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Short communication

Hydroxylated biphenyls as tyrosinase inhibitor: A spectrophotometric and electrochemical study

Paolo Ruzza [a, 1], Pier Andrea Serra [b, 1], Davide Fabbri [c], Maria Antonietta Dettori [c], Gaia Rocchitta [b], Giovanna Delogu [c, *]

[a] Istituto di Chimica Biomolecolare, Consiglio Nazionale Ricerche, Via Marzolo 1, I-35131 Padova, Italy
[b] Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi, Viale S. Pietro 43/b, I-07100 Sassari, Italy
[c] Istituto di Chimica Biomolecolare, Consiglio Nazionale Ricerche, Traversa La Crucca 3, I-07100 Sassari, Italy

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ABSTRACT

A small collection of C_2-symmetry hydroxylated biphenyls was prepared by straightforward methods and the capability to act as inhibitors of tyrosinase has been evaluated by both spectrophotometric and electrochemical assays. Our attention was focused on the diphenolase activity of this enzyme characterized by the absence of the characteristic lag time of enzymatic reaction of its monophenolase activity. To this purpose, we evaluated the capability of tyrosinase to oxidize a natural *o*-diphenol substrate to *o*-quinone analyzing the changes in the UV–Vis spectrum of a solution of caffeic acid and the reduction of the cathodic current in a tyrosinase-biosensor, respectively. Results of both the methods were comparable. Most of the compounds possessed higher inhibitory activity compared to compound **1**, a known hydroxylated biphenyl inhibitor of tyrosinase.

1. Introduction

Tyrosinase [EC 1.14.18.1] is a copper-containing enzyme involved in melanin biosynthesis, in unfavourable enzymatic browning of plant-derived foods, in pathological melanogenesis and in insects moulting process [1]. It catalyses the oxidation of both monophenols (monophenolase or cresolase activity) and *o*-diphenols (diphenolase or catecholase activity) to *o*-quinones (Fig. 1A).

Three forms of this enzyme (*oxy*-, *met*-, and *deoxy*-tyrosinase) with different copper structures of the active site are identified [2]. The monophenolase activity is catalyzed by the *oxy* form (less than 15%) that is transformed in the *deoxy* form in the successive oxidation to *o*-quinone, while in the diphenolase cycle both the *oxy* and *met* forms are involved. The *oxy* form oxidizes *o*-diphenol to *o*-quinone, yielding the *met* form, and this latter form transforms another *o*-diphenol molecule into *o*-quinone and is reduced to the *deoxy* form, the only one capable of reacting with molecular oxygen. For this reason, the monophenolase activity presents a characteristic lag time that exists until a sufficient amount of catechol is produced [3]. The length of the lag time is strictly connected to the enzyme source and concentration, the concentration of monophenol and the presence of catalytic amounts of *o*-diphenol or transition metal ions, which completely abolish the lag period [4].

Investigation of tyrosinase inhibitors may lead to development of novel skin whitening agents, medicinal products, antibrowning substances or insect control compounds [5]. Many natural occurring compounds have been identified for those targets whose structure has inspired the preparation of several collections of synthetic tyrosinase inhibitors [6]. The most studied chemical scaffolds of tyrosinase inhibitors belong to the polyphenols group. In spite of a large number of tyrosinase inhibitors only a few of these are generally used.

4,4′-Dihydroxy-biphenyl **1**, a natural biphenol, was found to be a potent tyrosinase inhibitor, more effective than kojic acid and arbutin [7]. Hydroxylated biphenyls are a class of polyphenols widely present in nature [8], some of them manifest high biological activity like ellagitannins, vancomicin, biphenomicins, others, structurally less sophisticated, are natural occurring dimers of 2-methoxy phenols and phenols. Compared to phenols, hydroxylated biphenyls manifest higher antioxidant activity and generally they are less toxic than the corresponding phenolic monomer [9,10]. There is an increasing interest in using the hydroxylated

* Corresponding author.
E-mail address: giovanna.delogu@icb.cnr.it (G. Delogu).
[1] These authors contributed equally to this work.

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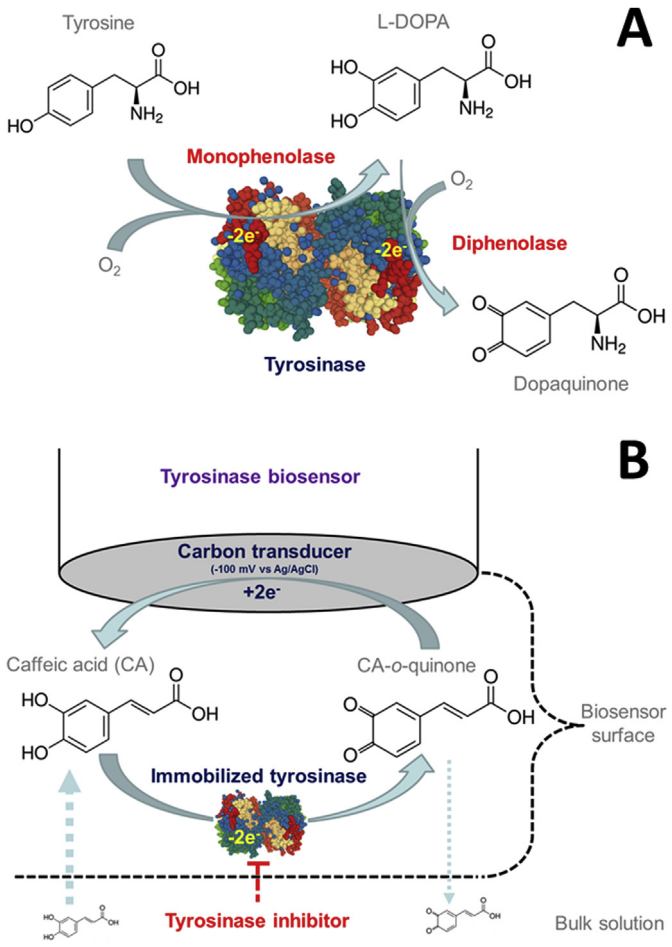

Fig. 1. (A) Schematic representation of the reactions catalyzed by tyrosinase. (B) Caffeic acid is oxidized to the corresponding quinone by the enzyme and reduced-back at carbon surface by applying a reducing potential of −100 mV vs Ag/AgCl reference electrode. The first reaction, inhibited by the compounds synthetized in this study, resulted in a significant reduction of the cathodic current.

biphenyl unit as building block to prepare bioactive molecules involved in human pathologies and disorders because most of them manifest interesting pharmacological and biological activities such as neuroprotective and antiproliferative properties [11].

In our continuing search on synthesis of hydroxylated biphenyls with interesting stereochemical and biological features [12–15]. we have prepared a small collection of C_2-symmetry biphenols and evaluated their capability to act as inhibitors of tyrosinase by both spectrophotometric and electrochemical assays.

2. Results and discussion

2.1. Chemistry

Taking in account the high level of specificity of hydroxylated biphenyl structure in protein binding in comparison to other aromatic compounds [16], we have considered C_2-symmetry isomer of biphenyl **1** (eg. compound **2**) and its derivatives by introducing hydroxylated functionalities in key positions (eg. compounds **3**, **4**) as depicted in Fig. 2A.

It is generally demonstrated that the presence of hydroxylated groups in a phenol structure improves tyrosinase activity [17–19]. According to those considerations, a small collection of biphenyls was selected. Different hydroxylated functionalities are present in

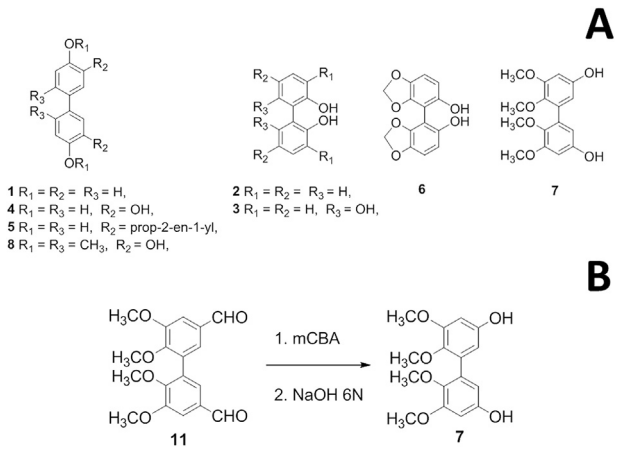

Fig. 2. (A) Hydroxylated biphenyl structures. (B) Preparation scheme of biphenyl **7**.

compounds **6** and **7** whereas compounds **5** and **8** are lipophylic derivatives of biphenol **1**. Compound **6** possesses a benzodioxole moiety in a biphenylic structure that reminds sesamol structure, recently studied for the effective activity as tyrosinase inhibitor [20].

Except compound **7**, all compounds studied in this work are known in literature, though some of them were achieved in scarce yields or as side-products. Yields of compounds **5** and **8** were increased and new straightforward synthetic procedures improved the sustainability of the process compared to those described in literature (Supplementary Data). Claisen rearrangement of the corresponding 2-(prop-2-en-1-yl)phenol derivative gave compound **5** in high yield following two microwave procedures carried out in aqueous solvents. Compounds **3**, **4**, **6**–**8** were obtained by coupling reactions of the corresponding monomer with different reaction conditions according to substituents in the phenolic ring. Synthesis and spectroscopic data of compounds **3**–**6** and **8** have been reported in the Supplementary Data. Compound **7** was obtained by oxidation of veratraldehyde C_2-symmetry dimer **9** in the presence of *m*-chloroperbenzoic acid (mCPBA) (Fig. 2B). Due to substitutions in *ortho-ortho′* and *ortho-meta* positions, biphenyls **3** and **6**–**8** are conformationally hindered. Biphenyls **6**–**8** were tested in racemic form.

2.2. Biological evaluation

With the aim to explore the biological properties of compounds **1**–**8**, we evaluated the capability of these molecules to inhibit the tyrosinase activity. In particular, our attention was focused on the diphenolase activity of this enzyme [21]. To this purpose, we evaluated the capability of tyrosinase to oxidize a natural *o*-diphenol substrate to *o*-quinone analyzing the changes in the UV–Vis spectrum of a solution of caffeic acid. The spectrum recorded before mixing the substrate and enzyme solutions, strongly immediately changes after the mixing and the absorbance at 280 and 311 nm, characteristic of caffeic acid, decrease in time-dependent mode, while a band at 413 nm attributable at the *o*-quinone appeared [22]. The time-course of caffeic acid oxidation, obtaining monitoring the absorbance values at 311 nm at different time, has been reported in Fig. 3 and is characterized by the absence of the characteristic lag time of enzymatic reaction catalyzed by tyrosinase due to its monophenolase activity.

Any significant contribute of compounds **1**–**8** in the examined UV–Vis region of caffeic acid, in presence or not of tyrosinase, in the experimental conditions was detected (data not shown). With the