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Structural characteristics that determine the inhibitory role of phenolic compounds on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) formation

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ABSTRACT

In an attempt to understand the structural characteristics of phenolic compounds that favour the inhibition of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) formation, this study analyzes the role of twenty-five phenolic compounds on the PhIP produced in phenylalanine/creatinine/oxidised lipid reaction mixtures. The results showed that phenols having two hydroxy groups at *meta* positions of the aromatic ring were the most efficient inhibitors. The presence of alkyl or carboxylic groups as additional substituents in the aromatic ring slightly reduced the inhibitory effect. On the other hand, the introduction of additional hydroxy and amino groups mostly cancelled the inhibitory effect, which was also mostly absent in *ortho* and *para* dihydroxy derivatives. In complex phenols, the presence of several rings with opposite effects produced a reduced inhibitory effect. All these results suggest that it is possible to predict if a phenolic derivative will inhibit the formation of PhIP, or not, based on its structure.

1. Introduction

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is one of the most abundant heterocyclic aromatic amines (HAAs) formed during thermal processing of proteinaceous foods, such as cooked beef, pork, chicken, and fish (Skog, Johansson, & Jägerstad, 1998). This HAA produces colon, prostate, and mammary gland tumors in rodents (Alaejos, Pino, & Afonso, 2008; Cheung, Loy, Li, Liu, & Yang, 2011; Choudhary, Sood, Donnell, & Wang, 2012), and the International Agency for Research on Cancer (IARC) has concluded that there is sufficient evidence in experimental animals for PhIP carcinogenicity (IARC, 1993). In addition, this compound is also considered as possibly carcinogenic to humans (IARC, 1993).

At present, PhIP is believed to be mainly produced from phenylalanine, creati(ni)ne and carbohydrates as a by-product of the Maillard reaction (Shioya, Wakabayashi, Sato, Nagao, & Sugimura, 1987). The reaction takes place in several steps, among which the formation of phenylacetaldehyde by phenylalanine degradation and the later reaction of the produced phenylacetaldehyde with creati(ni)ne seem to be key steps (Murkovic, Weber, Geiszler, Fröhlich, & Pfannhauser, 1999). Because conversion of phenylalanine into phenylacetaldehyde is not only produced by carbohydrates, but also by other reactive carbonyls such as those formed in the course of lipid oxidation (Hidalgo & Zamora, 2004), recent studies have shown that oxidised lipids are also able to contribute to PhIP formation (Zamora, Alcon, & Hidalgo, 2012, 2013a). Furthermore, the carbonyl compounds produced by thermal decomposition of some amino acids also contribute to PhIP formation (Zamora, Alcon, & Hidalgo, 2013b).

Inhibition of PhIP formation has been the objective of numerous studies, and many authors have shown that the use of phenolic compounds (and plant extracts rich in them) decreases the PhIP formed (see, for example, Damasius, Venskutonis, Ferracane, & Fogliano, 2011; Gibis & Weiss, 2012; Janoszka, 2010; Murkovic, Steinberger, & Pfannhauser, 1998; Quelhas et al., 2010). However, the inhibition of PhIP formation has not been well correlated with

Abbreviations: EC50, median effective concentration; HAAs, heterocyclic aromatic amines; IARC, International Agency for Research on Cancer; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

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the antioxidant/free radical-scavenging capacity of phenolic compounds and spice extracts (Cheng, Chen, & Wang, 2007; Damasius et al., 2011), which suggests that an antioxidant-independent mechanism should be playing a role in the inhibition of PhIP by phenolic compounds.

In an attempt to understand the structural characteristics of phenolic compounds that favour the inhibition of PhIP formation, this study analyzes the role of twenty-five phenolic compounds on the PhIP produced in phenylalanine/creatinine/oxidised lipid reaction mixtures. This model system was selected because it is an efficient PhIP producer (Zamora et al., 2012, 2013a).

2. Materials and methods

2.1. Materials

Twenty-five phenolic compounds were employed in this study. They are displayed in Fig. 1. As can be observed, most of them were simple compounds, having two or three hydroxy groups at different positions of the benzene ring. In addition, alkyl, methoxy, amino, and carboxylic groups were also present in some derivatives. Furthermore, some complex phenolic compounds, having more than one aromatic ring with hydroxy groups, were also studied for comparison purposes. To facilitate the study of the assayed phenols, these compounds have been classified into five groups: *o*-dihydroxy derivatives and analogues, *m*-dihydroxy derivatives and analogues, *p*-dihydroxy derivatives and analogues, trihydroxy derivatives, and complex phenols. All these compounds were purchased from commercial sources (see below).

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) was purchased from Toronto Research Chemicals (North York, Ontario, Canada). 4-Oxo-2-nonenal was prepared from 2-pentylfuran according to Shimozu, Shibata, Ojika, and Uchida (2009). All other chemicals were purchased from Aldrich (Milwakee, WI, USA), Sigma (St. Louis, MO, USA), Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany), and were of analytical grade.

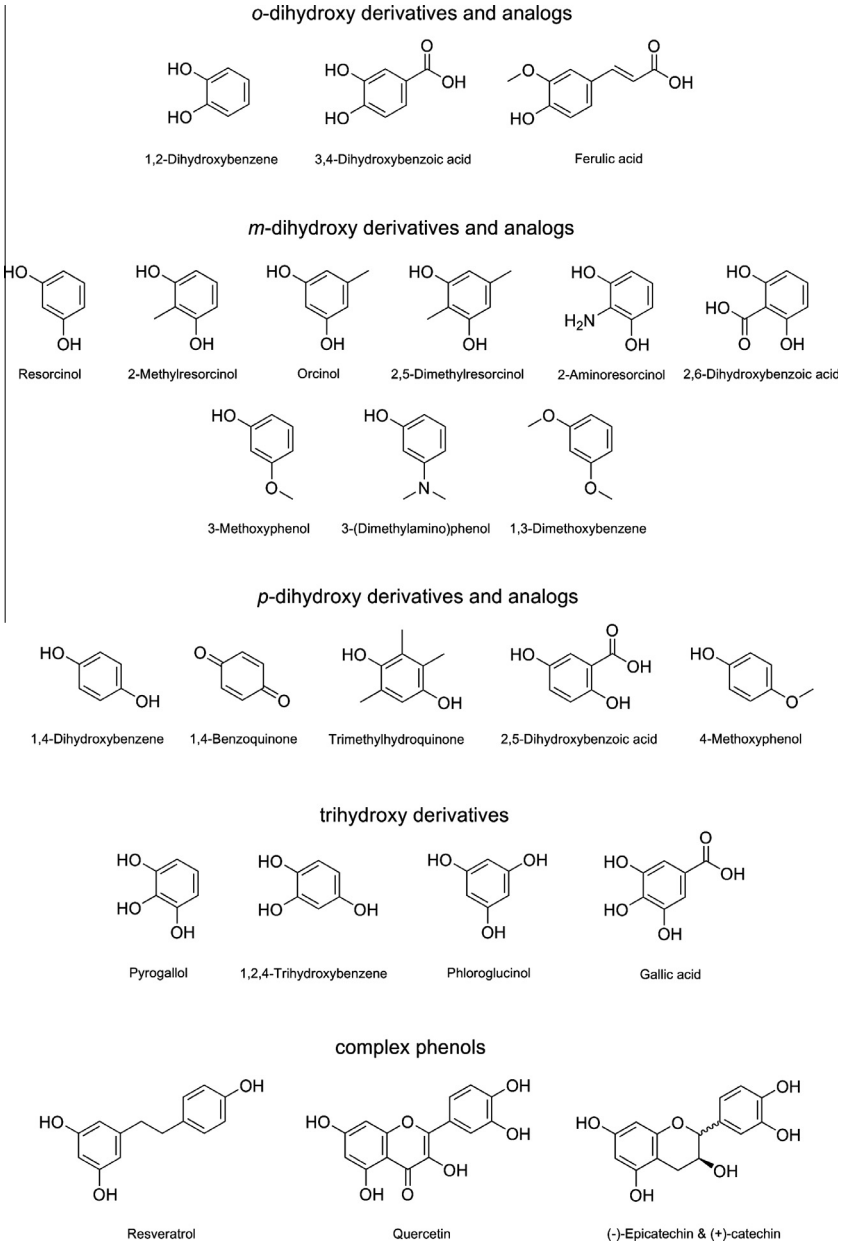

Fig. 1. Chemical structures of the phenolic compounds employed in this study. They have been classified according to the number and position of hydroxy groups in the benzene ring.