Synthesis of a serie of 2-phenylbenzofurans. Structures with rigid *trans*-resveratrol's core and their tyrosinase activity

Giovanna Delogu[2]*, Antonella Fais[1], Carmen Picciau[2], Silvia Serra[2], Stefania Utzeri[1], Benedetta Era[1], Maria João Matos[3], and Marcella Corda[1]

[1]*Dipartimento di Scienze della Vita e dell'Ambiente, Università degli Studi di Cagliari, Macrosezione Biomedica Complesso Universitario di Monserrato, S.S.554, 09042, Monserrato (Cagliari), Italy*.
[2]*Dipartimento di Scienze della Vita e dell'Ambiente, Università degli Studi di Cagliari, Macrosezione di Scienze del Farmaco, Via Ospedale 72, 09124 Cagliari, Italy*.
[2]*Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain.*

Email: giovannadelogu@hotmail.it

Abstract: Tyrosinases, better termed polyphenoloxidases (EC 1.14.18.1; monophenol, *o*-diphenol: oxygen oxidoreductase), are copper-containing monooxygenases catalyzing the *o*-hydroxylation of monophenols to the corresponding catechols (monophenolase activity) and the oxidation of catechols to the corresponding *o*-quinones (diphenolase activity).
Tyrosinase is responsible for melanisation in animals and browning in plants and fungi. Moreover, it is known that tyrosinase is involved in abnormal accumulation of melanin pigments (hyperpigmentation).

In previous studies, phenylbenzofurans were isolated from Morus lhou, belonging to the family of Moraceae, plant that is one of the most ubiquitous traditional herbal medicines in East Asia. Interestingly, a phenylbenzofuran *Moracin M* displayed significant inhibitory activities against tyrosinase. Based on this research, we describe the synthesis of a new series of 2-phenylbenzofurans suitably modified. These compounds included in their structure the resveratrol's nucleus, always in their *trans* configuration.

Resveratrol is a natural substance produced by several plants, as grapes. A number of beneficial health effects are associated to this compound. Anticancer, antiviral, anticoagulant, neuroprotective, antiaging, anti-inflammatory and life-prolonging effects have been reported.
With the aim of finding out structural features in the tyrosinase inhibitory activity, in the present work, we investigated the effect of nine phenylbenzofurans on the diphenolase activity of mushroom tyrosinase.
The synthesized compounds were evaluated *in vitro* as mushroom tyrosinase inhibitors. The results show that phenylbenzofurans act as activators or inhibitors of the diphenolase activity in relation not only by replacing the chemical group but also by its relative position. This work highlights how the introduction of different substituents in the phenylbenzofuran ring can influence the tyrosinase activity.

Keywords: tyrosinase, benzofuran, resveratrol

Introduction

Tyrosinase (EC 1.14.18.1) is a copper-containing enzyme that catalyzes two distinct reactions of melanin synthesis: the hydroxylation of tyrosine by monophenolase action and the oxidation of 3,4-dihydroxyphenylalanine (L-DOPA) to *o*-dopaquinone by diphenolase action.[1]

Tyrosinase is known to be a key enzyme in melanin biosynthesis, involved in determining the color of mammalian skin and hair. Various dermatological disorders, such as melasma, age spots and sites of actinic damage, arise from the accumulation of an excessive level of epidermal pigmentation.[2-3]

Plant sources of tyrosinase inhibitors are numerous, among these the *Morus lhou* (S.) Koidz. is well renowned. This is a polyphenol-rich plant, one of the most ubiquitous traditional herbal medicines in East Asia. This species, belonging to the family of *Moraceae*, may be considered to be a nontoxic natural therapeutic agent. It has been proven that many of them present antibrowning, hypoglycemic, antinephritis and anti-inflammatory properties. Additionally, previous workers

reported that this species contains flavanones, flavones, stilbenes and phenylbenzofurans with tyrosinase inhibiting activity.[4,5]

Benzofuran derivatives are an important class of organic compounds that occur in natural products because of their biological activities, including antitumoral properties. They can be used as inhibitors of 5-lipoxygenase, antagonists of the angiotensin II receptor, blood coagulation factor Xa inhibitors, ligands of adenosine A1 receptor and so forth.[6]

Resveratrol is a natural substance produced by several plants, as grapes. A number of beneficial health effects are associated to this compound. Anticancer, antiviral, anticoagulant, neuroprotective, antiaging, anti-inflammatory and life-prolonging effects have been reported.[7]

Recently, 3-phenylcoumarins that present also the *trans*-resveratrol core in their structure, (Figure 1) have showed an antioxidative, anticancer, and enzymatic inhibition properties. Some phenylcoumarins proved to be tyrosinase inhibitors.[8,9]

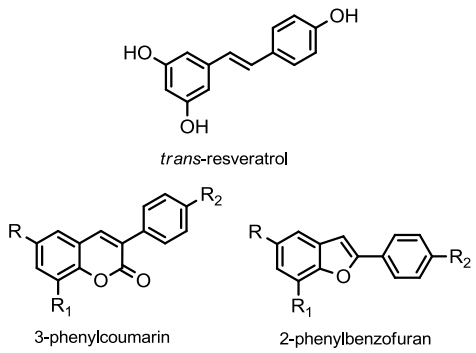


Figure 1

With the aim of finding out structural features in the tyrosinase inhibitory activity, in the present work, we describe the synthesis of 2-phenylbenzofurans suitably modified and investigate the effect on the diphenolase activity of mushroom tyrosinase.

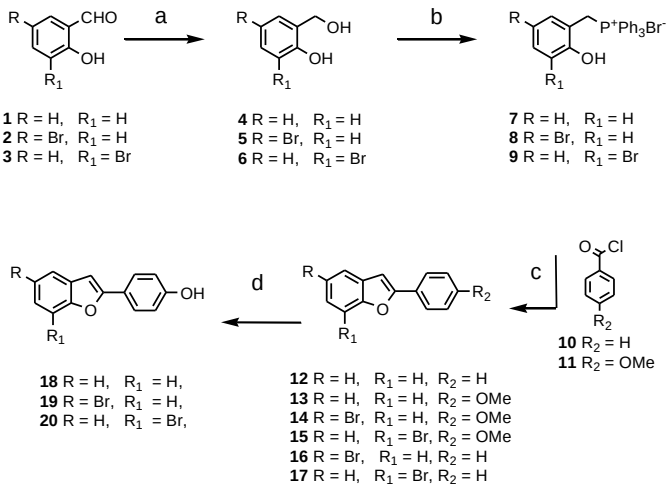
These compounds included in their structure the resveratrol nucleus, in *trans* configuration (Figure 1).

Results and discussion

The key step for the formation of the benzofuran moiety was achieved by an intramolecular Wittig reaction between triphenylphosphonium salts **7-9** and the appropriate benzoyl chlorides **10-11**.[10,11] The desired Wittig reagent, was readily prepared from the conveniently substituted 2-hydroxybenzyalcohols **4-6** and triphenylphosphine hydrobromide.[12-14]

The *p*-methoxy derivatives **13**, **14**, and **15**, were hydrolyzed with hydriodic acid, in the presence of acetic acid and acetic anhydride, at 110 °C, for 3 h.[11,14-17]

The compounds **4**, **5** and **7** are commercially available from Aldrich (Scheme 1 and Table 1).



Scheme 1: *Reagents and conditions*: (a) $NaBH_4$, EtOH, 0 °C to r. t., 2 h; (b) PPh_3 HBr, CH_3CN, 82 °C, 2 h; (c) toluene, NEt_3, 110 °C, 2 h; (d) HI, AcOH, Ac_2O, 0°C to reflux, 3h.

Compounds	Yield	Mp
12	75 %	118-120 °C
13	69 %	148-149 °C
14	50 %	166-167 ° C
15	51 %	185-187 °C
16	50 %	159-161°C
17	35 %	162-164 °C
18	33 %	197-199 °C
19	65 %	199-202 °C
20	70 %	131-133 °C

Table 1: Yields and Mp obtained for compound **12-20**

The tyrosinase inhibitory activity of compounds **12-20** was evaluated *in vitro* by the measurement of the enzymatic activity of mushroom tyrosinase enzyme extracted from the mushroom specie *A. bisporus*. The IC_{50} values for inhibitory effects of the new compounds were calculated (Table 1). In this table is included the kojic acid as a positive control.

Compounds Tested	***IC_{50}* (mM) (L-DOPA 0.5 mM)**
12	0.582 ± 0.023
13	1.027 ± 0.12
14	1.640 ± 0.25
15	0.370 ± 0.065
16	> 5.0
17	0.248 ± 0.078
18	0.472 ± 0.068
19	0.496 ± 0.014
Kojic acid	9.5×10^{-3}

Table 1: Inhibitory effects of compounds **12-19** and kojic acid on mushroom tyrosinase activity.

The 2-phenylbenzofuran **12** shows moderate inhibitory activity. The introduction of the methoxy group in *p*-position led to an increase in IC_{50} value (compound **13**). The compound **14** having a bromo atom in five position compared to the compound **13**, shows a decrease of the inhibitory activity. When the bromo substituent is in seven position, the inhibitory activity improves considerably (compound **15**). Curiously, the compounds **16** and **17** that present only one bromo atom respectively in five and seven position show anomalous comportment. The compound **17** is the best of the tested series (IC_{50} = 0.248 mM), on the contrary, its isomer **16** presents the worst result (IC_{50} > 5.0 mM).

Benzofurans hydroxilated **18**-**20** show an atypical behavior. The compound **20** presents a surprising functional activity compared to its isomer **19**. The increase of the tyrosinase activity on L-DOPA and the lag time phenomenon, when tyrosinase acts on the monophenol, suggest that compound **20** could be an activator.

General experimental procedure

Starting materials and reagents were obtained from commercial suppliers and were used without purification. Melting points (mp) are uncorrected and were determined with a Reichert Kofler thermopan or in capillary tubes in a Buchi 510 apparatus. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded with a Varian Inova 500 spectrometer using DMSO-δ6 or $CDCl_3$ as solvent. Mass spectrometry was carried out with a Saturn 2000 ion-trap coupled with a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA) operating under EI conditions (electron energy 70 eV).

Chemistry

*General procedure for the preparation of 2-hydroxy-benzylalcohol **6***: sodium borohydride (6.60 mmol) was added to a stirring solution of 2-hydroxybenzaldehyde (6.60 mmol) in ethanol (20 mL) in an ice bath. The reaction mixture was stirred at room temperature for 1 h. After the solvent was removed, 1 N aqueous HCl solution (40 mL) was added to the residue and extracted with diethyl ether. The solvent was evaporated under vacuum to give the desired compound without further purification.

*General methodology for the preparation of 2-hydroxy-benzyltriphenylphosphonium bromide **8*** and ***9***: a mixture of 2-hydroxybenzyl alcohol (2.46 mmol) and triphenylphosphine hydrobromide (2.46 mmol) in acetonitrile (30 mL) was stirred under reflux for 2 h. The solid formed was filtered and washed with acetonitrile to give the desired compound without further purification.

*General methodology for the preparation of 2-phenylbenzofurans**12-17***: a mixture of 2-hydroxy-benzyltriphenylphosphonium bromide (1.10 mmol) and benzoyl chloride (1.11 mmol) in a mixed solvent (toluene 20 mL and triethylamine 0.5 mL) was stirred under reflux for 2 h. The precipitate was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel chromatography (hexane/ethyl acetate 9:1) to give the desired compound.

*General methodology for the preparation of hydroxylated 2-phenylbenzofurans **18-20***: a solution of the corresponding methoxy-2-phenylbenzofuran (0.50 mmol) in acetic acid (5.0 mL) and acetic anhydride (5.0 mL), at 0 °C, was prepared. Hydriodic acid 57% (10.0 mL) was added drop-wise. The mixture was stirred under reflux temperature for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by FC (hexane/ethyl acetate 8:2) to give the desired compound.

Biological assays

The tyrosinase inhibitory activity of compounds **12**-**20** was evaluated *in vitro* by the measurement of the enzymatic activity of mushroom tyrosinase enzyme extracted from the

mushroom specie *A. bisporus*. The inhibitory activity of each compound was determined measuring the IC_{50}.

Pre-incubation with the enzyme: 6.6 mM phosphoric acid buffer solution (pH 6.8, 1.8 mL), an aqueous solution of mushroom tyrosinase (1000 U/mL, Sigma Chemical Co., 0.1 mL) and DMSO (0.1 mL) with or without the sample. The mixture was incubated at 25°C for 10 min. Then, a 1.5 mM of L-3,4-dihydroxyphenylalanine (L-DOPA) solution was added and the reaction was monitored at 475 nm, for 5 min. The percent of tyrosinase activity inhibition was calculated as: inhibition (%) = (A-B)/A x 100, where A represents the difference in the absorbance of control sample between 0.5 and 1.0 min, and B represents the difference in absorbance of the test sample between 0.5 and 1.0 min. The IC_{50} value, a concentration giving 50% inhibition of tyrosinase activity, was determinate by interpolation of dose–response curves. The mushroom tyrosinase activity was determinate by spectrophotometric assays (Instrument Cary 50, Varian).

Conclusion

These preliminary results indicate that the type and the position of the substituent groups play an important role in determining the kind of activity. In fact, the presence of only a bromo atom (compounds **16** and **17**), or the addition of another substituent group (compounds **16** and **19**, **17** and **20**) lead to different or even opposite effects (activation or inhibition).

The diverse activities of these compounds that differ in only one group require further study.

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References

1. Á. Sánchez-Ferrer, J. N. Rodríguez-López, F. García-Cánovas, and F. García-Carmona, *Biochim. Biophys. Acta*, **1995**, *1247*, 1.
2. Y. Mishima, *Pigment Cell Res.*, **1994**, *7*, 376.
3. E. Beradesca, N. Cameli, G. Primavera and M. Carrera, *Dermatol. Surg.*, **2006**, *32*, 526.
4. T-S. Chang, *Int. J. Mol. Sci.*, **2009**, *10*, 2440.
5. S. H. Jeong, Y. B. Ryu, M. J Curtis-Long, H. W. Ryu, Y. S. Baek, J. E. Kang, W. S. Lee and K. H. Park, *J. Agric. Food Chem.*, **2009**, *57*, 1195.
6. Y. Jiang, B. Gao, W. Huang, Y. Liang, G. Huang and Y. Ma, *Synthetic Commun.*, **2009**, *39*, 197.
7. F. Orallo, *Curr. Med. Chem.*, **2008**, *15*, 1887.
8. A. Fais, M. Corda, B. Era, M. B. Fadda, M. J. Matos, E. Quezada, L. Santana, C. Picciau, G. Podda and G. Delogu, *Molecules*, **2009**, *14*, 2514.
9. M. J. Matos, L. Santana, E. Uriarte, G. Delogu, M. Corda, M. B. Fadda, B. Era and A. Fais, *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 3342.
10. A. Hercouet and M. Le Corre, *Tetrahedron Lett.*, **1979**, *23*, 2145.
11. L. J. Twyman, D. Allsop, *Tetrahedron Lett.*, **1999**, *40*, 9383.
12. C. Meier, C. Ducho, H. Jessen, D. Vukadinović-Tenter and J. Balzarini, *Eur. J. Org. Chem.*, **2006**, 197.
13. M. L. Belyanin, V. D. Filimonov, E. A. Krasnov, *Russian J. Appli. Chem.*, **2001**, *74*, 103.
14. M. Ono, M. P. Kung, C. Hou and H. F Kung, *Nucl. Med. Biol.*, **2002**, *29*, 633.
15. G. W. Kabalka, L. Wang and R. M. Pagni, *Tetrahedron*, **2001**, *57*, 8017.
16. M. Begala, G. Delogu, E. Maccioni, G. Podda, G. Tocco, E. Quezada, E. Uriarte, M. A. Fedrigo, D. Favretto and P. Traldi, *Rapid Commun. Mass Spectrom.*, **2001**, *15*, 1000.
17. H. D. Choi, P. J. Seo, B. W. Son and B. W. Kang, *Arch. Pharm. Res.*, **2004**, *27*, 19.