QSAR study of the potential clastogenic activity of phenolic acids

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Many phenolic acids are plant metabolites widely distributed throughout the vegetal kingdom. Recent interest in phenolic acids stems from their potential as bioactive constituents and their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases like neurodegenerative diseases and cancer. This communication aims to identify structural alerts associated with clastogenic activity that may present the phenolic acids. It was performed a QSAR study (*Quantitative structure-activity relationships*) that allowed to use a structure *vs* clastogenic activity relationship model, which encodes topological information to a substructural level, according to the TOPS-MODE approach (*Topological substructural molecular design*). The predictions were made using the technique of linear discriminant analysis. The used software was STATISTIC and MODESLAB. The model has presented an adequate probability of good classification for the external prediction set. It has been identified the relevant structural features to the activity under study, such as: amount and position of hydroxyl and/or methoxy groups. These structural modifications represented an indicator of the toxicity of these compounds and provide a strategy for the design of new derivatives devoid of this activity. This study can be of interest to better understand the properties of natural substances in food, and also in the development of functional foods or nutraceuticals and drug design.

Keywords: phenolic acids; QSAR study; clastogenic activity; nutraceuticals.

Introduction

Phenolic acids are bioactive non-nutrients presented in food that may display different activities, highlighting the antioxidant capacity (1-3). However, several studies show conflicting results, agreeing that the type, dose and the support matrix may be factors that impact on the balance between benefit or adverse health effects of these natural compounds (4). The reported pro-oxidant activity of these compounds under certain conditions (5), has generated the need for the study of such activity (6). These phenolic compounds can be incorporated into the human body through food, as nutraceutical (which may be part of *design foods*, such as functional foods) or as pharmaceuticals (as dietary supplements). To be used as nutraceuticals or pharmaceuticals in the treatment or prevention of chronic diseases such as cancer, degenerative diseases, etc., studies are needed to provide evidence of their benefit (7). In this sense, its security aspects, such as its bioavailability and metabolism, must be clarified (8). Due to its high structural variability, studies are needed to reveal the relationship between chemical structure and activity (9).

Structure-activity or toxicity studies so-called QSAR/QSTR methods (*Quantitative Structure-Activit/Toxicity Relationship*) are an alternative that enable reliable prediction of the activity or toxicity of a particular compound. In this field, there are few QSAR studies associated with the pro-oxidant activity of polyphenols and phenolic acids, which also use different molecular descriptors (10-14). Our working group has studied the relationship between: the pro-oxidant activity and possible DNA damage (by clastogenic activity) and chemical structure of the type of flavonoid polyphenolic compounds, because of its importance in the development of different pathologies such as cancer (9, 15). The present study aims to identify structural alerts associated with clastogenic activity and DNA damage that can present reported pro-oxidant phenolic acids, specifically benzoic acid derivatives, using topological information in a substructural level, by TOPS-MODE approach (*Topological Substructural Molecular Desing*).

Methods

It was prepared a database of chemical compounds with benzoic acid derivatives presenting pro-oxidant activity, which allowed, in a second stage, the development of the QSAR study. It was used the TOPS-MODE approach through the following methodology:

1. Obtaining molecular descriptors from molecular graphs, using software MODESLAB.

2. Theoretical statistical clastogenic model selection.

In this case, it was selected the model (Equation 1) of structure-activity clastogenic relationship proposed by Estrada *et al.* (2006), which has been internally and externally validated (9, 15, 16).

$$AC = 0.0091\,[\Omega(\mu_1^{PS})] - 1.5520 \times 10^{-4}\,[\Omega(\mu_5^{vdW})] + 0.148\,[\Omega(\mu_4^{Ch})] - 0.0021\,[\Omega(\mu_2^{PS})] + 2.6261 \times 10^{-4}\,[\Omega(\mu_3^{PS})] - 3.8422 \times 10^{-5}\,[\Omega(\mu_4^{PS})] + 1.1520 \times 10^{-4}\,[\Omega(\mu_4^{MR})] + 1.2011 \times 10^{-6}\,[\Omega(\mu_5^{PS})] - 9.8202 \times 10^{-5}\,[\Omega(\mu_5^{MR})] - 3.8263 \times 10^{-5}\,[\Omega(\mu_8^{H})] - 0.0626\,[\Omega(\mu_2^{Pol})] + 1.6689\,[\Omega(\mu_1^{Pol})] - 0.0078\,[\Omega(\mu_5^{Ch})] + 0.1123\,[\Omega(\mu_3^{Ch})] - 0.6517 \quad (1)$$

Legend: AC represents the clastogenic activity, Ω inside the brackets indicates that the variable is orthogonalized relative to the rest of the variables included in the model, μn are the spectral moments (topological molecular descriptors) and their exponents are the properties of links which have been weighted. Their corresponding statisticians are: *Wilks-λ* = 0.629; $F(14.194) = 8.142$; $D^2 = 2.353$; $p < 0.0000$.

3. Prediction of clastogenic activity of pro-oxidant compounds (virtual screening), which was performed by linear discriminant analysis (LDA) implemented in software STATISTIC. Classification in "inactive" or "active" was expressed as the percentage of the highest probability of belonging to either group (*posterior probability*).

4. Identification of structural alerts associated with clastogenicity.

This study was performed by analyzing the effects of substituents and structural features that characterize the benzoic acid derivatives and their probability of being active. It was necessary to design a series of 18 new compounds that allowed the analysis of the influence of the amount and position of each analyzed substituent. **Figure 1** shows a diagram with the methodology carried out in performing this work.

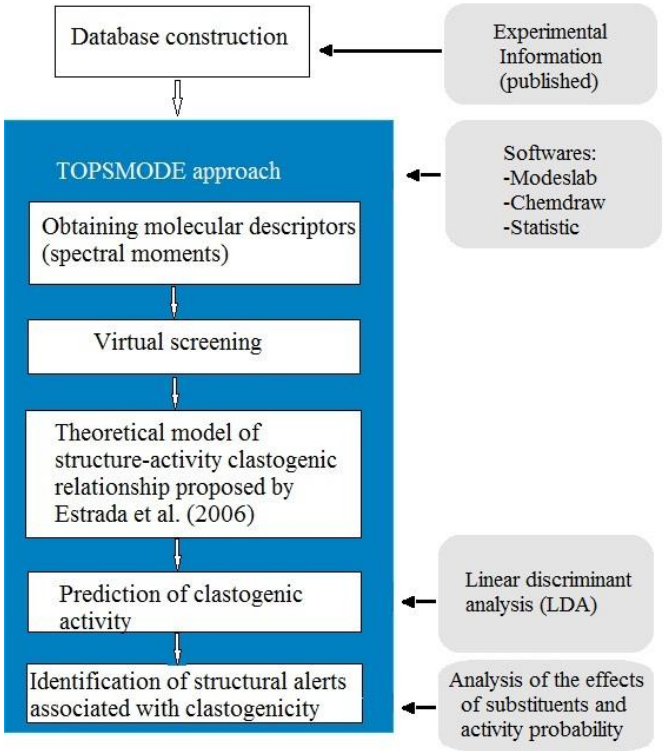


Fig. 1. Diagram sequences of the QSAR studies

Results and Discussion

Below in Tables 1 and 2 it is shown the database of benzoic acids with reported pro-oxidant and the prediction.

Table 1. Benzoic acids with reported pro-oxidant activity.

Name	CAS number	Smile	References*
salicylic acid	69- 72- 7	OC1=CC=CC=C1C(O)=O	(17)
m-hydroxybenzoic acid	99- 06- 09	O=C(O)C1=CC=CC(O)=C1	(17, 18)
p-hydroxybenzoic acid	99- 96- 7	O=C(O)C1=CC=C(O)C=C1	(17, 18)
protocatechuic acid	99- 50- 3	OC1=CC(C(O)=O)=CC=C1O	(18)
gallic acid	149- 91- 7	OC1=CC(C(O)=O)=CC(O)=C1O	(18)
vanillic acid	306- 08- 1	COC1=CC(C(O)=O)=CC=C1O	(17, 18)
syringic acid	530- 57- 4	COC1=CC(C(O)=O)=CC(OC)=C1O	(17, 18)
ellagic acid	476- 66- 4	O=C2OC1=C(C3=C2C=C(O)C(O)=C3OC4=O)C4=CC(O)=C1O	(18)

* extracted from (5).

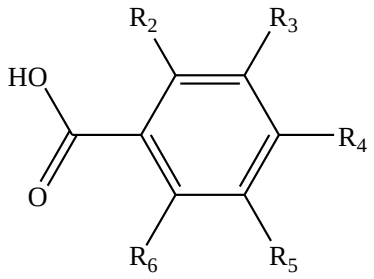
Benzoic acid derivatives with pro-oxidant report are not a numerous group of compounds. This may be because usually when discussing about the polyphenolic compounds in plants, the most described are flavonoids (19), accounting that they represent two-thirds of the phenols from the diet (20). However, although the phenolic acids are part of other more abundant phenolic compounds, interest has been to study the antioxidant activity by the health benefits they could bring.

The predicted compounds as active: vanillyl acid, syringic, gallic acid and ellagic could be cause chromosomal aberrations and DNA damaging clastogens. This prediction may be a way to explain the possible DNA damage of pro-oxidant substances. In the case of inactive compounds, it could be assumed that its pro-oxidant activity and the possible DNA damage cannot be explained by clastogenicity itself, but by other ways that have been described, such as cytotoxicity and mutagenicity (21). Model validation was performed from gallic acid, since it was in vivo demonstrated that this compound cause chromosomal aberrations (22), like the obtained prediction (54.66%). It means that the model has a 100% good rating for the series of prediction used.

To identify the structural alerts, different aspects were analyzed: a) effect of the amount of hydroxyl groups on the benzene ring, b) effect of the amount of methoxy groups in the ring, c) effect of the

position of the hydroxyl and methoxy groups in the ring (*ortho*, *meta* and *para*), d) effect of hydroxyl and methoxy groups isolated or accumulated, d) effect of the replacement of hydroxyl by methoxy substituents on the benzene ring. It was identified that the main structural feature of the benzoic acid derivatives which are associated with a maximum clastogenicity is the amount of hydroxyl and methoxy groups. It was observed that the active probability is proportional to the number of hydroxyl groups present in ring. From 3 hydroxyl groups, it is observed a positive classification of the compound (gallic acid). Methoxy groups will further increase clastogenic activity regardless of how they are near and far apart. Only in the presence of a methoxy group the compound is classified as positive (vanillic acid). As a result, the effect of the methoxyl groups can be associated with an increase in the clastogenic activity. The above behavior of hydroxyl and methoxy groups can be explained from electron charge parameter, which is represented by two molecular descriptors in Equation 1 ($^{-0.0078\left[\Omega\left(\mu_5^{Ch}\right)\right]}\,e^{+0.1123\left[\Omega\left(\mu_3^{Ch}\right)\right]}$), obtaining a positive differential load (+0.1045) which favors the methoxy group by having a greater availability of electronic charge on the oxygen atom by the presence of the methyl group.

Table 2. Prediction of benzoic acids in active and inactive in test sets.



Name	Substituents					Classification [a]	Probability [b] (%)
	R_2	R_3	R_4	R_5	R_6		
salicylic acid	OH	H	H	H	H	– 1	74.12
m-hydroxybenzoic acid	H	OH	H	H	H	– 1	76.43
p-hydroxybenzoic acid	H	H	OH	H	H	– 1	76.60
protocatechuic acid	H	OH	OH	H	H	– 1	63.81
gallic acid	H	OH	OH	OH	H	+1	54.66
vanillic acid	H	OCH_3	OH	H	H	+1	59.19
syringic acid	H	OCH_3	OH	OCH_3	H	+1	91.60
ellagic acid [c]	-	-	-	-	-	+1	93.22

[a] classification generated by LDA, where +1 corresponds to the group of active compounds and– 1 to the group of inactive compounds. [b] posterior probability using TOPS-MODE classification model (equation 1). [c] Polianular compound – benzoic acid derivative.

Conclusions

Important amounts of benzoic acid derivatives are distributed in some edible plants and processed food, although their reports as pro-oxidants are scarce. Regarding possible clastogenic activity when they behave as pro-oxidants, it can be summarized that the main structural feature of the benzoic acid derivatives which are associated with the clastogenicity effect is the amount of hydroxyl and methoxy groups in the structure. The number of methoxy groups required for the activity is less than hydroxyl groups. There are need 3 or 4 hydroxyl substitutions to improve the activity, whereas two methoxy substitutions increase this activity. The effect of the position of the hydroxyl and methoxy groups in the ring (*ortho, meta* and *para*), and the effect of hydroxyl and methoxy groups isolated or accumulated were important for the study. The position of the substituents did not affect for the clastogenic activity. Methoxy groups will increase clastogenic activity of the scaffold, regardless of how they are near or far apart. These structural modifications represent an indicator of the toxicity and also a good strategy for the design of new derivatives which do not exhibit this activity. This study represents an interesting tool to better understand the properties of natural substances in food. Therefore, it is also helpful in the development of functional foods or nutraceuticals and drug design.

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