euroclone S.p.A., Pero, Italy), 2 mM glutamine (Euroclone S.p.A., Pero, Italy), 100 U/mL of penicillin, and 0.1 mg/mL of streptomycin (Euroclone S.p.A., Pero, Italy). Human hepatocellular liver carcinoma (HepG2) cell line was purchased from the American Type Culture Collection (ATCC). HepG2 cells were cultured in Eagle's Minimum Essential Medium (MEM, Euroclone), supplemented with 10% FBS, 2 mM glutamine (Euroclone), 100 U/mL penicillin, 0.1 mg/mL streptomycin (Euroclone S), and 1% Non–Essential Amino Acids (NEAA, Euroclone). Cultured cells were maintained at 37 °C in an atmosphere containing 95% of air and 5% of CO_2. Cells were sub-cultivated every 48 h by a trypsine–EDTA solution [37].

Determination of cell growth was determined using the MTT assay at 24 h. On day 1, 10,000–15,000 cells/well were seeded into 96-well plates in a volume of 100 μL. On day 2, the various compound concentrations (1 μM–100 μM) were added. After the established incubation time with compounds (24 h), MTT (0.5 mg/mL, Sigma Aldrich, Darmstadt, Germany, # M2003) was added to each well, and after 3–4 h incubation at 37 °C, the supernatant was removed. Formazan crystals were solubilized using 100 μL of DMSO/EtOH (1:1) and absorbance values at 570 nm were determined on the microplate reader Victor 3 from PerkinElmer Life Sciences (Waltham, MA, USA).

5. Conclusions

The present study paves the way for the potential enhancement of agri-food waste by transforming it into high-value biological products. Hydroxytyrosol and tyrosol have been transformed into benzoic esters derivatives by a sustainable Pd-catayzed alkoxycarbonylative coupling that avoids dangerous CO and volatile and toxic solvents and occurs in short times (4–8 h) at relatively low temperatures (80 °C). All the synthesized compounds were found to be safe and exhibited good antioxidant activity. In addition, two compounds also showed fair potency in inhibiting α-glucosidase in the micromolar range. This synthetic strategy provides an innovative pathway to investigate biologically significant chemical space, connecting natural products with eco-friendly drug development.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal15030202/s1: Table S1: Optimization of the model reaction between tyrosol and iodobenzene; Table S2: Biological activities of esters **3bb**, **3bc**, **3be**, **3bf**, **3bg**; Spectroscopic data of tyrosol (**1a**) and hydroxytyrosol (**1b**); Spectroscopic data of esters **3ab**, **3ac**, **3ad**, **3bb**, **3bc**, **3be**, **3bf**, **3bg**, **3bh**; Copies of ^{1}H- and ^{13}C-NMR spectra of esters **3ab**, **3ac**, **3ad**, **3bb**, **3bc**, **3be**, **3bf**, **3bg**, **3bh**.

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Data Availability Statement: The data supporting the findings of this study are available within the article and its Supplementary Materials.

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