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Journal:	*Journal of Medicinal Chemistry*
Manuscript ID:	jm-2015-006699.R2
Manuscript Type:	Perspective
Date Submitted by the Author:	13-Jul-2015
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Hydroxytyrosol-derived Compounds: a Basis for the Creation of New Pharmacological Agents for Cancer Prevention and Therapy

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ABSTRACT: Hydroxytyrosol [2-(3,4-dihydroxyphenyl)ethanol, HTyr] is a phenolic compound found in olive leaves and fruits and extra-virgin olive oil, which has well-known strong antioxidant and radical-scavenging properties. Recently, it has received particular attention for its antiproliferative and apoptotic activities and its anti-inflammatory properties. During the last few years, more efforts have been focused on synthesizing HTyr-derived compounds with enhanced biological activities for their potential use in different chronic degenerative diseases. In this paper, we report a dissertation on the current knowledge of selected synthetic HTyr derivatives and analogs and their potential use in cancer prevention and therapy, which are related to their antioxidant, antiproliferative/apoptotic and anti-inflammatory properties. Based on the perspective of using HTyr-derived compounds as anti-cancer agents, we have taken into account only studies that were performed in experimental cell-based models.

Keywords: Hydroxytyrosol derivatives; hydroxytyrosol analogs; antioxidant activity; antiproliferative activity; apoptotic activity; anti-inflammatory activity

INTRODUCTION

Extra-virgin olive oil is the major fat component of the Mediterranean diet. Epidemiological studies demonstrated that its daily consumption is associated with a reduction of risk factors for coronary heart diseases, the prevention of some types of cancer and the modulation of immune and inflammatory responses.[1,2] Extra-virgin olive oil is characterized by a high nutritional value due to the presence of major components, mainly triacylglycerols, which constitute more than 98% of the total oil weight, and more than 200 minor secondary metabolites, including phytosterols, lipophilic and hydrophilic phenols, constituting approximately 2% of the total oil weight.[3-7] While lipophilic phenols such as tocopherols can also be found in other vegetable oils and fats, some hydrophilic phenols are present exclusively and abundantly in extra-virgin olive oil, conferring its high-value sensory and nutritional properties.[8,9]

Among the hydrophilic phenols, hydroxytyrosol [2-(3,4-dihydroxyphenyl)ethanol, HTyr] is one of the most representative compounds in extra-virgin olive oil and is present mainly as secoiridoid derivatives together with minor amounts of the free form (Figure 1). During olive ripening and processing, endogenous β-glucosidases release HTyr from the secoiridoid derivatives by hydrolytic mechanisms, conferring to the extra-virgin olive oil its typical rich and complex flavor.[10,11] Considering its strong hydrophilic character, HTyr is present also in by-products of the olive oil industry, in particular in liquid wastes named olive mill waste waters (OMWW).

They are annually produced in large volumes in a few months and represent a serious environmental problem in the Mediterranean area for their organic matter content and toxicity.[12,13]

Several *in vitro* and *in vivo* studies that were performed using HTyr in the free form reported a wide range of biological activities, including antimicrobial, hypotensive, hypoglycemic, anti-platelet aggregation, cardioprotective, antioxidant, antiproliferative and anti-inflammatory activities.[14-16]

Based on these attractive functions, an increasing number of research groups have focused their efforts to both synthesize HTyr [17-22] and recover it from wastes.[23-26] However, HTyr is unstable unless it is preserved and dried in the absence of air, and it has limited solubility in lipid media. Therefore, the search for novel lipophilic derivatives and analogs with greater stability and enhanced biological properties is of interest in both the pharmaceutical and food industries.

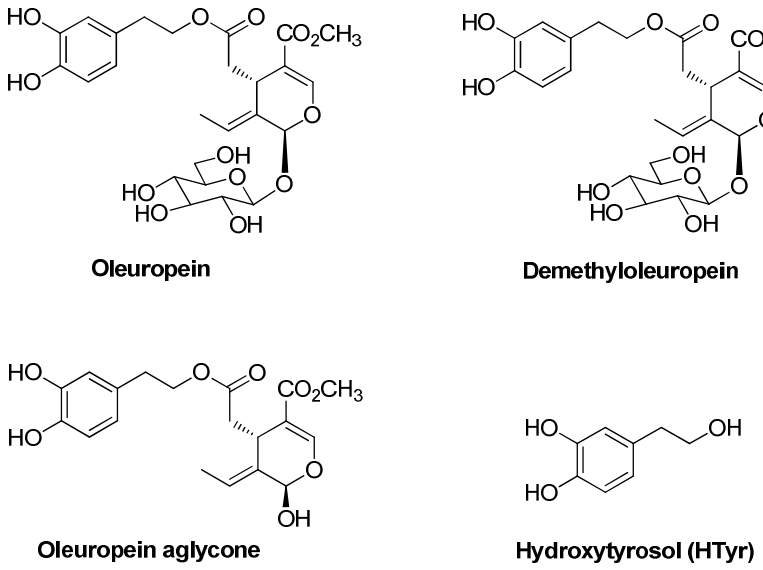


Figure 1. Oleuropein derivatives and HTyr present in extra-virgin olive oil.

In the last few years, a large number of HTyr-derived compounds have been synthesized, and several of them have been described in recent reviews.[27-29] In particular, many experiments have been performed to synthesize HTyr derivatives and analogs with a better hydrophilic/lipophilic balance (HLB) to increase their availability and to join HTyr to other biologically active compounds to enhance its biological functions. The biological activities of HTyr-derived compounds have been evaluated in cell-free and cell-based models, and, as reported by Manna et al., the results obtained from the use of the two different experimental models were not always comparable. This suggests that the compounds tested in cellular models may be metabolized by the cell, leading to biological activities due to their metabolic product(s).[30]

Therefore, based on the perspective of using HTyr-derived compounds for cancer prevention and therapy, we report here a dissertation on the current knowledge of selected HTyr derivatives and analogs that exhibit antioxidant, antiproliferative, apoptotic and anti-inflammatory activities in experimental cell-based biological models. Therefore, we exclude all studies performed in cell-free systems. These compounds include HTyr esters and analogs, alkyl ethers, thioderivatives and isochromans, which are synthesized by different methods to take advantage of the reactivity of the alcohol functional group without derivatizing the *ortho*-dihydroxy substitution of HTyr that is responsible for its antioxidant properties.[14]

DISCUSSION

1. Chemistry: synthesis of HTyr-derived compounds

1.1 Synthesis of HTyr esters. The most studied HTyr-derived compounds are lipophilic esters showing saturated and unsaturated aliphatic chains of different length, generally from 2 to 18 carbons. Some synthetic biologically relevant derivatives are depicted in Figure 2. The simplest ester is HTyr acetate **1**, which has been recently found in extra-virgin olive oil [31] in amounts that depend on the olive varieties used, olive ripeness, climate, location, type of crushing machine and oil extraction procedures.[32] It is a biophenol of interest because of its lipophilic property and higher antioxidant activity compared with HTyr.[33]

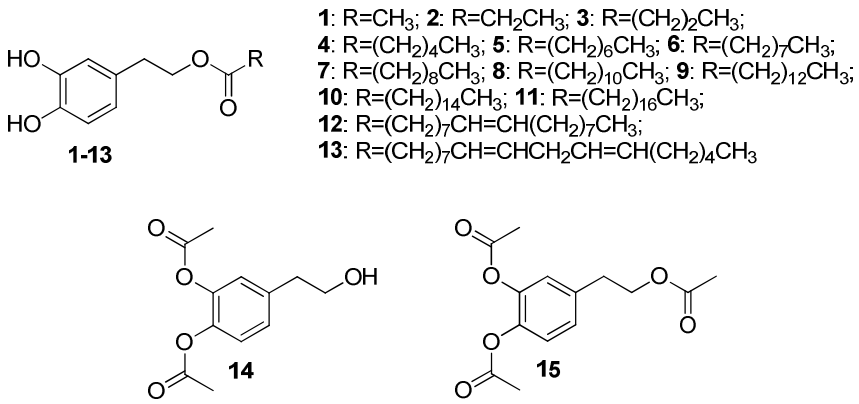
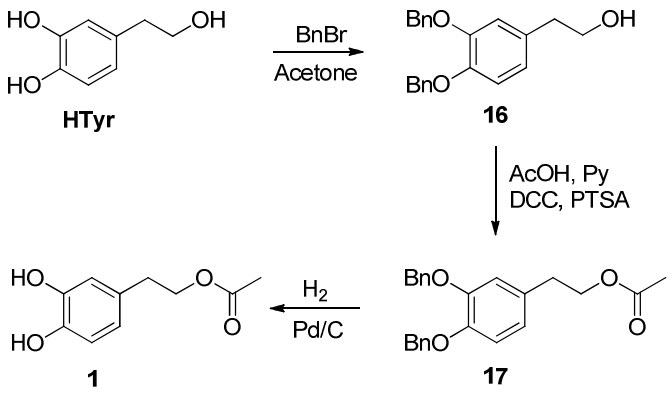


Figure 2. Lipophilic HTyr esters.

Synthetic samples of esters **1-13** were obtained from HTyr as starting material by different procedures. The direct use of HTyr, although obvious, is difficult for several reasons: high price, instability, and competition of the phenolic hydroxyl groups in the esterification reaction. However, when HTyr was treated with acetic anhydride, a

mixture of the diacetyl and triacetyl derivatives **14** and **15** was obtained.[34] Based on these considerations, several groups focused their attention on the search for procedures that are able to selectively insert the alcohol functional group of HTyr under mild conditions. To achieve this aim, many multi-step procedures were optimized, including a preliminary protection of the phenolic hydroxyl groups of HTyr before the acylation reaction and a final deprotection.

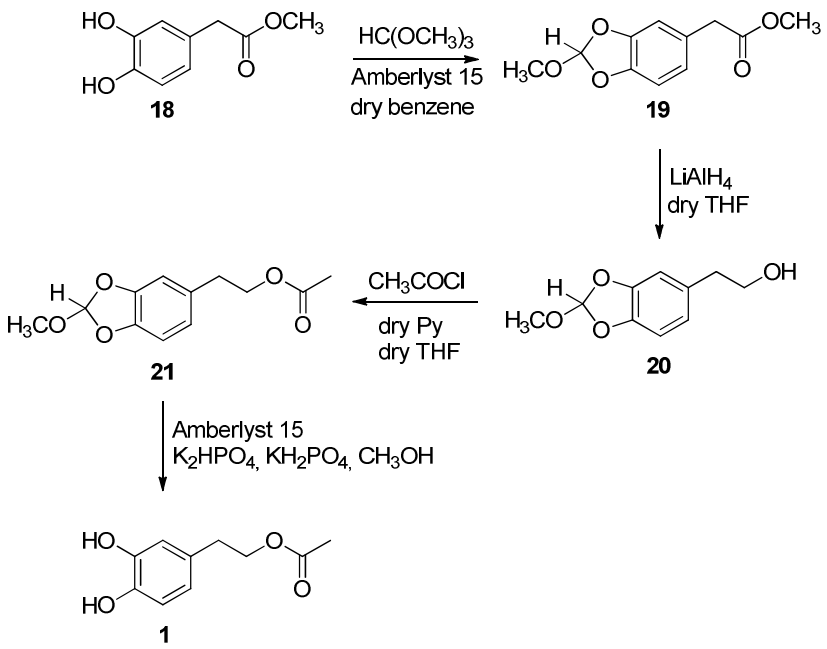
According to Scheme 1, Gordon's group protected the two phenolic groups of HTyr with benzyl bromide under basic conditions to afford the corresponding derivative **16**. When acetic acid, dicyclohexylcarbodiimide (DCC) and *p*-toluenesulfonic acid (PTSA) were added to a solution of **16** in pyridine, the acetate **17** was obtained. Finally, removal of the benzyloxy groups by catalytic hydrogenation with palladium-on-carbon and 1,4-cyclohexadiene produced HTyr acetate **1** at a 24% overall yield.[33]



Scheme 1. Synthesis of HTyr acetate **1** via benzylation of the catecholic moiety.[33]

An alternative synthetic strategy was proposed from Gambacorta et al. using (3,4-dihydroxyphenyl)acetic acid methyl ester **18** as the starting material (Scheme 2). The

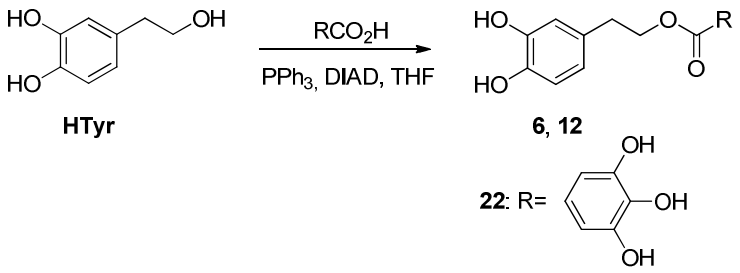
protection of the catecholic group was performed with trimethylorthoformate and Amberlyst 15. Then, the corresponding orthoformate derivative **19** was reduced with $LiAlH_4$ to give compound **20**; the subsequent acetylation with acetyl chloride in pyridine and deprotection of the orthoformate moiety with Amberlyst 15 produced HTyr acetate **1**. The overall yield of compound **1** was excellent (87%), but the synthesis required four steps, the use of dry solvents and a careful control of the reductive step to avoid the formation of by-products.[35]



Scheme 2. Synthesis of HTyr acetate **1** via methyl orthoformate-protection of the catecholic moiety.[35]

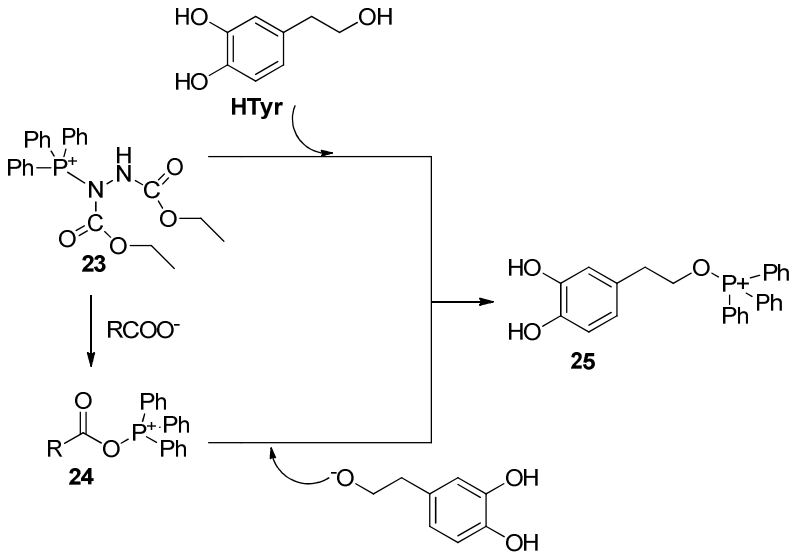
In 2002 and 2005, Appendino et al. proposed two different approaches for the synthesis of HTyr esters **6** and **12**: the Mitsunobu reaction [36] and the cerium(III) chloride ($CeCl_3$) acylation reaction.[37] The Mitsunobu reaction was performed by

treating HTyr with nonanoic and oleic acids in the presence of triphenylphosphine (PPh_3) and diethyl azodicarboxylate (DIAD) in THF (Scheme 3). The corresponding HTyr esters **6** and **12** were isolated at 41 and 34% yields, respectively. Under similar conditions, HTyr was also condensed with gallic acid at a 48% yield to produce the ester **22**.[36]



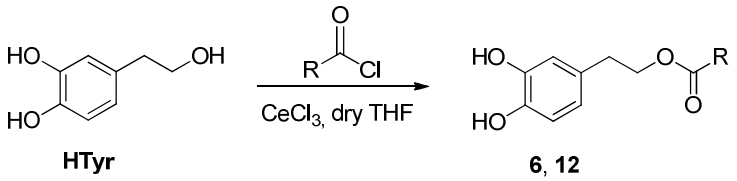
Scheme 3. Synthesis of lipophilic HTyr esters **6**, **12** and **22** using the Mitsunobu reaction conditions.[36]

Even if the mechanism of the Mitsunobu reaction is still controversial, the chemoselectivity of the esterification was explained by the formation of the alkoxyphosphonium intermediate **25** from the reaction of HTyr with compound **23** instead of the alcoholate displacement of the acyloxyphosphonium ion **24** (Scheme 4), demonstrating the role of the phenolic groups as "inert spectators" in this reaction, which proceeded by a S_N2 mechanism.[36]

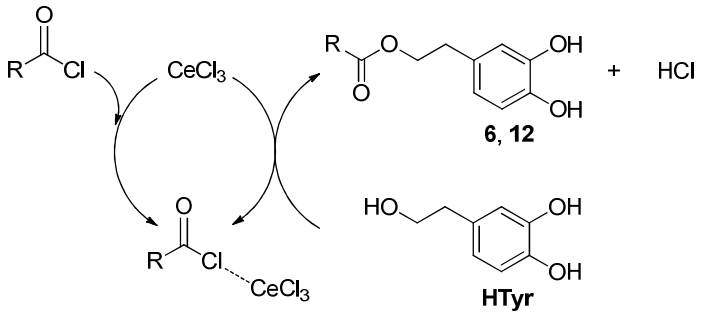


Scheme 4. Mechanism of the Mitsunobu reaction with HTyr.

As an alternative approach, Appendino et al. described the $CeCl_3$-promoted chemoselective acylation of HTyr and other selected phenols present into dietary vegetables.[37] This reaction with HTyr was performed in dry THF with nonanoyl and oleyl chloride activated by catalytic $CeCl_3$ and afforded the corresponding esters **6** and **12** at 53 and 60% yields, respectively (Scheme 5), indicating a slight improvement in terms of the yields compared with the Mitsunobu esterification. The proposed mechanism involves the formation of an electrophilic Lewis acid adduct between acyl chlorides and $CeCl_3$, which is quenched by the more nucleophilic alkyl hydroxyl group of HTyr to produce the expected esters and regenerate cerium(III) chloride (Scheme 6).



Scheme 5. $CeCl_3$-promoted chemoselective esterification of HTyr.[37]



Scheme 6. Mechanism of $CeCl_3$-promoted chemoselective esterification of HTyr.

Finally, the HTyr esters were obtained by direct catalyzed-esterification/transesterification reactions from HTyr. Compounds **1**, **3**, **8**, **10**, **11**, **12** and **13** were isolated in satisfactory yields (62-86%) by heating a solution of HTyr with the corresponding ethyl or methyl ester containing a catalytic amount of PTSA.[38,39]

In the last few years, enzymes and, in particular, lipases have been widely employed in non-aqueous solvents for the lipophilization of phenolic compounds as an alternative to the use of chemical catalysts.[40, 41] They give rise to environmentally friendly processes with lower energy consumption and fewer waste products, and offer several advantages: mild reaction conditions, selectivity, specificity, minimization of side reactions and by-products, and few purification steps.

Grasso and colleagues performed a complete study to optimize the synthesis of HTyr acetate **1** using vinyl acetate as the reagent and *t*-butyl methyl ether as the solvent with different lipases from *Aspergillus niger, Candida antarctica*, *Candida cylindracea, Chromobacterium viscosum, Mucor miehei, Mucor javanicus, Pseudomonas cepacia, Pseudomonas fluorescens, Rhizopus arrhizus, Rhizopus niveus,* porcine pancreas and wheat germ. The best results for HTyr acetate **1** in terms of reaction time, chemoselectivity and yield were obtained using *Candida antarctica* lipase (CAL-B).[42] Then, this enzyme was selected for the acylation of HTyr with other vinyl esters (propionate, butyrate, decanoate and stearate). The corresponding HTyr esters **2**, **3**, **7** and **11** were isolated with very good yields (92-96%) in 35-180 min reactions.

The sustainability of the enzymatic process increases in reactions with immobilized enzymes because these enzymes can be used for several runs with economic and environmental benefits. Buisman et al. first investigated the esterification of HTyr with octanoic acid in hexane in the presence of immobilized lipases from *Candida antartica* (CAL-B). These authors reported that the success of the reaction was highly dependent on the solvent, varying from a 20% yield using chloroform, dichloromethane and THF to an 85% yield using diethyl ether.[43,44]

Several years ago, Torres de Pinedo et al. reported the preparation of HTyr saturated fatty acid esters and mono- and poly-unsaturated fatty acid esters using immobilized *C. antarctica* (Novozym® 435) under vacuum in a solventless reaction. The final

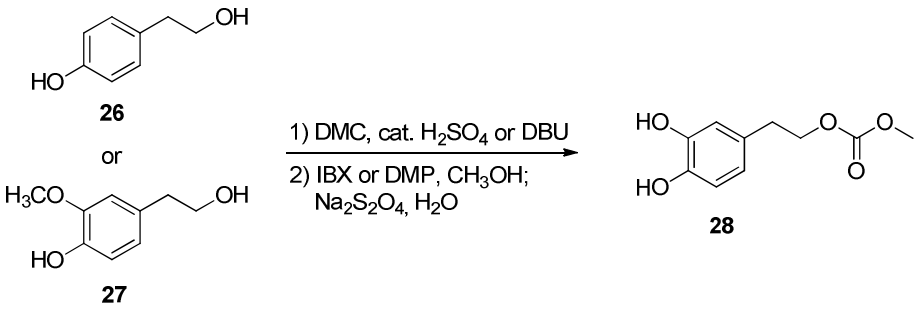
yields were 59-98% for the saturated fatty acid esters and 32-97% for the mono- and poly-unsaturated fatty acid esters.[45]

As an alternative to the use of expensive HTyr, lipophilic HTyr esters **1**, **4**, **10**, **12** and **13** were prepared from cheaper and commercially available starting materials such as tyrosol **26** and homovanillyl alcohol **27**. The synthesis involved a two-step high-yield procedure (Scheme 7).[19] The first step was the chemoselective protection of the alcohol functional group of tyrosol **26** or homovanillyl alcohol-**27** with a small excess of acyl chlorides (acetyl, hexanoyl palmitoyl, oleyl and linoleoyl chloride) in dimethyl carbonate (DMC), an ecofriendly solvent. The reaction was performed without a catalyst under no-dry conditions. The corresponding esters were isolated with good to excellent yields (60-98%). The observed chemoselectivity was explained by both the probable *in situ* generation of a trace amount of hydrochloric acid derived from the hydrolysis of the acyl chloride and the greater nucleophilicity of the aliphatic hydroxyl group compared with the phenolic group. Afterward, the esters were oxidized with 2-iodobenzoic acid (1-hydroxy-1-oxo-1*H*-1λ^5-benz[*d*][1,2]iodoxol-3-one, IBX) or with the corresponding 1,1,1-triacetoxy derivative named Dess-Martin periodinane (DMP). These reagents are well known in the literature for their ability to efficiently perform the oxidative demethylation of phenolic methyl aryl ethers and the oxidation of phenols, producing *ortho*-quinones with a selectivity similar to that of a polyphenol oxidase.[46,47] The subsequent *in situ* reduction with sodium dithionite ($Na_2S_2O_4$) produced catecholic compounds.[48] Therefore, by combining the use of IBX or DMP and $Na_2S_2O_4$, tyrosol **26** and

homovanillyl alcohol **27** were converted into the corresponding HTyr derivatives **1**, **4**, **10**, **12** and **13** under mild conditions with satisfactory yields (62-89%). Generally, IBX and DMP showed a comparable efficiency; the oxidation of tyrosol esters proceeded with higher yields compared with those of the homovanillyl derivatives. Both procedures are patented.[49, 50]

Scheme 7. Synthesis of lipophilic HTyr esters **1**, **4**, **10**, **12** and **13** by IBX oxidation/$Na_2S_2O_4$ reduction.[19,49,50]

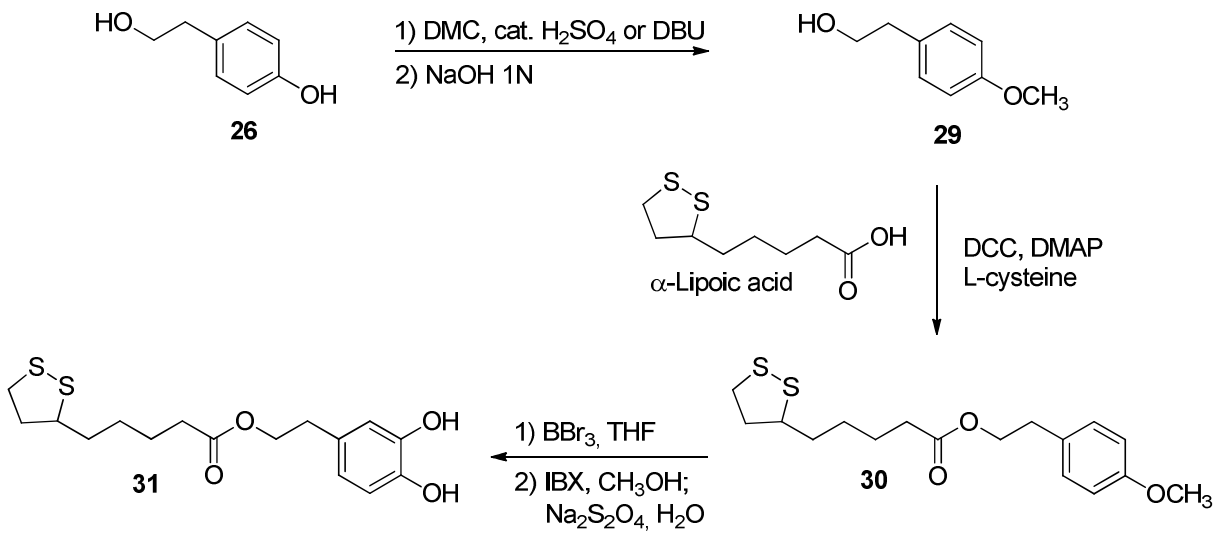
Similarly, a novel HTyr lipophilic derivative, HTyr methyl carbonate **28**, was prepared at a good yield (Scheme 8).[19] Compounds **26** or **27** were selectively derivatized on the alcohol functional group using dimethyl carbonate (DMC) in combination with catalytic sulfuric acid or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) after 7 h at the reflux temperature.[51] The following oxidation/oxidative demethylation and *in situ* reduction with the IBX or DMP/$Na_2S_2O_4$ system afforded the catecholic methyl carbonate derivative **28** at an 85% yield.[19]



Scheme 8. Synthesis of HTyr methyl carbonate **28** by IBX oxidation/$Na_2S_2O_4$ reduction.

In addition to lipophilic derivatives, in the last few years, novel HTyr-derived compounds were synthesized by joining HTyr to other biologically active molecules to enhance its biological properties. In this context, a novel derivative was synthesized in combination with α-lipoic acid, a non-phenolic antioxidant present in food, mainly wheat, potatoes and red meat, that exhibits many beneficial effects on human health.[52] Two procedures were described to isolate the final ester (overall yields: 62 and 40%). The most efficient procedure consists of five steps and is described in Scheme 9. First, both the alcohol and phenol groups of tyrosol **26** were protected with dimethyl carbonate, which was used as the solvent, methylating and carboxymethylating reagent, in the presence of DBU. The corresponding tyrosol carbonate methyl ether was isolated with quantitative yield after 24 h at the reflux temperature. After the selective deprotection of the carbonate moiety under basic conditions, the methyl derivate **29** was esterified with α-lipoic acid under Steglich conditions in the presence of L-cysteine to avoid the polymerization reaction of α-lipoic acid. The isolated ester was demethylated with boron tribromide in dry THF to

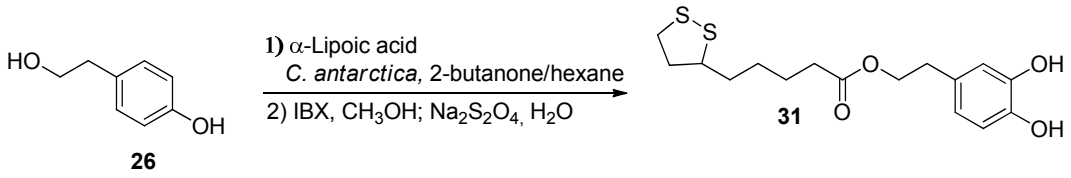
produce the phenolic derivative; the final hydroxylation reaction with the IBX/$Na_2S_2O_4$ system afforded the catecholic ester **31**.



Scheme 9. Esterification of HTyr with α-lipoic acid.[52]

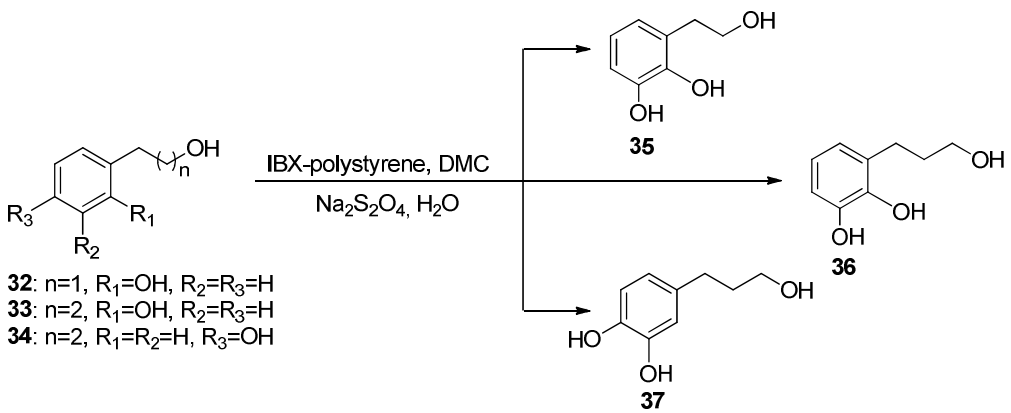
After one year, Kaki et al. synthesized several esters of α-lipoic acid with natural phenolic compounds, including HTyr, by a chemo-enzymatic procedure (Scheme 10).[53] The esterification consists of only two steps. The use of enzymes as catalyst in the reaction between tyrosol **26** and α-lipoic acid were responsible for the advantageous reduction of steps. The esterification was performed with immobilized lipase B from *Candida Antarctica* in 2-butanone/hexane as the reaction medium, which were both able to completely dissolve the substrates and maintain the activity of the lipase. The reaction proceeded smoothly at room temperature, affording the

expected ester exclusively. The final step resulted in the hydroxylation reaction performed by the IBX/$Na_2S_2O_4$ system.



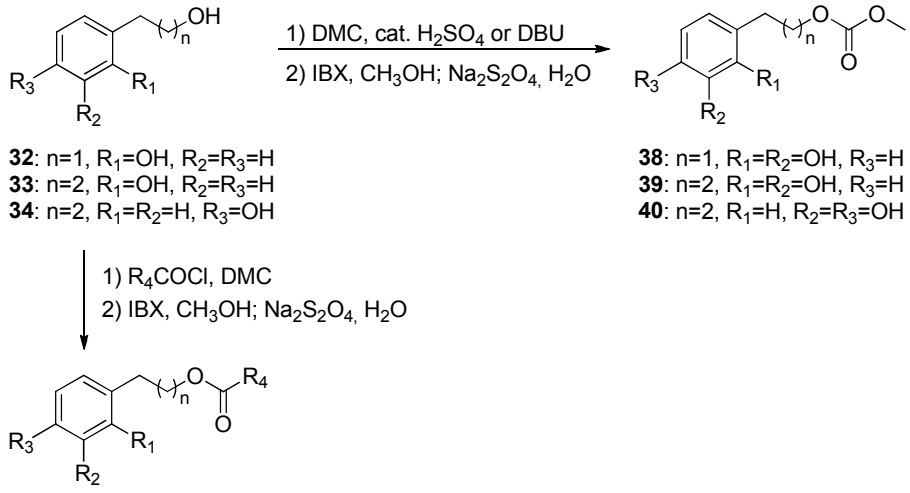
Scheme 10. Alternative synthesis of ester **31**.[53]

1.2 Synthesis of HTyr analogs. Three catecholic compounds that are structural analogs of HTyr, with a different pattern of substitution on the aromatic ring and/or a different length of the alcoholic chain, were synthesized in 65-80% yields. These compounds, 2-(2,3-dihydroxyphenyl)ethanol **35**, 3-(2,3-dihydroxyphenyl)-1-propanol **36** and 3-(3,4-dihydroxyphenyl)-1-propanol **37**, were obtained by direct hydroxylation reactions of the alcohol precursors **32**, **33** and **34** with IBX-polystyrene, and subsequent reduction with an aqueous solution containing $Na_2S_2O_4$ (Scheme 11).[54]



Scheme 11. Novel HTyr analogs **35**-**37**.[54]

The corresponding methyl carbonate derivatives **38-40** and lipophilic esters **41-73** were synthesized according to a two-step procedure: 1) derivatization of the alcohol chain with DMC/DBU and acyl chlorides (acetyl, butyryl, hexanoyl, octanoyl, decanoyl, dodeanoyl, lauryl, myristoyl, palmitoyl, oleyl and linoleoyl chloride) of the phenolic precursors **32-34**; and 2) oxidation/reduction of the methyl carbonate and ester derivatives with the IBX/$Na_2S_2O_4$ system (Scheme 12).[54] Interestingly, acylation of catechol **37** was performed with ethyl palmitate, stearate and lipase in acetonitrile, affording the corresponding esters in quantitative yields.[45]



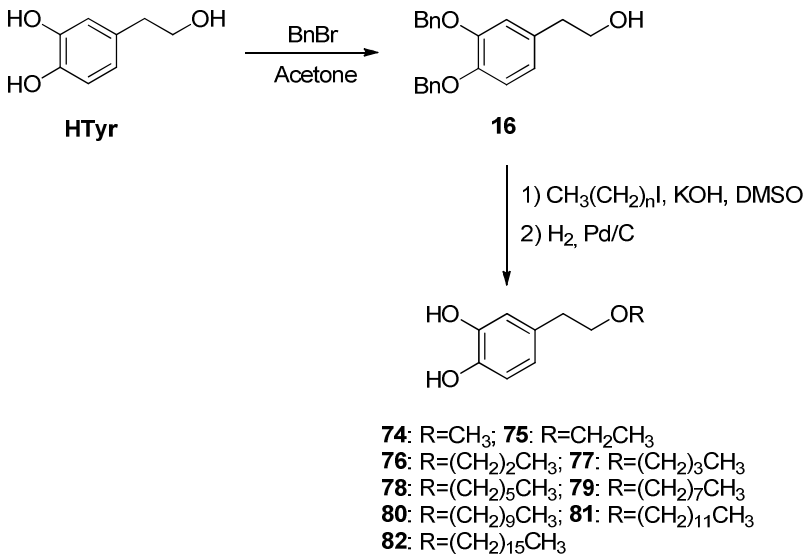
32: n=1, R_1=OH, R_2=R_3=H
33: n=2, R_1=OH, R_2=R_3=H
34: n=2, R_1=R_2=H, R_3=OH

38: n=1, R_1=R_2=OH, R_3=H
39: n=2, R_1=R_2=OH, R_3=H
40: n=2, R_1=H, R_2=R_3=OH

41: n=1, R_1=R_2=OH, R_3=H, R_4=CH_3; **42**: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_2CH_3$;
43: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_4CH_3$; **44**: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_6CH_3$;
45: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_8CH_3$; **46**: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_{10}CH_3$;
47: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_{12}CH_3$; **48**: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_{14}CH_3$;
49: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_{16}CH_3$; **50**: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_7CH{=}CH(CH_2)_7CH_3$;
51: n=1, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_6(CH_2CH{=}CH)_2(CH_2)_6CH_3$;
52: n=2, R_1=R_2=OH, R_3=H, R_4=CH_3; **53**: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_2CH_3$;
54: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_4CH_3$; **55**: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_6CH_3$;
56: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_8CH_3$; **57**: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_{10}CH_3$;
58: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_{12}CH_3$; **59**: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_{14}CH_3$;
60: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_{16}CH_3$; **61**: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_7CH{=}CH(CH_2)_7CH_3$;
62: n=2, R_1=R_2=OH, R_3=H, R_4=$(CH_2)_6(CH_2CH{=}CH)_2(CH_2)_6CH_3$
63: n=2, R_1=H, R_2=R_3=OH, R_4=CH_3; **64**: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_2CH_3$;
65: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_4CH_3$; **66**: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_6CH_3$;
67: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_8CH_3$ (96%); **68**: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_{10}CH_3$;
69: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_{12}CH_3$; **70**: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_{14}CH_3$;
71: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_{16}CH_3$; **72**: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_7CH{=}CH(CH_2)_7CH_3$;
73: n=2, R_1=H, R_2=R_3=OH, R_4=$(CH_2)_6(CH_2CH{=}CH)_2(CH_2)_6CH_3$

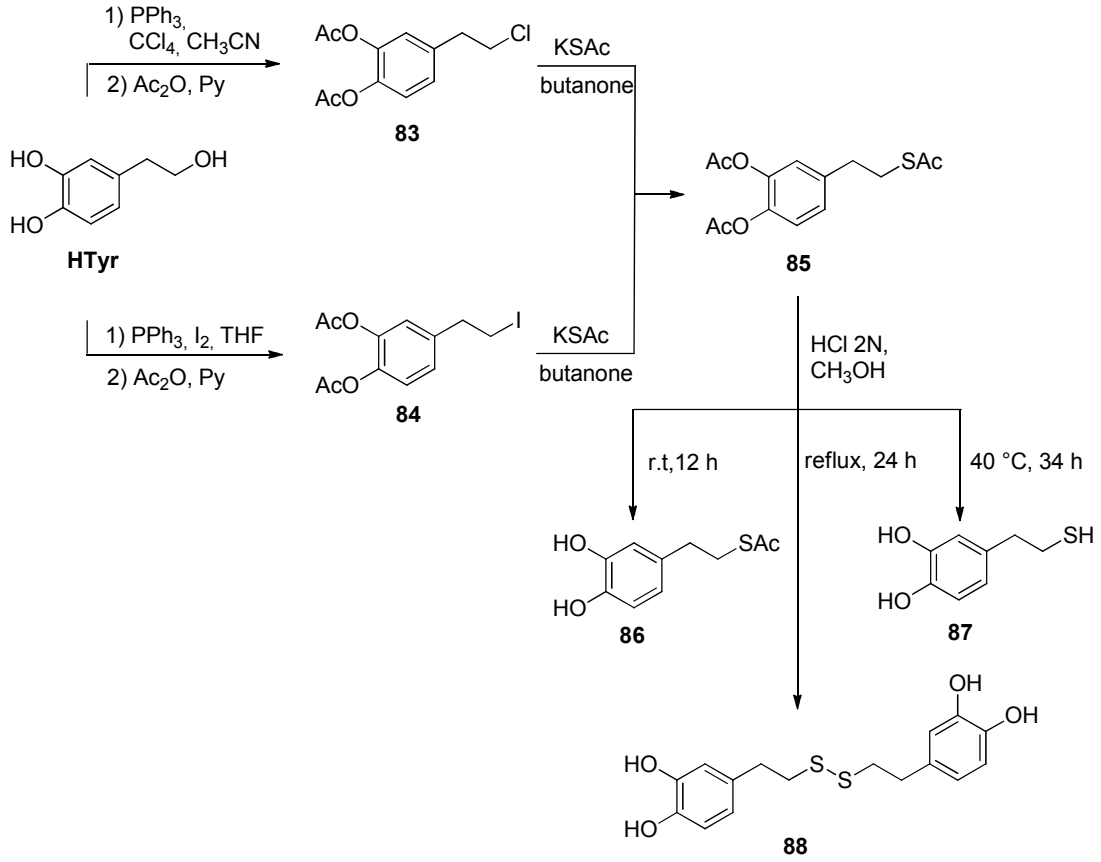
Scheme 12. Synthesis of lipophilic HTyr analogs **38-73**.[54]

1.3 Synthesis of HTyr alkyl ethers. Madrona et al. synthesized a new class of lipophilic HTyr derivatives, the HTyr alkyl ethers **74-82**, by a three-step procedure using HTyr recovered from OMWW as the starting material.[55] As depicted in Scheme 13, HTyr was preliminarily protected on the phenolic hydroxyl groups with benzyl bromide/potassium carbonate in acetone to afford the corresponding dibenzyl derivative **16**. Then, the alkylation of the free alcohol group with alkyl iodides of different length produced the corresponding derivatives in good to excellent yields. Finally, the hydrogenolytic cleavage of the protecting benzyl group with palladium over charcoal afforded alkyl ethers **74-82** at 82-98% yields.



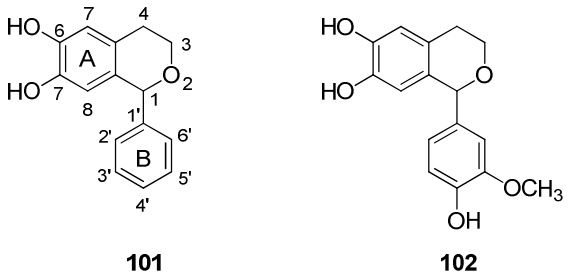
Scheme 13. Synthesis of lipophilic HTyr alkyl ethers **74-82**.[55]

1.4 Synthesis of HTyr thioderivatives. Novel HTyr derivatives **86-88** containing thioacetate, thiol and disulfide groups were synthesized by Sepporta et al.[56] The synthesis was performed according to the procedure depicted in Scheme 14. First, HTyr was converted into the corresponding 3,4-dihydroxyphenethyl chloro and iododerivatives by using PPh_3 and CCl_4 in CH_3CN and a mixture of PPh_3, I_2 and imidazole. The subsequent acetylation of the catechol moiety produced the corresponding di-O-acetyl derivatives **83** and **84** in satisfactory yields (79 and 75%, respectively). In the presence of potassium thioacetate in refluxing butanone, they were both converted into the thioacetate derivative **85** with 48 and 76% yields, respectively. The different results depend on the nature of the leaving group in the nucleophilic substitution reaction, as iodide is a better leaving group than chloride. Finally, the acid hydrolysis in HCl 2N at room temperature or 40 °C afforded thioderivatives **86** and **87** at 86 and 98% yields, whereas under reflux temperature, it produced disulfide **88** (42% yield).



Scheme 14. Synthesis of HTyr thioderivatives **86-88**.[56]

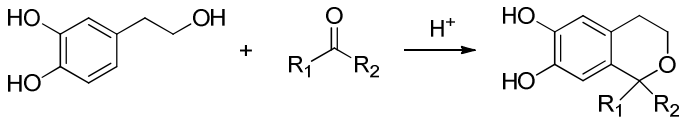
1.5 Synthesis of HTyr-derived isochromans. The isochromanic (3,4-dihydro-1*H*-benzo[*c*]pyran) nucleus is a ubiquitous structural motif present in many bioactive natural products, drugs and agrochemicals.[57] In 2001, Bianco et al. identified 1-phenyl-6,7-dihydroxy-isocroman **101** and 1-(4'-hydroxy-3'-methoxy)phenyl-6,7-dihydroxyisochroman **102** (Figure 3) in extra-virgin olive oil methanolic extracts, in amounts depending on the olive varieties (20-1400 ng/Kg).[58]



101 **102**

Figure 3. Isochromans present in extra-virgin olive oil.

Their presence was confirmed by comparison of the High Performance Liquid Chromatography (HLPC-MS/MS) spectra of the methanolic extracts with those of synthetic hydroxy isochromans obtained by the oxa-Pictet-Spengler reaction between HTyr and the corresponding aldehydes, benzaldehyde **89** and 4-hydroxy-3-methoxybenzaldehyde **90**, respectively (Scheme 15).[59] The synthesis was performed under mild conditions in the presence of PTSA or oleic acid as the catalyst; 1-phenyl-6,7-dihydroxy-isocroman **101** and 1-(4'-hydroxy-3'-methoxy)phenyl-6,7-dihydroxyisochroman **102** were isolated in satisfactory yields (60% and 76%).

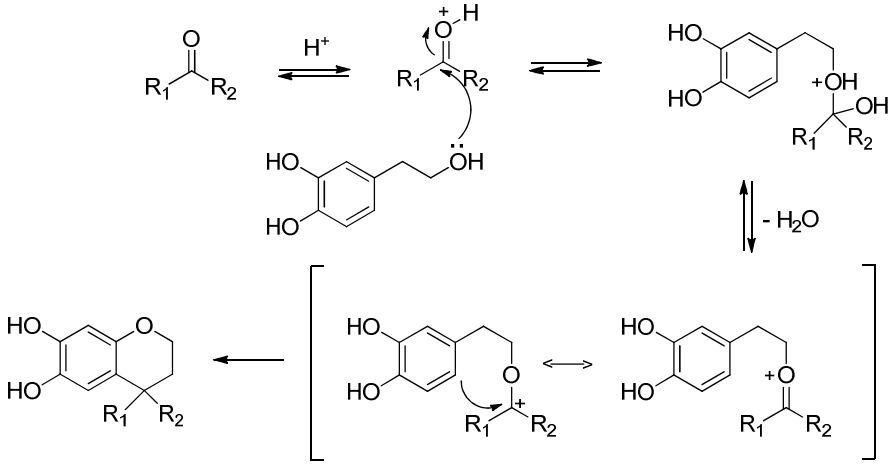


HTyr **89-100** **101-112**

89, **101**: R_1=H, R_2=phenyl
90, **102**: R_1=H, R_2=4-OH, 3-OCH_3-phenyl
91, **103**: R_1=R_2=CH_3
92, **104**: R_1=CH_3, R_2=$CH_2CH_2CH_3$
93, **105**: R_1=CH_3, R_2=$CH(CH_3)_3$
94, **106**: R_1=H, R_2=CH_2CH_3
95, **107**: R_1=H, R_2=$(CH_2)_3CH_3$
96, **108**: R_1=H, R_2=$(CH_2)_7CH_3$
97, **109**: R_1=H, R_2=*o*-OH-phenyl
98, **110**: R_1=H, R_2=*m*-OH-phenyl
99, **111**: R_1=H, R_2=*p*-OH-phenyl
100, **112**: R_1=H, R_2=*o*-OCH_3-phenyl

Scheme 15. The oxa-Pictet-Spengler reaction for the synthesis of HTyr-derived isochromans.[59]

The reaction mechanism is depicted in Scheme 16. The first step involves the formation of a hemiacetalic bond between the alcohol functional group of HTyr and the carbonyl group. The subsequent loss of water and the cyclization reaction produce the final isochromans. Due to the facility of the synthesis, this procedure was extended to several substituted aldehydes and ketones to produce the corresponding isochromans **103-112** with 42-80% yields (Scheme 15). The experimental results showed that aldehydes generally reacted faster than ketones; hindered ketones gave the lowest yields; and aromatic aldehydes produced the corresponding isochromans with higher yields than aliphatic aldehydes.



Scheme 16. Mechanism of the oxa-Pictet-Spengler reaction.

Based on the reported mechanism, the reaction was performed by adding a dehydrating agent (anhydrous sodium sulfate or molecular sieves).[60] Generally, the use of a dehydrating agent gave higher yields of all synthesized isochromans; in addition, molecular sieves were the most efficient (90-98% *versus* 60-90%). As an

alternative, 1-phenyl-6,7-dihydroxy-isocroman **101** and 1-(4'-hydroxy-3'-methoxy)phenyl-6,7-dihydroxyisochroman **102** were obtained at more than 80% yields by the oxa-Pictet-Spengler reaction of dimethyl carbonate from tyrosol **26** and homovanillyl alcohol **27** followed by oxidation/reduction with the IBX/$Na_2S_2O_4$.[61]

2. Biological properties of HTyr-derived compounds in cell-based models

2.1 Antioxidant activity. Reactive Oxygen Species (ROS), which are continuously formed as the result of metabolic processes in the organism, may cause oxidation and damage cellular macromolecules. ROS contribute to the development of chronic degenerative diseases, such as atherosclerosis, diabetes, rheumatoid arthritis and cancer. Many natural compounds, including phenolic compounds with antioxidant activity, may be capable of preventing the onset of these diseases or even curing them.[62] In particular, oxidative stress and redox signaling have been implicated in carcinogenesis, and ROS can affect cancer initiation, progression and responsiveness to therapy. However, even if the role of antioxidants in the prevention of many types of cancers is well accepted, their beneficial effect on cancer progression and therapy is more controversial.[63] In some circumstances, due to their enhanced metabolism, cancer cells produce high level of ROS and oxidative stress as by-products, which may contribute to cellular mutation and cancer cell growth. In contrast, in other situations, ROS can slow cellular proliferation and render cancer cells more vulnerable to therapeutic interventions that act by further augmenting oxidant generation. Indeed, the use of antioxidants such as vitamin E or N-acetylcysteine can

increase tumor cell proliferation by attenuating ROS, DNA damage and p53 expression.[64]

Among the natural antioxidants, HTyr has received particular attention based on its remarkable antioxidant activities.[14] Numerous studies have shown that dietary HTyr is able to reduce the risk of cancer due to its ability to inhibit ROS generation, attenuating DNA damage and lipid peroxidation.[15]

In vitro studies were performed in normal cells and cancer cells to investigate the antioxidant activity of different synthetic HTyr esters. In 2007, Grasso et al. analyzed the antioxidant effects of HTyr and its esters, such as HTyr acetate **1**, propionate **2**, butyrate **3**, caprate **7** and stearate **11**, on whole blood cells, both in the presence and absence of H_2O_2 pre-treatment, by the atypical Comet assay.[42] Interestingly, when all compounds were used at the same dosage (50 μM) and assayed for H_2O_2-induced DNA damage, a significant protective effect was observed for HTyr, HTyr acetate **1** and propionate **2**, a moderate effect for HTyr butyrate **3** and no effect for HTyr caprate **7** and stearate **11**. These results outlined that the length of the acyl chain in the HTyr esters plays a fundamental role in protecting DNA from damage and that a longer chain does not improve the antioxidant ability of the compounds in a cell-based assay.[42] However, these results also demonstrated that even if HTyr acetate **1** and propionate **2** exerted a significant protective effect against DNA damage, their efficacy was not higher than that of the parental HTyr.

According to these results, Tofani et al. showed that a large series of HTyr esters of C2-C18 fatty acids (10 μM) decreased cumene hydroperoxide-induced oxidation in

L6 rat muscle cells using the standard dichlorofluorescein (DCF) assay.[65] The data also showed that the antioxidant activity of HTyr esters followed a general sigmoid curve in a direct relationship with the length of the acyl chain. For short to medium acyl chains (C2-C10), the antioxidant activity rose as the lipophilicity increased, giving values that were always higher than HTyr. However, elongation over 12 carbons did not play a favorable role and the activity dropped for esters carrying C12-C18 acyl chains.[65]

Concerning the ester derivatives, Bouallagui et al. showed that non-cytotoxic concentrations (100 μM) of HTyr acetate **1** and HTyr oleate **12** had a significant effect in preventing iron-reactive oxidative stress in the HeLa human cervical cancer cell line, resulting in a reduction of approximately 36% and 38%, respectively, in thiobarbituric acid reactive substance (TBARS) production. These results indicate a modest but significant augmentation of the antioxidant activity of these acyl esters compared with HTyr, which reduced iron-reactive oxidative stress by approximately 30%. The authors suggested that the enhanced antioxidant activity was due in part to an increased bioavailability of both HTyr derivatives and also to their enzymatic conversion by the cell into the parental HTyr compound.[44]

A more recent study by Bernini et al. reported the synthesis of HTyr catecholic analogs **35-37** and a large panel of their fatty acid esters **38-73**.[54] In this study, the antioxidant activity of these compounds (10 μM) was evaluated in L6 rat muscle cells through the DCF assay. The results showed that the catecholic analogs **35-37** exerted an antioxidant activity similar to that of HTyr, indicating the penetration of these

compounds into the cells and the subsequent quenching of peroxide radicals. On the other hand, fatty acid esters behaved differently, according to the length of the chain. In fact, while for short to medium acyl chain the antioxidant activity was similar to that of the free catechols, esters with a chain longer than eight carbons showed a cut-off effect, almost completely losing their antioxidant activity. This effect could be explained by the assumption that, at a certain level of lipophilicity, the easy diffusion of esters into the cells could become unproductive by their entrapment into the plasma membrane, which is caused by the higher affinity of long acyl chains for phospholipids or for hydrophobic proteins inside the bilayer.

In vitro studies have also been performed to investigate the antioxidant activity of different synthetic HTyr alkyl ethers. In 2011, Pereira-Caro at al. showed that physiological concentrations (0.5-10 μM) of HTyr methyl, ethyl, propyl and butyl ethers **74**-**77** dose-dependently reduced ROS generation, GSH depletion and MDA formation in HepG2 human hepatoma cells treated with tert-butyl hydroperoxide.[66] These results were compared with those obtained using HTyr, which exerted similar protective effects to the HTyr methyl **74** and ethyl **75** ethers, but less than the more lipophilic HTyr propyl **76** and butyl ethers **77**. An interesting evaluation that emerges from this paper is that the lipophilic nature of the HTyr alkyl ethers is relevant for the establishment of their antioxidant efficacy. In fact, HTyr methyl and ethyl ethers **74** and **75** were less effective than the HTyr propyl and butyl ethers **76** and **77**, particularly at higher doses. Similar results were reported by Guerrero A. et al., who showed that 1, 10, or 100 μM concentrations of HTyr ethyl **75**, butyl **77**, hexyl **78**,

octyl **79** and dodecyl **81** ethers dose-dependently inhibited lipid peroxidation and reduced GSH depletion in rat brain slices, where oxidative stress was induced by hypoxia and reoxygenation. Moreover, the maximal antioxidant effects were found for the C4-C8 alkyl ether derivatives.[67]

All of the results reported here were performed in cell-based models to analyze the antioxidant activity of both HTyr esters and ethers and show that the activity of these compounds largely depends on the length of their chain. Medium-sized chains, in the range of C4-C10, exerted antioxidant activity higher than that of HTyr, whereas the use of C12-C18 chains exhibited a sharp decrease of the antioxidant effect. These results are in agreement with the revisited theory of the polar paradox by Laguerre et al.[68] The original theory of the polar paradox by Porter and his colleagues stated that polar antioxidants were more active in bulk lipids than their nonpolar homologs, whereas nonpolar antioxidants were more effective in oil-in-water emulsions, liposomes or even in tissues.[69] Although this theory was supported by many data, not all data fit the theory. The hydrophobicity of the compound was not always correlated with its antioxidant activity, particularly in cellular systems. Moreover, as mentioned above, a nonlinear trend was found in many studies.[70, 71] Indeed, a very recent paper by Laguerre et al. challenges the original theory of the polar paradox and suggests additional theories.[68] In this paper, the authors establish a relationship between the nonlinear trend and the cut-off phenomenon observed in cell systems and put forward three putative mechanisms for the cut-off effect: the "reduced mobility", "internalization" and "self-aggregation" hypotheses. The "reduced mobility"

hypothesis is linked to the idea that the mobility of the lipophilic antioxidant decreases with the elongation of its alkyl chain, which modifies its ability to both move toward the numerous oxidation sites and bind with higher affinity phospholipids or hydrophobic proteins inside the bilayer of the plasma membrane. The "internalization" hypothesis assumes that the elongation of the chain, from medium to long, drives away the antioxidant into emulsion droplet core, where antioxidant activity is reduced. Finally, the "self-aggregation" hypothesis speculates that the cut-off is due to antioxidant self-aggregation and that long chain phenolipids primarily exist as aggregates.[68] In addition, we can suppose that the different HTyr-derived compounds, although more lipophilic, stable and thus with increased bioavailability compared to HTyr, once enter the cells, might encounter different molecular fates. For example, HTyr esters can be hydrolyzed by cell lipases and esterases, thus possibly generating both the parental HTyr, which is largely responsible for the observed antioxidant activity, and a novel molecule that might be responsible for different activities, which might or might not interfere with those of HTyr (Figure 4). On the other hand, HTyr ethers are stable compounds, which can act in a similar or different way compared to that of the parental HTyr (Figure 5). In any case, on the basis of all the results reported, the above-mentioned HTyr derivatives maintain the antioxidant activity of their parental compound that, in some cases, is also slightly but significantly higher than that of HTyr.

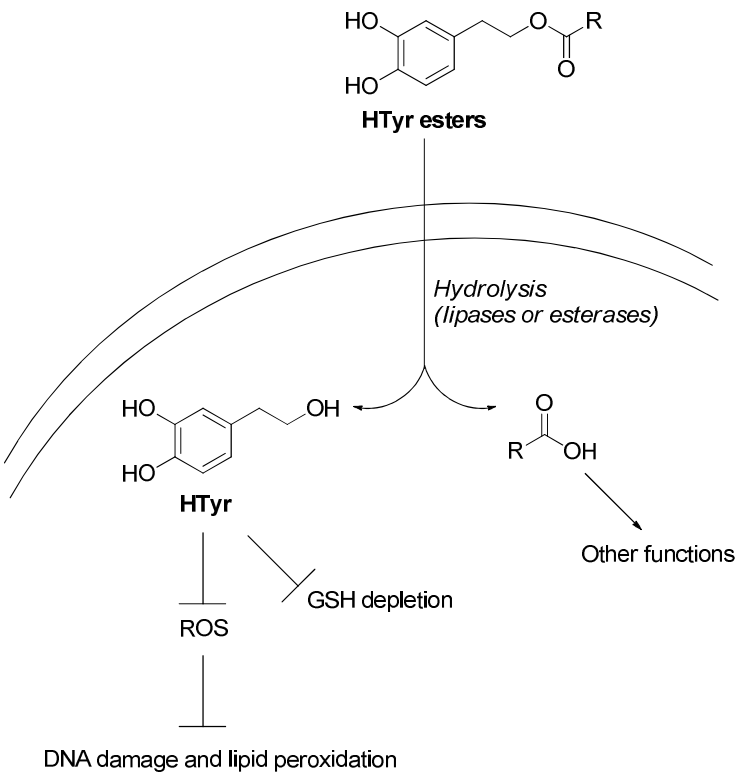


Figure 4. Effects on antioxidant activity of HTyr esters.

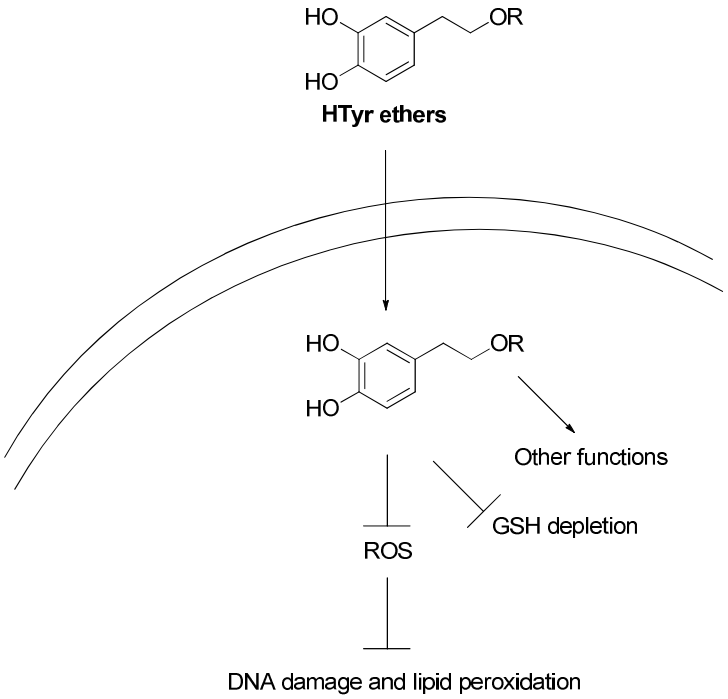


Figure 5. Effects on antioxidant activity of HTyr ethers.

2.2 Anti-proliferative and apoptotic activities. As stated by Hanahan and Weinberg, among the hallmarks of cancer, uncontrolled proliferation and the resistance to apoptosis represent the distinctive and complementary capabilities that enable tumor growth and metastatic dissemination.[72] In fact, as the balance between cellular proliferation and apoptosis is crucial for normal development and tissue size homeostasis in the adult organism, the deregulation of this balance can lead to tumorigenesis and sustain cancer growth. Cellular proliferation is the ability of cells to go through the different phases of cell cycle. Progression in the cell cycle is strictly regulated by heterodimers formed by cyclin-dependent kinases (CDKs) and their regulatory partner proteins, the cyclins, and by the CDK inhibitors (CDKIs). Intensive research has identified the use of CDKIs, which are capable of arresting proliferation and inducing apoptosis in neoplastic cells, as a promising strategy for cancer treatment. One CDKI is p21 (CIP1/WAF1) that, when overexpressed, leads to G1 and G2 or S-phase arrest. Moreover, p21 appears to have a dual role in that, although it was identified as a CDKI and originally considered as a cell context-specific negative regulator of the cell cycle and tumor suppressor, it can also act as an oncogene and promote tumorigenesis by inducing cell migration and proliferation.[73] In addition to controlled proliferation, programmed cell death by apoptosis is an extremely important preventive mechanism against cancer and it interferes with the transformation of a normal cell into a malignant cell. The apoptotic machinery is composed of both upstream and downstream effector components that induce the

"apoptotic trigger", which is controlled by pro- and anti-apoptotic members of the Bcl-2 family proteins.[74] In this scenario, up- or down-regulation of BH3-only proteins (Bcl-2 family proteins, effectors of canonical mitochondrial apoptosis) by damage signals may result in cell survival or death. Tumor cells have evolved a variety of strategies to limit apoptosis, such as the increased expression of survival proteins (Bcl-2, Bcl-xL) the decreased expression of BH3-only proteins and the inhibition of Bak and Bax, pro-apoptotic proteins that are required for mitochondrial outer membrane permeation.

In the last few years, a number of studies have investigated the anti-proliferative and apoptotic activities exerted by some HTyr-derived compounds and compared their activity to that of HTyr. In 2011, Bernini et al. showed that the novel ester **31** was able to induce a more potent cell growth inhibition than HTyr at all doses used (100, 150 and 300 μM) in the HT-29 human colorectal adenocarcinoma cell line.[52] In fact, 150 μM compound **31** treatment inhibited cancer cell growth by 69.9%, which was similar to HTyr at 300 μM; HTyr at 150 μM inhibited growth by 37.5%. Moreover, according to results obtained with HTyr, it was suggested that the antiproliferative effect exerted by compound **31** was due to the induction of cell cycle arrest in the G2/M phase.[52]

Then, Mateos et al. investigated the anticancer activity of HTyr acetate **1** in CaCo-2/TC7 human colon adenocarcinoma cells, analyzing both the proliferative response and the expression of genes related to the cell cycle and apoptosis. After having established that both HTyr and HTyr acetate **1** were not toxic for CaCo-2/TC7 cells at

concentrations of up to 50 μM, they found that significant (20%) cell growth inhibition could be obtained with 5 μM HTyr acetate **1** and it reached the half maximal inhibitory concentration (IC_{50}) at 32 μM.[75] The inhibition of cell proliferation correlated with the inhibition of cell cycle progression. At concentrations of up to 50 μM, HTyr acetate **1** provoked an arrest in S phase, while, at higher doses (100 and 200 μM), the arrest was in G0/G1 phase. The up-regulation of cyclin p21 and cyclin G2 and the reduction of cyclin B1 confirmed the ability of this compound to interfere with cell cycle progression. Moreover, by using HTyr acetate **1** at 50 μM, the antiproliferative effect could be associated with apoptotic activity, as indicated by the up-regulation of pro-apoptotic proteins such as BNIP3, BNIP3L (mitochondrial proteins that induce apoptosis when transiently overexpressed), PDCD4 (Programmed Cell Death 4), ATF3 (Activating Transcription Factor 3), and caspase-3.[76]

Finally, Burattini et al. analyzed the effect of HTyr and HTyr laurate **8** on the viability of the U937 human myelomonocytic cell line and murine C2C12 myoblasts.[77] The authors showed that 20 μM HTyr and 5 μM HTyr laurate **8** did not influence cell viability as measured by the trypan blue exclusion assay. However, when the authors analyzed the effect of HTyr and HTyr laurate **8** in H_2O_2-induced apoptosis, pretreatment with 20 μM HTyr and 5 μM HTyr laurate **8** resulted in a strong anti-apoptotic activity. In addition, ultrastructural analysis suggested that not only apoptotic but also autophagic cell death could be inhibited by HTyr or HTyr laurate **8** because the autophagic vacuoles that appeared in C2C12 cells undergoing

H_2O_2-induced cell death were no longer detectable. This observation deserves further study because autophagy, a form of cell defense under stress conditions, may represent an alternative cell death pathway that is triggered by the use of these compounds in cancer therapy.[77] These three studies on HTyr ester derivatives highlight the capability of these different compounds to exert antiproliferative activity in different cell systems (Figure 6). Moreover, in all three systems, the antiproliferative effect induced by the three HTyr esters is higher than the parental HTyr. However, a larger number of studies are needed to i) confirm their activity; ii) analyze whether the chain length influences the antiproliferative function, similar to the antioxidant activity; and iii) investigate the molecular mechanisms underlying the antiproliferative activity.

Recently, studies on the anticancer activity of some HTyr ethers have been performed by Pereira-Caro et al. [66,78] and Calderón-Montaño et al.[79] In 2011, Pereira-Caro et al. showed that physiological concentrations (0.5-10 μM) of HTyr methyl **74**, ethyl **75**, propyl **76** and butyl **77** ethers did not influence the viability and proliferation of the HepG2 human hepatoma cell line.[66] However, differences in cell viability became evident at concentrations of up to 200 μM of the HTyr methyl **74**, 100 μM of HTyr ethyl **75**, 50 μM of HTyr propyl **76** and 20 μM HTyr of butyl **77** ethers. A significant arrest of HepG2 cell growth was observed when cells were treated with 200 μM HTyr methyl **74**, ethyl **75** and propyl **76** ethers, whereas HTyr butyl ether **77** significantly inhibited cell proliferation at 50 μM. These results highlight that alkyl HTyr ethers have an antiproliferative effect, whose efficacy increases with the

elongation of the alkyl chain. However, in this study, the antiproliferative effect was compared among the four ethers but not to HTyr. In a more recent work, Pereira-Caro et al. studied the anticancer activity of the HTyr ethyl ether **75** in CaCo-2/TC7 human colon adenocarcinoma cells by analyzing the proliferative response and the expression of genes related to the cell cycle and apoptosis.[78] A wide transcriptome analysis of gene expression induced by HTyr and his HTyr ethyl ether derivative **75** was performed by microarray analysis and validated by RT-PCR analysis, and it highlighted that 10 μM HTyr and 5 μM HTyr ethyl ether **75** caused a significant up-regulation of cyclin G2 (CCNG2) and cyclin p21 as well as a down-regulation of cyclin B1 (CCNB1). The interference with cell cycle progression was more extensively investigated by analyzing the cell cycle distribution. The analysis showed that both HTyr and HTyr ethyl ether **75**, at doses ranging from 50 to 200 μM, inhibited cell cycle progression by blocking cells at the G0/G1 phase. Apoptosis was induced by both compounds, as demonstrated by activation of caspase-3.[78] In this work, the advantage of using HTyr ethyl ether **75** versus the parental HTyr is highlighted well, and data on both proliferation and apoptosis are well supported.

An interesting study was performed by Calderón-Montaño and coworkers, who investigated the cytotoxic activity of a panel of HTyr alkyl ether derivatives towards the A549 human lung cancer cell line and the MRC5 non-malignant lung fibroblast cell line.[79] HTyr alkyl ethers **75**, **77**, **78**-**82** differed in the lengths of their side chains (ethyl, butyl, hexyl, octyl, decyl, dodecyl and hexadecyl) and the doses needed to detect their activity. HTyr had activity at up to 1000 μM; ethyl **75**, butyl **77**, hexyl **78**

at up to 320 μM; octyl **79**, decyl **80**, dodecyl **81** at up to 100 μM and hexadecyl **82** at up to 32 μM. All alkyl HTyr ethers were more cytotoxic than HTyr towards both the malignant and non-malignant cell lines, and a higher selective cytotoxic activity was observed for the A549 cancer cells compared with the MRC5 non-malignant cells. However, although HTyr hexadecyl ether **82** was the most cytotoxic for A549 cells, HTyr dodecyl ether **81** was the most selective, with an IC_{50} value for A549 cells 2.46-fold lower than that for the MRC5 cells (20 μM *versus* 49 μM, respectively). Furthermore, the authors showed that the combination of HTyr dodecyl ether **81** (10 μM) with the anticancer drug 5-fluorouracil (10 μM) induced a synergistic cytotoxicity in A549 cells but not in MRC5 cells. To further verify the selective cytotoxic activity of the HTyr dodecyl ether **81,** the authors used another experimental model, the MCF7 human breast cancer cell line and MCF10 normal breast epithelial cells. The results confirmed that the HTyr dodecyl ether **81** (1-30 μM) exerted a more potent and selective cytotoxic activity than HTyr, displaying a marked selective cytotoxicity for breast cancer cells compared with normal breast cells. This study also showed a linear correlation between the lipophilicity of the compounds and the cytotoxicity towards cancer cells. In fact, although the authors indicate that HTyr dodecyl ether **81** is the most efficient in inducing selective cytotoxicity in cancer cells, the HTyr ether with a longer alkyl chain, HTyr hexadecyl ether **82**, is more cytotoxic. This means that the antiproliferative activity is not related to the antioxidant function. Moreover, the use of low doses of HTyr ethers has the advantage of protecting the cell from oxidative stress-induced damage without

interfering with cancer cell proliferation, while the use of high doses can inhibit cell proliferation and induce cell death (Figure 7).[66, 77]

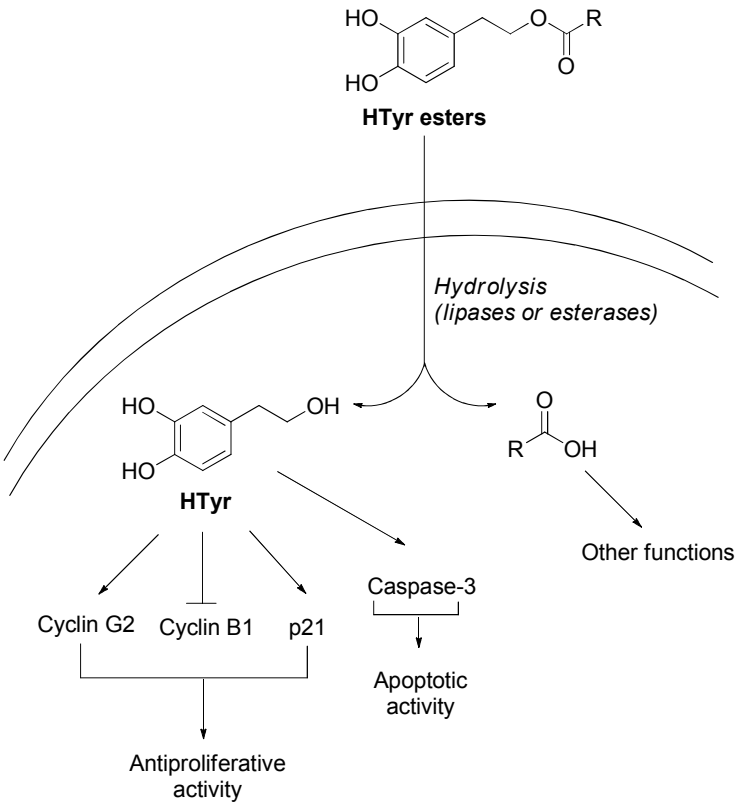


Figure 6. Effects on proliferative and apoptotic activities of HTyr esters.

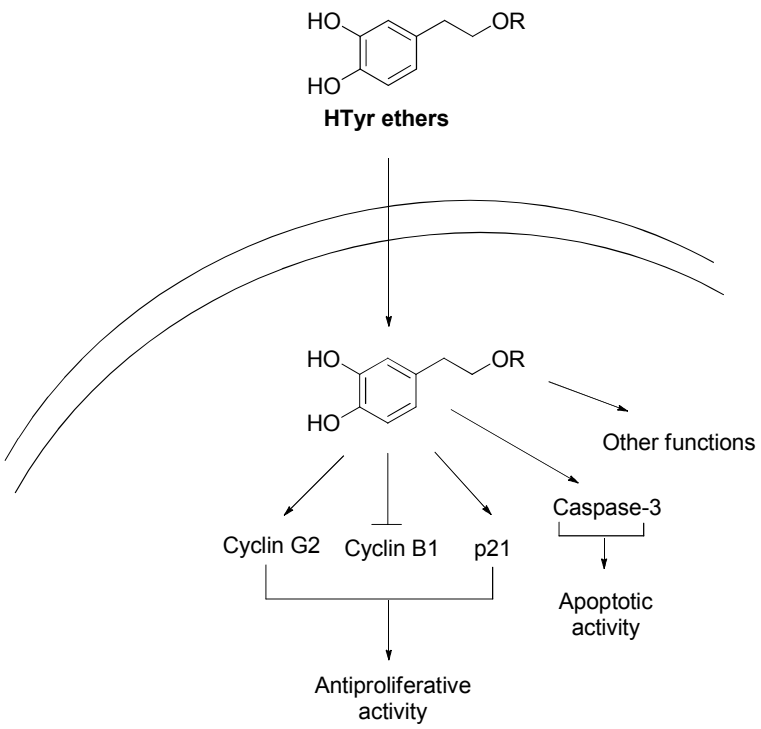


Figure 7. Effects on proliferative and apoptotic activities of HTyr ethers.

Finally, Sepporta et al. compared the anti-proliferative and pro-apoptotic activities of three thioderivatives of HTyr **86-88** in the HL60 human promyelocytic leukemia cell line and its multidrug-resistant HL60R variant.[56] All of the new compounds were more efficient than HTyr in inducing apoptosis in HL60R cells, and HTyr disulfide **88** was the one that triggered both apoptosis and necrosis in HL60 and HL60R cells. Indeed, apoptosis induced by 25 μM HTyr disulfide **88** was 6- and 3-fold higher than HTyr in HL60 and HL60R cells, respectively. The authors did not study the mechanisms underlying the induction of apoptosis by these compounds. However, they suggested that the mechanism was different from that of HTyr because all thioderivatives **86-88** did not induce H_2O_2 release in the culture medium, in contrast to HTyr. Although specific investigations are needed to characterize the molecular

mechanisms underlying HTyr disulfide activity, these results suggest a possible way forward for the development of new strategies to overcome the chemo-resistance of tumor cells.

2.3 Anti-inflammatory activity. The immune system is considered a "double-edge sword" for cancer. It protects the host by destroying tumor cells through immunosurveillance, and yet, paradoxically, it promotes and sustains cancer through different mechanisms, including the development of pro-tumorigenic immune responses.[80] Inflammation is a critical component of pro-tumorigenic immune responses by contributing to all of the carcinogenic steps, including cancer initiation (through innate immune cell-mediated oxidative cell damage), promotion (through induction of cell proliferation), and progression (through promotion of neo-angiogenesis and metastasis), as well as by suppressing anti-tumor immunosurveillance.[81] The pro-tumorigenic inflammatory responses contribute several components of the immune system, including platelets and macrophages.

Emerging evidence demonstrates that platelets, beyond playing a role in homeostasis and thrombosis, also function as immune and inflammatory effector cells.[82] Indeed, platelets play a central role in inflammation through either their direct interaction with leukocytes and endothelial cells or the release of many inflammatory mediators, including lipids such as thromboxane (TX) A2 (as the result of the activity of cyclooxygenase (COX)-1) and proteins such as a wide number of angiogenic and growth factors. Thus, in some contexts, platelet-mediated functions are protective

immune reactions, whereas in others, they contribute to adverse inflammatory outcomes. Enhanced platelet activation has been detected in cardiovascular diseases as well as in pro-tumorigenic inflammation and tumorigenesis in response to epithelial and endothelial injury. As immune cells, platelets are a component of the tumor microenvironment, and evidence is emerging on their important function in the mechanisms involved in all steps of carcinogenesis (including tumor growth, angiogenesis, metastasis) as well as in the modulation of tumor therapy.[83] Among the manifold mechanisms triggered by platelets, a key mechanism to trigger the complex biological cascade of molecular and cellular signals that mediate inflammation and promote all steps of cancerogenesis is their capacity to synthesize and release TXA_2 (via the concurrent activity of COX-1 and TXA synthase) and to promote the persistent and aberrant expression of the cytokine-inducible COX-2-dependent prostanoids (mainly prostaglandin (PG) E2) in endothelial cells, inflammatory leukocytes (e.g., monocytes), stromal cells and tumor cells.[84] In addition, retrospective analyses of randomized studies designed to assess the effect of the antiplatelet drug aspirin on cardiovascular events strongly suggest that the intake of long-term low-dose aspirin is associated with both decreased incidence of all cancers and the mortality in adenocarcinomas.[85,86] Although the exact mechanism for the anticancer effect of aspirin remains unknown, the efficacy of low-dose aspirin in the prevention of either vascular occlusion or cancer highlights its important role in the complete irreversible inhibition of the platelet COX-1 pathway, in particular TXA_2 biosynthesis,[87] while causing a limited and rapidly reversible inhibitory effect on

COX-2 expression in nucleated cells (such as macrophages, endothelial and cancer cells).[88] However, in contrast to its beneficial effects, long-term low-dose aspirin intake is also associated with a significant risk of bleeding.[89] Evidence from experimental and clinical studies indicates that, during inflammation, another population of inflammatory cells, such as monocytes and macrophages, can produce many mediators contributing to cancer initiation, promotion, progression and metastasis.[90] The transcription factor NF-kB is one of the master regulators of the inflammatory response in macrophages, inducing the expression of genes encoding key pro-tumorigenic inflammatory mediators, including prostanoids, such as TXA_2 (as the result of the COX-1 activity) and PGE2 (as the result of PGH synthase isoforms, constitutive COX-1 and the mostly inducible COX-2 enzyme activities), as well as cytokines such as tumor necrosis factor (TNF)-α. Based on these observations, the inhibition of pro-tumorigenic inflammation by anti-platelet and anti-inflammatory agents represents an attractive novel approach in the fight against cancer.

The majority of the studies on the anti-inflammatory activity mediated by HTyr-derived compounds have been focused on their effects on the physiopathological mechanisms of cardiovascular diseases, in particular on their capability to inhibit the function of platelets and other inflammatory cells involved in the development of atherosclerosis and atherothrombosis.[91] Indeed, the effects investigated included the inhibition of platelet aggregation and the stimulation of nitric oxide (NO) production, as well as the inhibition of oxidation and macrophage-mediated inflammation. In

2003, Togna et al. examined the *in vitro* antiplatelet activity of scalar doses (from 1 to 100 μmol/L) of two hydroxyl-isochromans, 1-phenyl-6,7-dihydroxy-isochroman **101** and 1-(3'-methoxy-4'-hydroxy-phenyl)-6,7-dihydroxy-isochroman **102**, in human derived platelet-rich plasma (PRP).[92] Both compounds inhibited arachidonic acid- and collagen-induced platelet aggregation in a dose-dependent manner, starting from a concentration of 1 μmol/L, with complete inhibition at concentrations ranging between 10 and 20 μmol/L. Moreover, inhibition of platelet aggregation paralleled the inhibition of TXA_2 production by platelets. In contrast, neither isochroman affected platelet reactivity to ADP at doses up to 30 μmol/L. These data may indicate a contribution of the radical-scavenging activity of these compounds in the antiplatelet effects because ROS production has been reported to be more important during the initial phases of arachidonic acid- and collagen-induced platelet activation than the other agonists, such as ADP.[93] Moreover, isochromans **101** and **102** also inhibited arachidonic acid mobilization from platelet membrane phospholipids that were induced by thrombin and to a greater degree by collagen, suggesting a direct inhibition of phospholipase A2 (PLA2) by these isochromans. Because thrombin induces arachidonic acid mobilization by directly stimulating PLA2 without ROS production (unlike collagen),[94] the greater level of collagen-induced inhibition may be the result of an additional indirect effect of the compounds on PLA2 that is mediated by their scavenging activity. Based on the data reported in the literature, isochromans **101** and **102** appeared to be more active than HTyr in inhibiting collagen-induced platelet aggregation, and less active in modifying the platelet

response to ADP. In fact, previous studies reported a 50% IC_{50} on collagen-induced platelet aggregation and ADP by 67 μmol/L and 27 μmol/L of HTyr, respectively, and a complete inhibition of collagen-induced aggregation by 400 μmol/L of HTyr.[95] Interestingly, the differences in the interference of platelet activity between isochromans **101** and **102** *vs* HTyr did not seem to be dependent on the higher antioxidant power of these compounds because both HTyr derivatives exerted lower radical scavenging activity than HTyr. More recently, the anti-inflammatory activity of the 1-phenyl-6,7-dihydroxy-isochroman **101** was also investigated *in vitro* on lipopolysaccharide (LPS)-stimulated human peripheral blood-derived adherent monocytes.[96] Compound **101** significantly inhibited the production of prostanoids, such as monocyte-derived TXA_2 and PGE_2, starting at concentrations of 1 and 10 μM, respectively. Because pre-treatment of monocytes with aspirin (inhibitor of COX-1 activity) did not significantly interfere with inhibition of prostanoid production, even at the lowest concentration assayed (1 μM), the authors suggested that the isochroman **101**-mediated inhibition was primarily due to its inhibitory effect on COX-2 activity. Moreover, isochroman **101** significantly decreased LPS-induced COX-2 and NF-kB protein expression at 100 μM. Therefore, because the inhibitory effect on COX-2-mediated prostanoid release was recorded even at 1 μM, while COX-2 expression was significantly reduced only at 100 μM, the effect of isochroman **101** appeared to be primarily dependent on the direct inhibition of COX-2 enzyme activity rather than the inhibition of COX-2 protein expression by suppressing the NF-kB signal transduction pathway. Furthermore, 0.5, 10 and 100

μM isochroman **101** treatment decreased TNFα production by activated monocytes by approximately 30, 60 and 80%, respectively. On the basis of the data reported in the literature, isochroman **101** appears to be more active than HTyr in inhibiting the pro-tumorigenic COX-2-PGE2 pathway and TNFα production, in that HTyr-mediated inhibition was only observed at doses ranging from 50 to 100 μM.[97]

Encouraging results have also been obtained from the evaluation of the anti-inflammatory activity of lipophilic HTyr acyl esters such as HTyr acetate **1**. Interestingly, the HTyr acetate **1** effects on platelet function were compared not only to HTyr but also to acetylsalicylic acid (ASA), in *in vitro* and *in vivo* experimental models.[98,99] González-Correa et al. showed that 1 to 1000 μM doses of HTyr acetate **1**, HTyr and ASA dose-dependently inhibited platelet aggregation induced by arachidonic acid, collagen and ADP in whole blood and in isolated platelets (PRP).[98] However, HTyr acetate **1** and ASA had a greater anti-aggregating effect than HTyr in whole blood, and their inhibitory activities were observed at ≤10 μM, whereas HTyr inhibition was found at doses ranging from 100 to 1000 μM. In collagen- or ADP-induced PRP, there were no significant differences between the three compounds, but when arachidonic acid was used as the inducer, the effect of HTyr acetate **1** or ASA was stronger than HTyr. Moreover, in collagen-induced PRP that was incubated with erythrocytes, the antiaggregating effects of HTyr, HTyr acetate **1** and ASA were not significantly different from PRP alone. However, in collagen-induced PRP that was incubated with leucocytes, the antiaggregating effect of HTyr acetate **1** and ASA (unlike HTyr) increased in comparison to the effect in PRP alone. These results,

together with the different antiaggregating effects exerted by the three compounds in whole blood *vs* PRP, may be due to enhanced NO production by neutrophils, as it has been reported for aspirin.[100] Of note, in all of the experiments, the profile of platelet aggregation inhibition by HTyr acetate **1** was similar to ASA. Therefore, the authors further investigated the antiaggregating activity of HTyr and HTyr acetate **1** in relation to the mechanism of ASA, which consists of inhibiting TXA_2 synthesis by platelets and enhancing NO production by leukocytes.[100] All of the compounds inhibited platelet production of TXA_2 in a concentration-dependent manner, but HTyr acetate **1** and ASA had a stronger inhibitory activity than HTyr. Moreover, HTyr acetate **1** and ASA exerted their inhibitory effect at $\leq$10 μM, whereas HTyr inhibited at doses ranging from 100 to 1000 μM. All three compounds stimulated calcium-induced NO production in whole blood in a concentration-dependent manner, but the effect of HTyr, even at 1000 μM, was significantly weaker than HTyr acetate **1** and ASA. Therefore, the authors proposed that HTyr acetate **1** exerts a greater antiaggregating effect because, in contrast to HTyr (whose antiplatelet activity primarily relies on TXA_2 synthesis inhibition) and similar to ASA, it acts both by inhibiting TXA_2 synthesis and by enhancing NO production. In addition, all three compounds inhibited the production of TNFα by LPS-stimulated leucocytes, with no significant differences between them. The analysis of the antioxidant effect by these compounds showed that the production of 3-nitrotyrosine (an indicator of peroxynitrite production) was inhibited only at 1000 μM, a concentration greater than that used to inhibit platelet function. This result indicates that HTyr acetate **1** does not

exert higher antioxidant activity than HTyr and that the antioxidant effect of these compounds does not have a direct relationship with their antiplatelet effect. In conclusion, HTyr acetate **1** exerts an anti-platelet aggregation effect that is greater than HTyr and similar to ASA. The latter observation is potentially very important because ASA is widely used to prevent cardiovascular diseases and cancer. Interestingly, the effect of HTyr acetate **1** on platelet aggregation was also investigated *in vivo* by oral administration (7 days) of this compound in healthy rats, and its activity was compared with that of HTyr and ASA.[76] The authors showed that HTyr, HTyr acetate **1** and ASA dose-dependently inhibited collagen-induced platelet aggregation in whole blood. By extrapolating the dose of each compound that inhibited platelet aggregation by 50% (ID50) of that in the control group, the resulting ID50 values were 16.05 mg/kg per day for HTyr acetate **1**, 48.25 mg/kg per day for HTyr, and 2.42 mg/kg per day for ASA, showing that the antiplatelet aggregating effect of HTyr acetate **1** was stronger than that of HTyr. In addition, the investigation of the antiaggregating activity of HTyr and HTyr acetate **1** in relation to the ASA mechanism showed that all compounds inhibited TXA_2 synthesis. However, the activity mediated by HTyr and HTyr acetate **1** was weaker than ASA, and the effect induced by HTyr acetate **1** was only slightly stronger (37%) than HTyr (30%). Therefore, in contrast to ASA, TXA_2 inhibition by HTyr and HTyr acetate **1** did not parallel the platelet aggregation inhibition, raising the possibility that an additional mechanism was involved in the antiaggregating ability of these two polyphenols. The analysis of vascular NO production by HTyr and HTyr acetate **1** showed an

enhancement of NO production, and the effect induced by HTyr acetate **1** was significantly stronger (66%) than HTyr (34.2%) and comparable to ASA (64%). In conclusion, these data indicate that HTyr acetate **1** is able to inhibit platelet aggregation *in vivo*, and its effect is stronger than that of HTyr. Moreover, the mechanisms underlying this effect may primarily include an increase in NO production and, to a lesser extent, a decrease in thromboxane synthesis. Although all of these *in vivo* results should be taken with caution because of the differences in the doses and pharmacokinetics of HTyr between rats and humans, they open new perspectives toward the potential use of HTyr acetate **1** as an alternative to ASA in the prevention of both arterial thrombotic events and tumors. More recently, HTyr acetate **1** has also been investigated for its anti-inflammatory activity both on murine macrophages [101] and in dextran sulfate sodium (DSS)-induced acute colitis in mice.[102] HTyr acetate **1** (50 and 100 μM) has been shown to inhibit COX-2 protein expression and NF-kB activation both in murine LPS-stimulated peritoneal macrophages and in an experimental model of inflammatory bowel disease associated with colon cancer, exerting thus a significant anti-inflammatory activity that might be exploited for the development of new strategies for the prevention of pro-tumorigenic inflammatory diseases.

Similar investigations to those mentioned above have been performed using five alkyl HTyr ether derivatives (ethyl **75**, hexyl **78**, octyl **79**, dodecyl **81** and hexadecyl **82**) in *in vitro* and *in vivo* experimental models, and the effects were compared with those of HTyr.[103,104] Reyes at al. showed that all five HTyr alkyl ethers, at doses ranging from

1 to 1000 μM, significantly and dose-dependently inhibited platelet aggregation by arachidonic acid and collagen, and, to a lesser extent, by ADP in human whole blood *in vitro*.[103] However, at 10 μM, some HTyr alkyl ethers exerted a greater antiaggregating effect than HTyr, depending on the carbon chain length. The inhibitory effect was biphasic, it increased from two to six carbons and it decreased from eight to twelve carbons. The comparison of the antiaggregating effect of HTyr hexyl ether **78** (IC_{50} in the 10^{-7}-10^{-6} M range) with the previously mentioned HTyr acetate **1** (IC_{50} in the 10^{-5} M range) shows that the inhibitory effect of HTyr hexyl ether **78** is stronger. Moreover, all HTyr ether derivatives could inhibit TXA_2 production in a concentration-dependent manner, but the effect was evident only at 100 μM for all compounds, and no quantitative significant difference was found between the compounds, indicating that the main mechanism of action of these compounds does not rely on this activity. On the other hand, HTyr alkyl ethers increased calcium-induced NO production in LPS-stimulated leukocytes in a concentration-dependent manner, suggesting that increased NO production may be one of the mechanisms underlying the inhibition of platelet function by these compounds. Again, the HTyr hexyl ether **78** showed a greater effect than HTyr. In addition, the investigation of the modulation of three biochemical pathways of tissue inflammation (including COX-2, inducible NO synthase (iNOS) and interleukin (IL)-1β production), showed that HTyr alkyl ether derivatives (mainly ethyl, butyl and hexyl derivatives **74**, **76** and **78**) suppress the production of LPS-induced inflammatory mediators, such as the COX-2-PGE2 pathway, iNOS and IL-1 β, in

whole blood, and their inhibitory effects were greater than those of HTyr. The effects of the five alkyl (ethyl **74**, butyl **76**, hexyl **78**, octyl **79** and dodecyl **81**) HTyr ether derivatives on platelet aggregation and TXA_2 production has also been investigated *in vivo* by oral administration (7 days) of these compounds or HTyr in healthy rats.[104] All five compounds dose-dependently inhibited collagen-induced platelet aggregation and TXA_2 production in whole blood. As for the *in vitro* studies, both inhibitory effects were non-linear in relation to the length of the carbon chain, in that compounds with four or six carbons had an increasing effect, whereas those with eight or twelve carbons had a decreasing effect. After the administration of 20 mg/kg/day of each compound, the greatest inhibitory effects were observed with the hexyl derivative **78** (58.7% inhibition for both parameters with respect to the control group). Comparing the results for TXA_2 production *in vitro* and *ex vivo*, the authors found that the butyl **76** and hexyl **78** derivatives, which had no effect *in vitro*, inhibited thromboxane production in whole blood. Moreover, calcium-induced NO synthesis by thoracic aorta was significantly increased by the hexyl ether derivative **78**. These findings suggest that the anti-platelet effect by HTyr ethers is a consequence of their ability to both inhibit thromboxane synthesis and enhance NO production. The investigation of their anti-oxidant activity showed that the plasma lipid peroxide concentration was significantly reduced after the administration of 20 mg/kg/day of the butyl **77**, hexyl **78**, octyl **79** and dodecyl **81** ether derivatives. As before, there was a non-linear inhibitory effect depending on the length of the carbon chain and inhibition was greatest with the hexyl ether derivative **78** (84.7% at 20

mg/kg/day). Furthermore, the GSH concentrations in red blood cells were increased in animals treated with 20 mg/kg/day of the hexyl **78**, octyl **79** and dodecyl **81** derivatives. These findings confirmed a decrease in the plasma concentration of lipid peroxides, as well as an increase in the concentration of GSH. Consistent with other observations, the non-linear effect with the lower dose (20 mg/kg/day) was maximal for the hexyl derivative **78** (cut-off effect). Taken together, the present findings suggest that the hexyl HTyr derivative **78** has the most favorable anti-platelet effect among all compounds tested. Thus, these results suggest that the cut-off theory recently developed by Laguerre et al., in which the critical chain length for HTyr lipophilized derivatives (alkyl esters) is 7±4 carbons,[70] may also be relevant for the antiplatelet activity exerted by HTyr alkyl ether derivatives.

All of these data indicate that the HTyr-derived compounds can be more effective than HTyr in inhibiting platelet function. Moreover, the mechanism underlying this activity seems to be independent of the antioxidant activity exerted by these compounds because most of them do not exert higher antioxidant activity than HTyr (Figures 8 and 9).

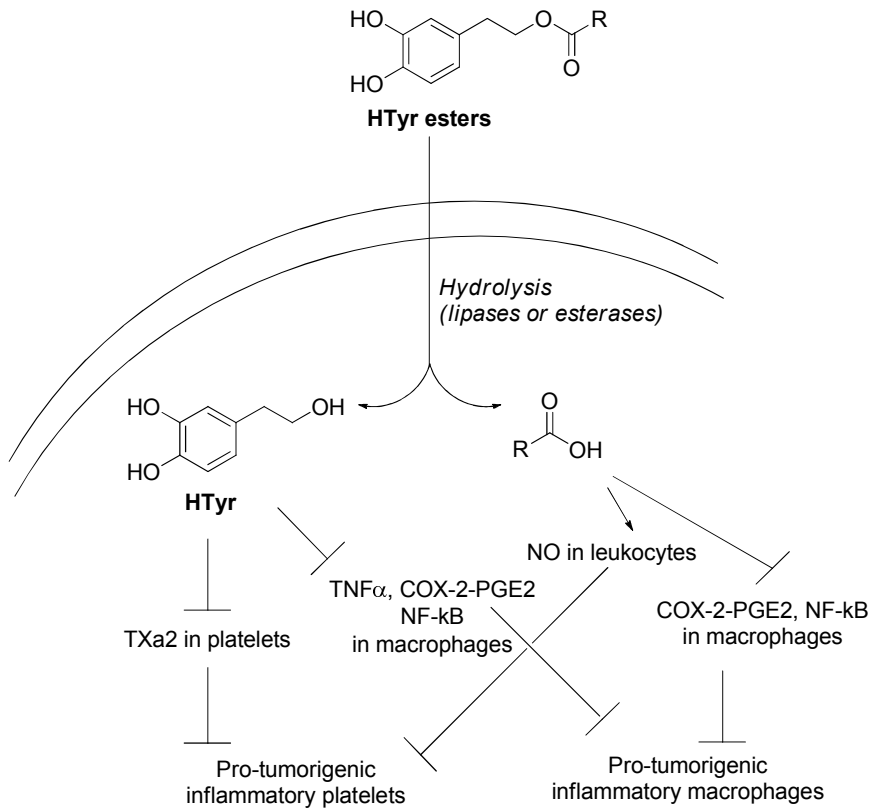


Figure 8. Effects on pro-tumorigenic inflammatory activity of HTyr esters.

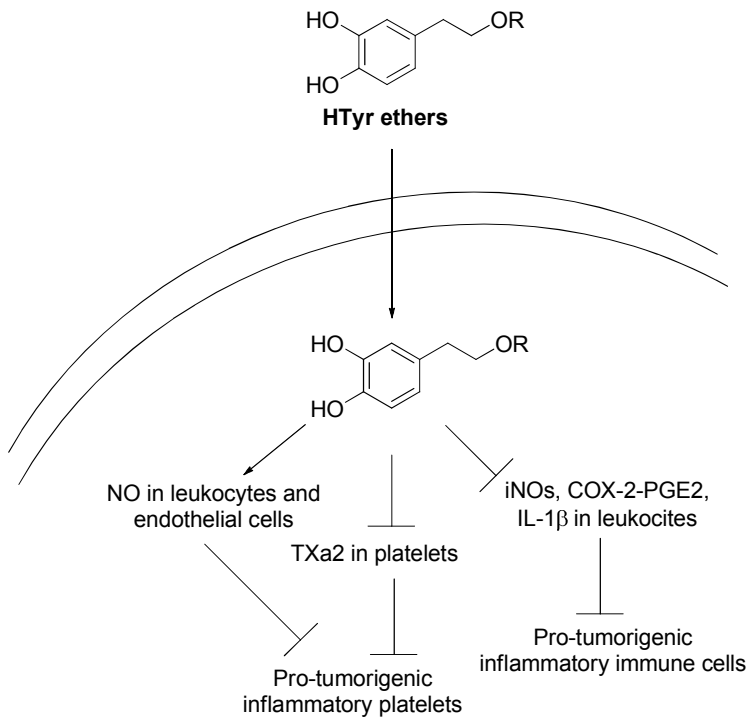


Figure 9. Effects on pro-tumorigenic inflammatory activity of HTyr ethers.

It appears that the enhanced anti-platelet aggregation activity may include an increase in NO production by leukocytes and endothelial cells associated with a decrease in thromboxane synthesis by platelets, whereas HTyr acts mainly on the inhibition of TXA2 production. These data suggest that different mechanisms of action are involved. In addition, HTyr derivatives appear more effective than HTyr in inhibiting monocyte/macrophage pro-tumorigenic inflammatory functions. The fact that the isochroman **101** seems more effective than HTyr in inhibiting key inflammatory mediators produced by human monocytes, such as TNFα and the COX-2-PGE2 pathway, is very important because TNFα acting on tumor and stromal cells is a major mediator of cancer-related inflammation. In contrast, PGE2 exerts pleiotropic effects on tumors, such as the promotion of tumor cell proliferation/survival and metastasis, the orchestration of neo-angiogenesis and the induction of immunosuppression. Together, these considerations suggest that isochroman **101** may play a central role in the suppression of tumorigenesis by inhibiting TNFα production as well as the COX-2-PGE2 pathway in macrophages. TNFα is now considered a therapeutic target in cancer treatment, and TNFα antagonists have been shown to exert therapeutic activity in Phase I and II clinical cancer trials.[105] Moreover, recent studies have been devoted to the development of new molecules that are inhibitors of COX-2 enzymatic activity.[106] Therefore, the inhibition of TNFα production and COX-2 activity by isochroman **101** may represent an alternative antitumor molecular

approach.

CONCLUSIONS

An explosion in the research into the synthesis of HTyr-derived compounds has occurred during the last few years. In particular, many studies have been performed to synthesize HTyr derivatives and analogs with a more lipophilic character than HTyr to increase their bioavailability, as well as in joining HTyr to another biologically active compound to enhance the beneficial health properties of the final compound. Thus, the search for novel HTyr-derived compounds is a fruitful field for organic chemists. The bioavailability and a certain number of biological properties of the different classes of HTyr derivatives have been demonstrated, and it has been established that some HTyr-derived compounds may be also largely more effective than parental HTyr, especially for their anti-proliferative and anti-inflammatory functions. However, most experimental designs lack experimental procedures that allow an in-depth evaluation of the relationship between the chemical structure variations and the modulation of biological responses. We think that future investigations would be focused on the analysis of the biological properties exerted not only by the final HTyr derivative compound, but also by its individual molecular components. This should also allow to better understand the interactions of HTyr derivatives with intracellular molecular targets and thus to develop more effective strategies for the synthesis of HTyr-derived compounds with enhanced beneficial health properties.

The studies mentioned above indicate that the introduction of a lipophilic chain in HTyr can increase its bioavailability and its stability to oxygen and air.[107] Therefore, considering the potential greater stability of some HTyr-derived compounds compared with HTyr, the use of these molecules is more attractive from a pharmacological point of view. However, based on the literature data and as discussed above, we can assume that the reported HTyr-derived compounds exert an almost equal or slightly higher antioxidant activity than the parental HTyr. Therefore, in terms of protection from oxidative damage, they exert almost the same effects as HTyr on cancer prevention and therapy.[15] Indeed, these compounds are able to reduce ROS generation, DNA damage, and lipid peroxidation in cellular models and, like HTyr, are potentially effective in cancer prevention. However, as reported by our group and others,[15,63] the use of antioxidants needs to be carefully evaluated in cancer therapy because the cytotoxic efficacy of several antineoplastic drugs currently used for cancer chemotherapy is based on the induction of high oxidative stress levels in cancer cells.

With regard to the anti-proliferative and apoptotic properties of the HTyr-derived compounds, we can conclude that all of the above-mentioned derivatives (such as esters, ethers and thioderivatives) exert higher anti-proliferative and pro-apoptotic activity than the parental compound; for this reason, they deserve further investigation. However, there are still a only a few studies in the literature, and these studies are not comparable to each other because they use different experimental systems. Therefore, it should be interesting to perform studies investigating a certain

number of HTyr esters with different chain lengths for their proliferative activity both on cancer cell lines and on their related non-malignant cell types. The information that would be obtained is not only useful to know whether the chain length influences the antiproliferative and apoptotic activities but also to understand whether the anti-proliferative and apoptotic functions are linked to the antioxidant activity exerted by these compounds. As mentioned before, the antioxidant activity of HTyr esters with C2-C18 fatty acids in L6 cells showed a sharp drop for long-chain esters (C12-C18).[65] Moreover, the results obtained with HTyr ethers showed that the cut-off effect observed in the studies on their antioxidant activity did not correlate with their antiproliferative function. In fact, Guerrero et al. reported that higher antioxidant effects were observed with a chain length in the range of C4-C8,[67] whereas Calderon-Montano et al. showed that the highest cytotoxic activity was exerted by the hexadecyl ether **82**.[79] These studies have been performed in different cell systems and this may be the reason of these discrepancies.

Concerning the anti-inflammatory activity exerted by HTyr-derived compounds, we can consider that all of the derivatives reported here, but some ethers, are more effective than HTyr in inhibiting both platelet function and monocyte/macrophage pro-tumorigenic inflammatory activities *in vitro* and *in vivo*. In *in vitro* studies, all of the effects reported require that the HTyr-derived compounds penetrate inside inflammatory cells such as platelets and leukocytes. Because good penetration has been demonstrated for some of these compounds through the biological membranes of some cell types,[107] it could be assumed that HTyr-derived compounds also pass the

cytoplasmic membranes of inflammatory cells. However, this point has not been studied for platelets and leukocytes and it requires specific studies. These studies are further needed because, interestingly, in the majority of the cases, the anti-inflammatory activity of the HTyr-derived compounds does not seem to be dependent on their antioxidant activity. These results suggest that the mechanisms underlying their anti-inflammatory activity may be different than those underlying oxidative damage protection and, ultimately, from those of HTyr. This is a very interesting point because, as previously reported by our group and others,[15,63] the antioxidant activity is not always useful for cancer therapy. Moreover, as mentioned before, we can speculate that once HTyr esters enter the cells, they may undergo hydrolysis and generate different metabolites that could be responsible for the different activities. Considering this hypothesis, we think that it should be interesting to investigate whether different metabolites from the different HTyr-derived compounds are generated inside the cell and determine which molecular pathways are triggered. In contrast, for *in vivo* studies, we can suppose that HTyr-derived compounds that are transferred across the enterocyte monolayer are not metabolized and are expected to remain unmodified when they reach the portal blood and, subsequently, the liver. However, the pharmacokinetic parameters for the administration of the HTyr derivatives remain to be established to postulate the importance of these compounds in the treatment of inflammatory related diseases. Finally, all of these *in vitro* and *in vivo* studies indicate that the synthetic HTyr derivatives may represent potential alternatives to natural HTyr as anti-inflammatory compounds. Of note, HTyr acetate

1 may be considered as potential alternative to aspirin, which is widely used as an anti-platelet aggregation and anti-inflammatory drug to prevent cardiovascular disease as well as cancer.

In conclusion, the findings reported here propose that some HTyr-derived compounds are powerful antioxidant, antiproliferative and anti-inflammatory agents, suggesting a more effective cancer chemopreventive and chemotherapeutic activity than that exerted by the parental HTyr. However, future *efforts would be extended* to the investigation of the direct relationship between chemical structure variations and the modulation of different biological properties. Moreover, further *in vitro* and *in vivo* studies are needed to elucidate the cellular and molecular targets of the HTyr-derived compounds clarifying their activities on the suite of the mechanisms that regulate tumorigenesis.

ABBREVIATIONS

ASA Acetylsalicylic acid

COX Cyclooxygenase

DBU 1,8-Diazabicyclo[5.4.0]undec-7-ene

DCC Dicyclohexylcarbodiimide

DIAD Diethyl azodicarboxylate

DMC Dimethyl carbonate

DMSO Dimethylsulfoxide

DMP Dess-Martin periodinane

HTyr	Hydroxytyrosol
IBX	2-Iodoxybenzoic acid (1-hydroxy-1-oxo-1*H*-1λ^5-benz[*d*][1,2]iodoxol-3-one)
NO	Nitric oxide
PG	Prostaglandine
PLA2	Phospholipase A2
PRP	Platelet-rich plasma
PTSA	*p*-Toluenesulfonic acid
ROS	Reactive Oxygen Species
THF	Tetrahydrofuran
TNF	Tumor necrosis factor
TX	Thromboxane

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REFERENCES

(1) Martin-Pelaez, S.; Covas, M. I.; Fito, M.; Kusar, A.; Pravst, I. Health effects of olive oil polyphenols: recent advances and possibilities for the use of health claims. *Mol. Nutr. Food Res.* **2013**, *57*, 760-771.

(2) Bendini, A.; Cerretani, L.; Carrasco-Pancorbo, A.; Gomez-Caravaca, A. M.; Segura-Carretero, A.; Lercker, G. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules* **2007**, *12*, 1679-1719.

(3) Montedoro, G. F.; Servili, M.; Badioli, M.; Miniati, E. Simple and hydrolizable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *J. Agric. Food. Chem.* **1992**, *40*, 1571-1576.

(4) Montedoro, G. F.; Servili, M.; Badioli, M.; Miniati, E. Simple and hydrolizable phenolic compounds in virgin olive oil. 2. Initial characterization of the hydrolizable fraction. *J. Agric. Food. Chem.* **1992**, *40*, 1577-1580.

(5) Montedoro, G. F.; Servili, M.; Badioli, M.; Selvaggini, R.; Miniati, E. Macchioni, A. Simple and hydrolizable phenolic compounds in virgin olive oil. 3.

Spectroscopic characterization of the secoiridoid derivatives. *J. Agric. Food. Chem.* **1993**, *41*, 2228-2234.

(6) Servili, M.; Baldioli, M.; Mariotti, F.; Montedoro, G. F. Phenolic composition of olive fruit and virgin olive oil: distribution in the constitutive parts of fruit and evolution during oil mechanical extraction process. *Acta Hort.* **1999**, *474*, 609-619.

(7) Mateos, R.; Espartero, J. L.; Trujillo, M.; Rios, J. J.; Leon-Camacho, M.; Alcudia, F.; Cert, A. Determination of phenols, flavones, and lignans in virgin olive oils by solid-phase extraction and high performance liquid chromatography with diode array ultraviolet detection. *J. Agric. Food Chem.* **2001**, *49*, 2185-2192.

(8) Servili, M.; Montedoro, G. F. Contribution of phenolic compounds to virgin olive oil quality. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 602-613.

(9) Amiot, M. J.; Fleuriet, A.; Macheix, J. J. Importance and evaluation of phenolic compounds in olive oil during growth and maturation. *J. Agric. Food Chem.* **1986**, *34*, 823-825.

(10) Briante, R.; La Cara, F.; Febbraio, F.; Barone, R.; Piccialli, G.; Carolla, R.; Mainolfi, P.; De Napoli, L.; Patumi, M.; Fontanazza, G.; Nucci, R. Hydrolysis of oleuropein by recombinant β-glycosidase from hyperthermophilic archaeon sulfolobus solfataricus immobilized on chitosan matrix. *J. Biotechnol.* **2000**, *77*, 275-286.

(11) Gambacorta, A.; Tofani, D.; Bernini, R.; Migliorini D. High-yielding preparation of a stable precursor of hydroxytyrosol by total synthesis and from the natural glycoside oleuropein. *J. Agric. Food Chem.* **2007**, *55*, 3386-3391.

(12) Della Greca, M.; Monaco, P.; Pinto, G.; Pollio, A.; Previtera, L.; Temussi, F. Phytotoxicity of low-molecular-weight phenols from olive mill waste waters. *Bull. Environ. Contam. Toxicol.* **2001**, *67*, 352-359.

(13) Fiorentino, A.; Gentili, A.; Isidori, A.; Monaco, P.; Nardelli, A.; Parrella, A.; Temussi, F. Environmental effects caused by olive oil mill wastewaters: toxicity comparison of low-molecular weight phenol components. *J. Agric. Food Chem.*, **2003**, *51*, 1005-1009.

(14) Visioli, F.; Galli, C. Olive oil phenols and their potential effects on human health. *J. Agric. Food Chem.* **1998**, *46*, 4292-4296.

(15) Bernini, R.; Merendino, N.; Romani, A.; Velotti, F. Naturally occurring hydroxytyrosol: synthesis and anticancer potential. *Curr. Med. Chem.* **2013**, *20*, 655-670 and references therein.

(16) Hu, T.; He, X. W.; Jiang, J. G.; Xu, X. L. Hydroxytyrosol and its potential therapeutic effects. *J. Agric. Food Chem.* **2014**, *62*, 1449-1455.

(17) Capasso, R.; Evidente, A.; Avolio, S.; Solla, F. A Highly convenient synthesis of hydroxytyrosol and its recovery from agricultural waste waters. *J. Agric. Food Chem.* **1999**, *47*, 1745-1748.

(18) Espin, J. C.; Soler-Rivas, C.; Cantos, E.; Tomas-Barberan, F. A.; Wichers, H. J. Synthesis of the antioxidant hydroxytyrosol using tyrosinase as biocatalyst. *J. Agric. Food Chem.* **2001**, *49*, 1187-1193.

(19) Bernini R.; Mincione, E.; Barontini, M.; Crisante F. Convenient synthesis of hydroxytyrosol and its lipophilic derivatives from tyrosol or homovanillyl alcohol. *J. Agric. Food Chem.* **2008**, *56*, 8897-8904.

(20) Piersanti, G.; Retini, M.; Espartero, J. L. Madrona, A.; Zappia, G. An efficient, economical synthesis of hydroxytyrosol and its protected forms via Baeyer-Villiger oxidation. *Tetrahedron Lett.* **2011**, *52*, 4938-4940.

(21) Zhang, Z.-L.; Chen, J.; Xu, Q.; Rao, C.; Qiao, C. Efficient synthesis of hydroxytyrosol from 3,4-dihydroxybenzaldehyde. *Synth. Comm.* **2012**, *42*, 794-798.

(22) Deffieux, D.; Gossart, P.; Quideau, S. Facile and sustainable synthesis of the natural antioxidant hydroxytyrosol. *Tetrahedron Lett.* **2014**, *55*, 2455-2458.

(23) Allouche, N.; Fki, I.; Sayadi, S. Toward a high yield recovery of antioxidants and purified hydroxytyrosol from olive mill wastewaters. *J. Agric. Food Chem.* **2004**, *52*, 267-273.

(24) Sabatini, N. Recent patents in olive oil industry: new technologies for the recovery of phenols compounds from olive oil, olive oil industrial byproducts and waste waters. *Recent Pat. Food Nutr. Agric.,* **2010**, *2*, 154-159.

(25) Pizzichini, M.; Russo, C. Process for recovering the components of olive mill wastewater with membrane technologies. WO 2005123603 A1; December 29, 2005.

(26) Pizzichini, D.; Russo, C.; Vitagliano, M.; Pizzichini, M.; Romani, A.; Ieri, F.; Pinelli, P.; Vignolini, P. Process for producing concentrated and refined

actives from tissues and by-products of *Olea europaea* with membrane technologies. EP 2338500 A1; June 29, 2011.

(27) Fernandez-Bolanos, J. G.; Lopez, O.; Fernandez-Bolanos, J.; Rodriguez-Gutierrez, G. Hydroxytyrosol and derivatives: isolation, synthesis and biological properties. *Curr. Org. Chem.* **2008**, *12*, 442-463.

(28) Chillemi, R.; Sciuto, S.; Spatafora, C.; Tringali, C. Hydroxytyrosol lipophilic analogues: synthesis, radical scavenging activity and human cell oxidative damage protection. In *Olives and Olive Oil in Health and Disease Prevention*. Preedy, V.; Watson, R.; Ed. Academic Press, 2010; pp. 1233-1243.

(29) Fernandez-Bolanos, J. G.; Lopez, O.; Lopez-Garcia, M. A.; Marset, A. Biological properties of hydroxytyrosol and its derivatives. In *Olive Oil - Constituents, Quality, Health Properties and Bioconversions*. Boskou, D. Ed. InTech Europe, 2012; pp. 375-396.

(30) Manna, C.; Migliardi, V.; Sannino, F.; De Martino, A.; Capasso, R. Protective effects of synthetic hydroxytyrosol acetyl derivatives against oxidative stress in human cells. *J. Agric. Food Chem.* **2005**, *53*, 9602-9607.

(31) Brenes, M.; Garcia, A.; Garcia, P.; Rios, J. J.; Garrido, A. Phenolic compounds in Spanish olive oils. *J. Agric. Food Chem.* **1999**, *47*, 3535-3540.

(32) Romero C.; Medina, E.; Vargas, J.; Brenes, M.; de Castro, A. In vitro activity of olive oil polyphenols against Helicobacter pylori. *J. Agric. Food Chem.* **2007**, *52*, 1973-1979.

(33) Gordon, M. H.; Paiva-Martins, F.; Almeida, M. Antioxidant activity of hydroxytyrosol acetate compared to that of other olive oil polyphenols. *J. Agric. Food. Chem.* **2001**, *49*, 2480-2485.

(34) Capasso, R.; Sannino, F.; De Martino, A.; Manna, C. Production of triacetylhydroxytyrosol from olive mill waste waters for use as stabilized bioantioxidant. *J. Agric. Food Chem.* **2006**, *54*, 9063.

(35) Gambacorta, A.; Tofani, D.; Migliorini, A. High-yielding synthesis of methyl orthoformate-protected hydroxytyrosol and its use in preparation of hydroxytyrosyl acetate. *Molecules*, **2007**, *12*, 1762-1770.

(36) Appendino G, Minassi A, Daddario N, Bianchi F, Tron G. C. Esterification of phenolic acids and alcohols. *Org. Lett.* **2002**, *4*, 3839-3841.

(37) Torregiani, E.; Seu, G.; Minassi, A.; Appendino, G. Cerium(III) chloride-promoted chemoselective esterification of phenolic alcohols. *Tetrahedron Lett.* **2005**, *46*, 2193-2196.

(38) Alcudia, F.; Cert, A.; Espartero, J. L.; Mateos, R.; Trujillo, M. Method of preparing hydroxytyrosol esters, esters thus obtained and use of same. PCT WO 2004/005237; March 25, 2004.

(39) Trujillo, M.; Mateos, R.; Collantes de Teran, L.; Espartero, J. L.; Cert, R.; Jover, M.; Alcudia, F.; Bautista, J.; Cert, A.; Parrado, J. Antioxidant activity in lipid matrices and biological systems. *J. Agric. Food Chem.* **2006**, *54*, 3779-3785.

(40) Figueroa-Espinoza, M.-C.; Villeneuve, P. Phenolic acids enzymatic lipophilization. *J. Agric. Food Chem.* **2005**, *53*, 2779-2787.

(41) Villeneuve, P. Lipases in lipophilization reactions. *Biotechol. Adv.* **2007**, *25*, 515-536.

(42) Grasso, S.; Siracusa, L.; Spatafora, C.; Renis, M.; Tringali, C. Hydroxytyrosol lipophilic analogues: enzymatic synthesis, radical scavenging activity and DNA oxidative damage protection. *Bioorg. Chem.* **2007**, *35*, 137-152.

(43) Buisman G. J. H; van Heleren, C. T.W.; Kramer, G. F. H.; Veldsink, J. W.; Derksen, J. T. P.; Cuperus F. P. Enzymatic esterifications of functionalized phenols for the synthesis of lipophilic antioxidants. *Biotechnol Lett.* **1998**, *20*, 131-136.

(44) Bouallagui, Z.; Bouaziz, M.; Lassoued, S.; Engasser, J. M.; Ghoul, M.; Sayadi, S. Hydroxytyrosol acyl esters: biosynthesis and activities. *Appl. Biochem. Biotechnol.* **2011**, *163*, 592-599.

(45) Torres de Pinedo, A.; Penalver, P.; Rondon, D.; Morales, J. C. Efficient lipase-catalyzed synthesis of new lipid antioxidants based on a catechol structure. *Tetrahedron* **2005**, *61*, 7654-7660.

(46) Ozanne, A.; Pouységu, L.; Depernet, D.; Francois, B.; Quideau, S. A Stabilized formulation of IBX (SIBX) for safe oxidation reactions including a new oxidative demethylation of phenolic methyl aryl ethers. *Org. Lett.* **2003**, *5*, 2903-2906.

(47) Magdziak, D.; Rodriguez, A. A.; Van De Water, R. W.; Pettus, T. R. R. Regioselective oxidation of phenols to *o*-quinones with *o*-iodoxybenzoic acid. *Org. Lett.* **2002**, *4*, 285-288.

(48) Bernini, R.; Fabrizi, G.; Pouysegu, L.; Deffieux, D.; Quideau, S. Synthesis of biologically active catecholic compounds via *ortho*-selective

oxygenation of phenolic compounds using hypervalent iodine(V) reagents. *Curr. Org. Synth.* **2012**, *9*, 650-669 and references therein.

(49) Bernini, R.; Mincione, E.; Barontini, M.; Crisante, F. Procedimento per la preparazione di derivati dell'idrossitirosolo e di idrossitirosolo. IT/0001381959; September 27, 2010.

(50) Bernini, R.; Mincione, E.; Barontini, M.; Crisante, F. Procedimento per la preparazione di derivati dell'idrossitirosolo e di idrossitirosolo via demetilazione ossidativa. IT/0001382586; December 22, 2010.

(51) Bernini, R.; Mincione, E.; Crisante, F.; Barontini, M.; Fabrizi, G.; Gentili, P. Chemoselective and efficient carboxymethylation of the alcoholic chain of phenols by dimethyl carbonate (DMC). *Tetrahedron Lett.* **2007**, *48*, 7000-7003.

(52) Bernini, R.; Crisante, F.; Merendino, N.; Molinari, R.; Soldatelli, M. C.; Velotti, F. Synthesis of a novel ester of hydroxytyrosol and α-lipoic acid exhibiting an antiproliferative effect on human colon cancer HT-29 cells. *Eur. J. Med. Chem.* **2011**, *46*, 439-446.

(53) Kaki, S. S.; Grey, C.; Adlercreutz, P. Bioorganic synthesis, characterization and antioxidant activity of esters of natural phenolics and α-lipoic acid. *J. Biotechnol.* **2012**, *157*, 344-349.

(54) Bernini, R.; Crisante, F.; Barontini, M.; Tofani, D.; Balducci, V.; Gambacorta, A. Synthesis and structure/antioxidant activity relationship of novel catecholic antioxidants structurally analogues to hydroxytyrosol and its lipophilic esters. *J. Agric. Food Chem.* **2012**, *60*, 7408-7416.

(55) Madrona, A.; Pereira-Caro, G.; Mateos, R.; Rodriguez, G.; Trujillo, M.; Fernandez-Bolanos, J.; Espartero, J. L. Synthesis of hydroxytyrosol alkyl ethers from olive oil waste waters. *Molecules* **2009**, *14*, 1762-1772.

(56) Sepporta, M. V.; Lopez-Garcia, M. A.; Fabiani, R.; Maya, I.; Fernandez-Bolanos, J. G. Enhanced chemopreventive activity of hydroxytyrosol on HL60 and HL60R cells by chemical conversion into thioderivatives. *Eur. J. Pharm. Sci.* **2013**, *48*, 790-798.

(57) Larghi E. L.; Kaufman T.S. The oxa-Pictet-Spengler cyclization: synthesis of isochromans and related pyran-type heterocycles. *Synthesis* **2006**, *2*, 187-220.

(58) Bianco, A.; Coccioli, F.; Guiso, M.; Marra, C. The occurrence in olive oil of a new class of phenolic compounds: hydroxy-isochromans. *Food Chem*. **2001**, *77*, 405-411.

(59) Guiso, M.; Marra, C.; Cavarischia, C. Isochromans from 2-(3,4-dihydroxy)phenylethanol. *Tetrahedron Lett.* **2001**, *42*, 6531-6534.

(60) Guiso, M.; Bianco, A.; Marra, C.; Cavarischia, C. One-pot synthesis of 6-hydroxyisochromans: the example of demethyl-oxa-coclaurine. *Eur. J. Org. Chem.* **2003**, 3407-3411.

(61) Bernini, R.; Crisante, F.; Fabrizi, G.; Gentili, P. Convenient synthesis of 1-aryl-dihydroxyisochromans exhibiting antioxidant activity. *Curr. Org. Chem.* **2012**, *16*, 1051-1057.

(62) Nasri, H.; Baradaran, A.; Shirzad, H.; Rafieian-Kopaei, M. New concepts in nutraceuticals as alternative for pharmaceuticals. *Int. J. Prev. Med*. **2014**, *5*, 1487-1499.

(63) Schumacker, P. T. Reactive oxygen species in cancer: a dance with the devil. *Cancer Cell.* **2015**, *27,* 156-157.

(64) Sayin, V. I.; Ibrahim, M. X.; Larsson, E.; Nilsson, J. A.; Lindahl, P.; Bergo, M. O. Antioxidants accelerate lung cancer progression in mice. *Sci. Transl. Med.* **2014**, *6,* 221-215.

(65) Tofani, D.; Balducci, V.; Gasperi, T.; Incerpi, S.; Gambacorta, A. Fatty acid hydroxytyrosyl esters: structure/antioxidant activity relationship by ABTS and in cell-culture DCF assays. *J. Agric. Food Chem.* **2010**, *58*, 5292-5299.

(66) Pereira-Caro, G.; Sarriá, B.; Madrona, A.; Espartero, J. L.; Goya, L.; Bravo, L.; Mateos, R. Alkyl hydroxytyrosyl ethers show protective effects against oxidative stress in HepG2 cells. *J. Agric. Food Chem.* **2011**, *59*, 5964-5976.

(67) Guerrero, A; De La Cruz J. P.; Munoz-Marin J.; Lopez-Villodres J. A.; Madrona A.; Espartero J. L.; Gonzalez-Correa, J. A. Neuroprotective effect of alkyl hydroxytyrosyl ethers in rat brain slices subjected to a hypoxia-reoxygenation model. *Food Chem*. **2012**, *134*, 2176-2183.

(68) Laguerre, M.; Bayrasy, C.; Panya, A.; Weiss, J.; McClements, D. J.; Lecomte, J.; Decker, E. A.; Villeneuve, P. What makes good antioxidants in lipid-based systems? The next theories beyond the polar paradox. *Crit. Rev Food Sci. Nutr.* **2015**, *55*, 183-201.

(69) Porter, W. L.; Black, E. D.; Drolet, A. M. Use of polyamide oxidative fluorescence test on lipid emulsions: contrast in relative effectiveness of antioxidants in bulk versus dispersed systems J. *Agric. Food Chem*. **1989**, *37*, 615-624.

(70) Laguerre, M.; Giraldo, L. J.; Lecomte, J.; Figueroa-Espinoza, M. C.; Baréa, B.; Weiss, J.; Decker, E. A.; Villeneuve, P. Chain length affects antioxidant properties of chlorogenate esters in emulsion: the cutoff theory behind the polar paradox. *J Agric Food Chem*. **2009**, *57*, 11335-11342.

(71) Laguerre, M.; López Giraldo, L. J.; Lecomte, J.; Figueroa-Espinoza, M. C.; Baréa, B.; Weiss, J.; Decker, E. A.; Villeneuve. P. Relationship between hydrophobicity and antioxidant ability of "phenolipids" in emulsion: a parabolic effect of the chain length of rosmarinate esters. *J Agric Food Chem.* **2010,** *58*, 2869-2876.

(72) Hanahan, D.; Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell*. **2011**, *144*, 646-674.

(73) Warfel, N. A; El-Deiry, W. S. p21WAF1 and tumourigenesis: 20 years after. *Curr. Opin. Oncol*. **2013**, *25*, 52-58.

(74) Adams, J. M; Cory, S. Bcl-2regulated apoptosis: mechanism and therapeutic potential. *Curr. Opin. Immunol*. **2007**, *19*, 488-496.

(75) Mateos, R.; Pereira-Caro, G.; Saha, S.; Cert, R.; Redondo-Horcajo, M.; Bravo, L.; Kroon, P. A. Acetylation of hydroxytyrosol enhances its transport across differentiated Caco-2 cell monolayers. *Food Chemistry* **2011**, *125*, 865-872.

(76) Mateos, R.; Pereira-Caro, G.; Bacon, J. R.; Bongaerts, R.; Sarriá, B.; Bravo, L.; Kroon, P. A. Anticancer activity of olive oil hydroxytyrosyl acetate in human adenocarcinoma Caco-2 cells. *J. Agric. Food Chem.* **2013**, *61*, 3264-3269.

(77) Burattini, S.; Salucci, S.; Baldassarri, V.; Accorsi, A.; Piatti, E.; Madrona, A.; Espartero, J. L.; Candiracci, M.; Zappia, G.; Falcieri, E. Anti-apoptotic activity of hydroxytyrosol and hydroxytyrosyl laurate. *Food Chem. Toxicol.* **2013**, *55*, 248-256.

(78) Pereira-Caro, G.; Mateos, R.; Traka, M. H.; Bacon, J. R; Bongaerts, R.; Sarriá, B; Bravo, L.; Kroon, P. A. Hydroxytyrosyl ethyl ether exhibits stronger intestinal anticarcinogenic potency and effects on transcript profiles compared to hydroxytyrosol. *Food Chem* **2013**, *138*, 1172-1182.

(79) Calderón-Montaño, J. M.; Madrona, A.; Burgos-Moron, E.; Orta, M. L.; Mateos, S.; Espartero, J. L.; Lopez-Lazaro, M. Selective cytotoxic activity of new lipophilic hydroxytyrosol alkyl ether derivatives. *J. Agr. Food Chem.* **2013**, *61*, 5046-5053.

(80) Dunn, G. P.; Bruce, A. T.; Ikeda, H.; Old, L. J.; Schreiber, R. D. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat. Immunol*. **2002**, *3*, 991-998.

(81) Coussens, L. M.; Werb, Z. Inflammation and cancer. *Nature* **2002**, *420*, 860-867.

(82) Morrell, C. N.; Aggrey, A. A.; Chapman, L. M.; Modjeski, K. L. Emerging roles for platelets as immune and inflammatory cells. *Blood* **2014**, *123*, 2759-2767.

(83) Carr, B. I. Platelets and cancer. *Semin Oncol*. **2014**, *41*, 300-301.

(84) Dovizio, M.; Alberti, S.; Guillem-Llobat, P.; Patrignani, P. Role of platelets in inflammation and cancer: novel therapeutic strategies. *Basic Clin. Pharmacol. Toxicol*. **2014**, *114*, 118-127.

(85) Rothwell, P. M.; Fowkes F. G.; Belch, J. F.; Ogawa, H.; Warlow, C. P.; Meade, T.W. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomized trials. *Lancet* **2011**, *377*, 31-41.

(86) Rothwell, P. M.; Wilson, M.; Price, J. F.; Belch, J. F.; Meade, T.W.; Mehta, Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet* **2012**, *379*, 1591-1601.

(87) Dovizio, M.; Bruno, A.; Tacconelli, S.; Patrignani, P. Mode of action of aspirin as a chemopreventive agent. *Recent Results Cancer Res*. **2013**, *191*, 39-65.

(88) Capone, M. L.; Tacconelli, S.; Sciulli, M.G.; Grana, M.; Ricciotti, E.; Minuz, P.; Di Gregorio, P.; Merciaro, G.; Patrono, C.; Patrignani, P. Clinical pharmacology of platelet, monocyte, and vascular cyclooxygenase inhibition by naproxen and low-dose aspirin in healthy subjects. *Circulation* **2004**, *109*, 1468-1471.

(89) Patrono, C.; García Rodríguez, L. A.; Landolfi, R.; Baigent, C. Lowdose aspirin for the prevention of atherothrombosis. *N. Engl. J. Med*. **2005**, *353*, 2373-2383.

(90) Noy, R.; Pollard, J. W. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* **2014**, *41*, 49-61.

(91) Bulotta, S.; Celano, M.; Lepore, S. M.; Montalcini, T.; Pujia, A.; Russo, D. Beneficial effects of the olive oil phenolic components oleuropein and hydroxytyrosol: focus on protection against cardiovascular and metabolic diseases. *J. Transl. Med.* **2014**, *12*, 219.

(92) Togna, G. I.; Togna, A. R.; Franconi, M.; Marra, C.; Guiso, M. Olive oil isochromans inhibit human platelet reactivity. *J. Nutr.* **2003**, *133*, 2532-2536.

(93) Pratico, D.; Iuliano, L.; Pulcinelli, F. M.; Bonavita, M. S.; Gazzaniga, P. P.; Violi, F. Hydrogen peroxide triggers activation of human platelets selectively exposed to nonaggregating concentrations of arachidonic acid and collagen. *J. Lab. Clin. Med*. **1992**, *119*, 364-370.

(94) Kramer, R. M.; Roberts, E. F.; Manetta, J. V.; Hylsop, P. A.; Jakubowski, J. A. Thrombin-induced phosphorylation and activation of Ca-sensitive cytosolic phospholipase A2 in human platelets. *J. Biol. Chem*. **1993**, *268*, 26796-26804.

(95) Petroni, A.; Blasevich, M.; Salami, M.; Papini, N.; Montedoro, G. F.; Galli, C. Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thromb. Res*. **1995**, *78*, 151-160.

(96) Trefiletti, G.; Togna, A. R.; Latina, V.; Marra, C.; Guiso, M.; Togna, G. 1-Phenyl-6,7-dihydroxy-isochroman suppresses lipopolysaccharide-induced pro-inflammatory mediator production in human monocytes. *Br. J. Nutr*. **2011**, *106*, 33-36.

(97) Zhang, X.; Cao, J.; Zhong, L. Hydroxytyrosol inhibits pro-inflammatory cytokines, iNOS, and COX-2 expression in human monocytic cells. *Naunyn Schmiedebergs Arch. Pharmacol*. **2009**, *379*, 581-586.

(98) González-Correa, J. A.; López-Villodres, J. A.; Asensi, R.; Espartero, J. L.; Rodríguez-Gutiérrez, G.; De La Cruz, J.P. Virgin olive oil polyphenol hydroxytyrosol acetate inhibits in vitro platelet aggregation in human whole blood: comparison with hydroxytyrosol and acetylsalicylic acid. *Br. J. Nutr.* **2009**, *101,* 1157-1164.

(99) González-Correa, J. A.; Navas, M. D.; Muñoz-Marín, J.; Trujillo, M.; Fernández-Bolaños, J.; de la Cruz, J.P. Effects of hydroxytyrosol and hydroxytyrosol acetate administration to rats on platelet function compared to acetylsalicylic acid. *J. Agric. Food Chem.* **2008**, *56*, 7872-7876.

(100) Madajka, M.; Korda, M.; White, J.; Malinski, T. Effect of aspirin on constitutive nitric oxide synthase and the bioavailability of NO. *Thromb. Res.* **2003**, *110*, 317-321.

(101) Aparicio-Soto, M.; Sánchez-Fidalgo, S.; González-Benjumea, A.; Maya, I.; Fernández-Bolaños, J.G.; Alarcón-de-la-Lastra, C. Naturally occurring hydroxytyrosol derivatives: hydroxytyrosyl acetate and 3,4-dihydroxyphenylglycol modulate inflammatory response in murine peritoneal macrophages. Potential utility as new dietary supplements. *J. Agric. Food Chem*. **2015**, 63, 836-846.

(102) Sánchez-Fidalgo, S.; Villegas, I.; Aparicio-Soto, M.; Cárdeno, A.; Rosillo, M.Á.; González-Benjumea, A.; Marset, A.; López, Ó.; Maya, I.; Fernández-Bolaños, J.G.; Alarcón de la Lastra, C. Effects of dietary virgin olive oil polyphenols: hydroxytyrosyl acetate and 3, 4-dihydroxyphenylglycol on DSS-induced acute colitis in mice. *J. Nutr. Biochem.* **2015**, 26, 513-520.

(103) Reyes, J. J.; De La Cruz, J. P.; Muñoz-Marin, J.; Guerrero, A.; Lopez-Villodres, J. A.; Madrona, A.; Espartero, J. L.; González-Correa, J.A. Antiplatelet effect of new lipophilic hydroxytyrosol alkyl ether derivatives in human blood. *Eur. J. Nutr*. **2013**, *52*, 591-599.

(104) Muñoz-Marín, J.; De la Cruz, J. P.; Reyes, J. J.; López-Villodres, J. A.; Guerrero, A.; López-Leiva, I.; Espartero, J.L.; Labajos, M. T.; González-Correa, J.A. Hydroxytyrosyl alkyl ether derivatives inhibit platelet activation after oral administration to rats. *Food Chem. Toxicol.* **2013**, *58*, 295-300.

(105) Mantovani, A.; Allavena, P.; Sica, A.; Balkwill, F. Cancer-related inflammation. *Nature* **2008**, 454, 436-444.

(106) Misra, S.; Sharma, K. COX-2 signaling and cancer: new players in old arena. *Curr Drug Targets*. **2014**, 15, 347-359.

(107) Pereira-Caro, G.; Sarriá, B.; Madrona, A.; Espartero, J. L.; Escuderos, M. E.; Bravo, L.; Mateos, R. Digestive stability of hydroxytyrosol, hydroxytyrosol acetate and alkyl hydroxytyrosol ethers. *Int. J. Food Sci. Nutr.* **2012**, *63*, 703-707.

(108) Barrera, G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. *ISRN Oncol.* **2012**, 137289, 21 pages.

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