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Antifumonisin activity of natural phenolic compounds A structure–property–activity relationship study

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ABSTRACT

Fumonisin B_1 (FB_1) is a *Fusarium* mycotoxin that has received considerable attention from food regulatory agencies, since it shows immunotoxic, neurotoxic, hepatotoxic, nephrotoxic and carcinogenic properties in animals. Although several publications have reported that some natural phenolic compounds can cause a reduction in mycotoxin production, little is known about the molecular properties related to their antitoxigenic activities. The objective of this work was to evaluate which of these molecular properties are important in antifumonisin activity, with this being the first structure–activity relationship study concerning the antimyctoxigenic activity of natural phenolic compounds. The results of the experimental determination of the FB_1 inhibition capacity for ten natural phenolic compounds revealed thymol, carvacrol, and isoeugenol followed by eugenol to be the most active antifumonisin compounds. Lipophilicity, molar refractivity and saturated area were demonstrated to be the molecular properties or descriptors which best explained the antifumonisin activity of these phenolic compounds. A mathematical expression, obtained by QSAR analysis, was able to predict the antifumonisin activity of other structurally related molecules. These findings could provide an important contribution in the search for new compounds with antifumonisin activity.

1. Introduction

Fungi of the genus *Fusarium* are widely found in plant debris and crop plants worldwide (Marasas et al., 1984), with several species from this genus being economically relevant because they can infect and cause tissue destruction in important crops such as corn, wheat and other small grains. Moreover, they can produce mycotoxins on the crops in the field and in stored grains. *Fusarium verticillioides* (Sacc.) Niremberg (e.g. *F. moniliforme* Sheldon) is one of the most common fungal pathogens associated with maize worldwide. Under favorable conditions, it can cause seedling diseases, root rot, stalk rot, ear rot and it is also considered to be an important producer of fumonisin mycotoxins in maize (Munkvold and Desjardins, 1997). Fumonisin B_1 (FB_1) is generally the most abundant fumonisin analogue (Leslie et al., 1992), and has received considerable attention from food regulatory agencies since it shows immunotoxic, neurotoxic, hepatotoxic, nephrotoxic and carcinogenic properties in animals. A strong correlation between the consumption of maize highly contaminated with fumonisins and a high-incidence of human esophageal cancer has been detected in various regions of the world (Stockmann-Juvala and Savolainen, 2008, and references therein). Consequently, fumonisin B_1 has been classified by the International Agency for Research on Cancer (IARC) as a Group 2B substance (potentially carcinogenic) (WHO-IARC, 2002).

The use of synthetic fungicides is a common storage technology to protect food products from deterioration by fungi and mycotoxin contamination. However, because most of the synthetic fungicides cause residual toxicity in grains and can contribute to the development of fungal resistance (Srivastava et al., 2008), consumer pressure to reduce or eliminate chemically synthesized additives has generated interest in the use of natural occurring compounds (Sofos et al., 1998) to reduce fungal growth and mycotoxin production. Nevertheless, despite several publications having reported that some phenolic compounds can reduce mycotoxin production (Dambolena et al., 2008; Samapundo, et al., 2007) little is known about the molecular properties related to their antitoxigenic activity.

The quantitative structure–activity relationship (QSAR) is a mathematical expression by which the chemical structure is quantitatively correlated with well defined processes, such as biological activity or chemical reactivity. For isolated receptors, cellular systems, and *in vivo* (Verma and Hansch, 2006), a variety of QSAR studies have been performed in an effort to predict the biological activity, establish the mechanisms involved and to understand the chemical–biological interactions in the drug-design process. QSARs describing the phenolic antifungal properties on wood-decaying fungi (Voda et al., 2004), of apoptotic properties on leukemia cells, cytotoxicity on ovarian cancer cells (Verma and Hansch, 2006) and antibacterial activities (Greenberg et al., 2008; Shapiro and Guggenheim, 1998)

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have also been reported. However, no QSAR studies on mycotoxin production have yet been performed.

The objective of this investigation was to evaluate which of the molecular properties of the natural phenolic compounds are important in antifumonisin activity. In the present work, we performed SAR and QSAR studies on the inhibition of fumonisin production by natural phenolic compounds, which might serve as a guide for the rational design of future inhibitors.

2. Material and methods

2.1. Material

The compounds 2-methyl-5-propan-2-ylphenol (carvacrol), 5-methyl-2-propan-2-ylphenol (thymol), 2-methylphenol (ortho-cresol), 3-methylphenol (meta-cresol), 4-methylphenol (para-cresol), 2-methoxy-4-prop-2-enylphenol (eugenol), 2-methoxy-4-[(E)-prop-1-enyl]phenol (isoeugenol), 2-methoxy-4-methylphenol (creosol), 2-methoxyphenol (guaiacol) and 4-hydroxy-3-methoxybenzaldehyde (vanillin) were purchased from Fluka-Kahlbaum-Germany (Fig. 1).

2.2. Fungal strain

A isolate of *F. verticillioides* MRC 4316 from the Program on Mycotoxins and Experimental Carcinogenesis, Tygerberg, Republic of South Africa (PROMEC), grown on carnation leaf agar by monosporic isolation, was used in all the experiments. This isolate is a good fumonisin producer in liquid culture (Vismer et al., 2004).

2.3. Effect of phenolic compounds on FB_1 production

A conidia suspension (1×10^6/ml) prepare with a *F. verticillioides* culture grown for 1 week in Czapek-dox agar and sterile water was used as the inoculum. An aliquot (0.5 ml) of this conidia suspension was inoculated into 50 ml Myro liquid medium (Blackwell et al., 1994) and incubated in the dark at 28°C for 21 days. The phenolic compounds were dissolved with DMSO, in order to obtain concentrations of 1.00 mM in the culture medium. As control, a compound-free medium with a 25 µl final concentration of DMSO was used. The solutions were added to the different flasks on the fifth day post-inoculation. Five replications of each treatment were performed, with this experimental procedure being repeated.

2.4. Fumonisin B_1 quantification

Samples (1000 µl) from the liquid cultures were centrifuged for 15 min at 9000 *g*. The supernatants obtained were diluted with acetonitrile (1:1), and the quantification of the samples was performed following a methodology proposed by Shephard et al. (1990). Briefly, an aliquot (50 µl) was derivatized with 200 µl of a solution prepared by adding 5 ml of 0.1 M sodium tetraborate and 50 µl of 2-mercaptoethanol to 1 ml of methanol containing 40 mg of *o*-phthaldialdehyde. The derivatized samples were then analyzed by means of a Hewlett Packard HPLC equipped with a fluorescence detector. The wavelengths used for excitation and emission were 335 nm and 440 nm, respectively. An analytical reverse phase column C18 (150 mm × 4.6 mm internal diameter and 5 µm particle size) connected to a precolumn C18 (20 mm × 4.6 mm and 5 µm particle size) was also used. The mobile phase was methanol and NaH_2PO_4 0.1 M (75:25), with the pH being set at 3.35 ± 0.2 with orthophosphoric acid and a flow rate of 1.5 ml/min used. The quantification of fumonisin B_1 was carried out by comparing the peak areas obtained from samples with those corresponding to the analytical standards of FB_1 (PROMEC, Program on Mycotoxins and Experimental Carcinogenesis, Tygerberg, Republic of South Africa).

2.5. Free radical-scavenging activity

The radical scavenger activity of the phenolic compounds was tested by means of a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Briefly, 350 µl of DPPH solution (0.022% in MeOH) was added to a range of solutions at different concentrations (fifteen serial 2-fold dilutions to give a final range of 200 to 0.015 mM) for each phenolic compound to be tested in methanol (1610 µl). Absorbance at 517 nm was determined 30 min after the addition of compound solutions and activity was calculated as a percentage. Gallic acid was used as the positive control (five serial dilutions to give a final range of 0.1 to 0.0063 mM) (Gálvez et al., 2005).

2.6. Partition coefficients by IAM–HPLC method

The determination of the Capacity factor by IAM–HPLC was performed following a methodology proposed by Reiner et al. (2009). Briefly, methanol solutions of 10 mM of each compound were diluted 1/10, with the solvent used as the mobile phase and injected in a IAMDD2 Fast Screen Mini Column (30mmlength and

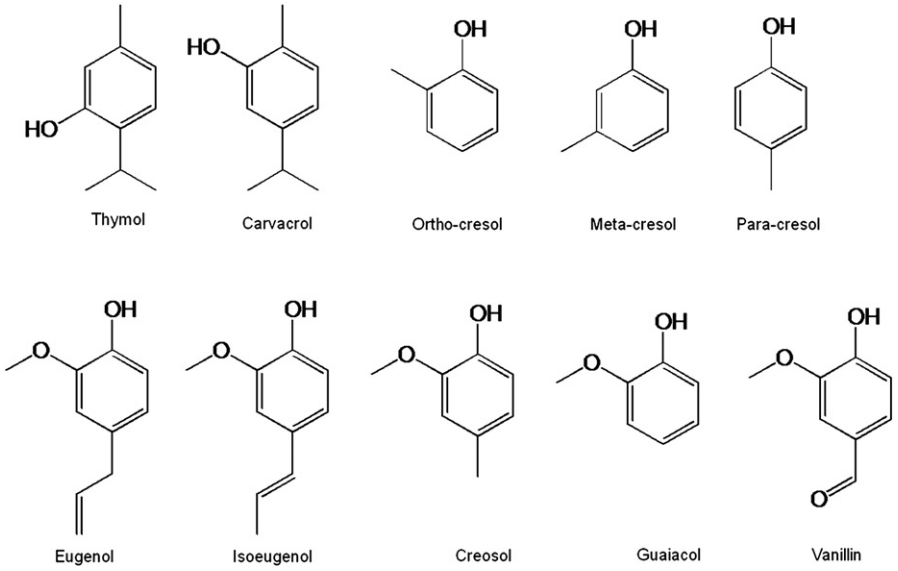

Fig. 1. Chemical structures of natural phenolic compounds studied in the present work.

4.6mmi.d., Regis Technology, Morton Grove, IL, USA), which contained 11-carboxylundecylphosphocholine covalently bound to the silica material and end-capped with the C_3- and C_{10}-alkyl groups. The capacity factors (k) were calculated from the retention times as follows: $k=(tr-t0)/t0$, where tr is the retention time of the compound and t0 is the dead time of the column (which is equivalent to the retention time of a substance which is not retained). The experiments were performed with two different mobile phases: pure water or acetonitrile (ACN) 10% (v/v), with a flow rate of 0.2 ml/min. All retention measurements by HPLC were made with a PerkinElmer Series 200HPLC apparatus (Shelton, CT, USA) equipped with a UV detector set at 280 nm. An injector with a 20 μl sample loop was used for all sample injections, with duplicate injections also being performed.

2.7. Molecular modelling and calculation of molecular parameters

Molecular structures of the natural phenolic compounds were constructed using the molecular modelling program CS Chem3D 3.5.1, and the lowest energy 3-D conformations of the molecules were determined using the energy minimization function of the program. The MM2 Force Field and semi-empirical quantum mechanics calculations of the molecular parameters were performed using the MOPAC 3.5.1 module with the MNDO potential function (Cambridge Soft Corporation, MA, USA).

After energy minimization, these structures were transferred to different programs (PCModels, ACD Laboratories, MOL Inspiration, Chemaxon and Cambridge Soft) and the following molecular parameters were calculated for each molecule: total area, polar area (PSA), unsaturated area and saturated area by PCModels; molecular volume (cubic angstroms), Log P, surface tension, parachor and Pka by ACD Laboratories; hydrogen bond acceptor capacity and hydrogen bond donor capacity by MOL Inspiration; molar refractivity (MR), dipole, dihedral angles (Dang) and electrostatic potential charges by Cambridge Soft; polarizability, Log P, Log D and Van der Waals surface force by CHEMAXON.

Log P is the logarithm of the octanol/water partition coefficient. Van der Waals surface force represents the energy for the through-space interaction of atoms separated by two atoms and the dipole measures the asymmetry in the molecular charge distribution as a vector in three dimensions. Molar refractivity is a constitutive additive property, which can be calculated by the Lorentz–Lorenz equation. It is related to the polarizability, the real volume of the molecule and the London dispension forces that act on the drug-receptor interaction (Padrón et al., 2002).

2.8. Statistical analysis

Multivariate methods of numerical analysis were applied here to investigate the relationship between the structure and the molecular properties with the antitoxigenic activity of the phenolic compounds.

Hierarchical cluster and K-Means cluster analyses were carried out based on the FB_1 production inhibition capacity, to evaluate possible similarities in the antifumonisin activities of the compounds. For this study, we used the "average linkage" clustering algorithm and the "Standard Euclidean distance" method.

Principal component analysis (PCA) was performed on the molecular property data to determine which of these parameters contributed most to the conformation of the groups, and these molecular parameters were then standardized.

Canonical correlations were calculated to investigate relationships between the linear combinations of the primary (antifumonisin activity) and secondary (molecular property data) variables, which were evaluated using the chi-square test.

Multiple Linear regression analyses (MLR) were calculated in order to examine quantitative relationships between linear combinations of the dependent variable (antifumonisin activity) and the predictor variables (structure and molecular properties). In the MLR equations, N is the number of data points, r is the correlation coefficient between the observed values of the dependent variable and the values calculated from the equation, and r^2 is the square of the correlation coefficient and represents the goodness of fit. Results with p values <0.05 were considered significant. All statistical analyses were calculated by using the InfoStat software Professional 2010p.

3. Results

3.1. Effect of phenolic compounds on FB_1 production

The results of the experimental determination of FB_1 inhibition capacity for the 10 natural phenolic compounds revealed thymol, carvacrol, and isoeugenol followed by eugenol to be the most antifumonisin active compounds (Table 1). On the other hand, m-cresol, p-cresol, creosol and guaiacol did not show any significant effects on FB_1 production. The FB_1 inhibition data obtained were then used as the dependent variables in the cluster analysis, to investigate similarities in the antifumonisin activities of the compounds. The hierarchical cluster and K-Means cluster analyses (Fig. 2) showed that three distinct groups existed for the 10 phenolic compounds based on their levels of their activity against FB_1 production by *F. verticillioides*. These groups were designated:

Group I. Carvacrol, thymol and isoeugenol.

Group II. Eugenol, o-cresol and vanillin.

Group III. m-Cresol, p-cresol, creosol and guaiacol.

3.2. Structure/activity relationships (SAR)

A PCA was carried out in order to determine which of the molecular properties were the main contributors to the differences found in the three groups and to evaluate how well these variables were able to discriminate, thus giving some indication of their overall importance. In PCA, the contribution of each molecular parameter to a specific component is determined by the associated loading value (e), with the highest loading values indicating a strong influence on the component characteristics.

Some of the calculated molecular parameters, such as dihedral angles (Dang) and electrostatic potential charges, were omitted from PCA because no differences were found between the compounds. The plot of PCA showed that the antifumonisin group (group 1) with the

Table 1
Effects 10 natural phenolic compounds on FB_1 production.

Phenolic compounds*	FB_1 (μg/ml)**	Biomass (g dry wt/ml)**	FB_1/biomass**
Thymol	7.35(1.29)[a]	0.0077(0.0005)[a]	938.4 (126.6)[a]
Carvacrol	7.28(0.73)[a]	0.0082(0.0006)[a]	924.6(126.5)[a]
Ortho-cresol	41.73(5.81)[b]	0.011(0.0003)[b]	3768.2(437.2)[c]
Meta-cresol	57.41(4.48)[c]	0.0121(0.0004)[b]	4754.4(207.4)[d]
Para-cresol	71.69(7.73)[c]	0.0139(0.0004)[c]	5149.5(416.6)[d]
Eugenol	20.24(3.01)[a]	0.0081(0.0004)[a]	2418.4(255.1)[b]
Isoeugenol	6.19(1.00)[a]	0.0072(0.0004)[a]	860.5(12.0)[a]
Creosol	62.06(6.04)[c]	0.0121(0.0006)[b]	5546.2(235.4)[d]
Guaiacol	64.56(5.28)[c]	0.0116(0.0001)[b]	5135.4(453.7)[d]
Vanillin	42.23(5.32)[b]	0.0115(0.0010)[b]	3645.6(154.4)[c]
Control	64.59(5.31)[c]	0.0116(0.0003)[b]	5600.5(512.0)[d]

*The phenolic compounds were evaluated at final concentrations of 1.00 mM and were applied on the fifth day post-inoculation. Five replications were done for each treatment. Values having different letters are significantly different from each other according to DGC multiple range test at $P<0.05$ ($n=5$).
**Each cell in the table is formatted according to the scheme mean (standard error of the mean).

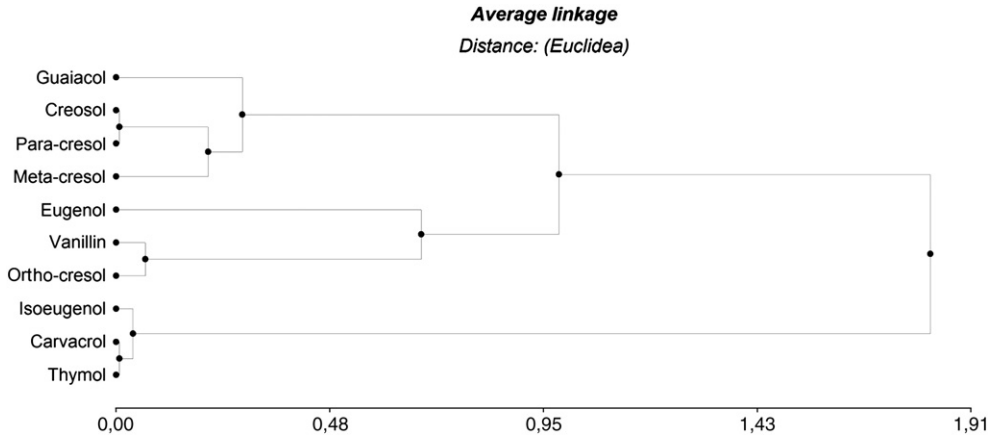

Fig. 2. Hierarchical clustering of 10 natural phenolic compounds based on the FB_1 production inhibition capacity. The "average linkage" clustering algorithm and "Standard Euclidea distance" were used.

most activity was separated from the rest of the groups by the first component (PC1), which explained 59.25% of the total variance (Fig. 3). This component was mainly influenced by volume (e = 0.32), polarizability (e = 0.32), molar refractivity (MR) (e = 0.34), parachor (e = 0.34), saturated area (e = 0.36), phospholipid interaction capacity (K′) (e = 0.36) and the van der Waals surface area (vdWSA) (0.34).

A second PCA was carried out in order to determine if the structural and molecular properties that were able to discriminate group 1 in the first PCA permitted the separation of thymol, carvacrol and isoeugenol (group 1) from the rest of the phenolic compounds. In Fig. 4, the first two components of PCA accounted for 88.4% of the total variance (cofenetic Correlation = 0.979). The plot of PCA showed that the antifumonisin compounds (thymol, carvacrol and isoeugenol) with the most activity were well apart from the rest, with this separation being due to PC1 (68.7%). This component was mainly influenced by molar refractivity (e = 0.51), parachor (e = 0.46), saturated area (e = 0.51) and phospholipid interaction capacity (K′) (e = 0.49). The plot also showed that thymol, carvacrol and isoeugenol were separated from eugenol by the second component (PC2), which explained 19.7% of the total variance, with PC2 being mainly influenced by vdWAS (e = 0,92). To corroborate the relationships between antifumonisin activity and the selected molecular properties, a canonical correlation analysis was performed. The results obtained allowed us to conclude that 95% of the antifumonisin activity variability could be explained by the selected molecular properties ($R^2 = 0.95$; p value = 0.01), with the phospholipid interaction capacity (1.11), molar refractivity (0.80) and saturated area (−0.70) being the most important molecular parameters in this relationship.

3.3. Quantitative structure/activity relationships (QSAR)

MLR analyses were performed in order to investigate the quantitative relationships between antifuminisin activity and structure and the molecular properties. The resulting equation produced a model representing 99.0% of the variance ($R^2 = 0.99$), demonstrating a good correlation between inhibition activity and molecular parameters, and is given by the expression:

$$\%\ FB_1\ \text{inhibition} = -2.88(K`)^2 + 34.66(K`) + 2.25(MR) - 0.63(Satarea)$$

$$N = 10,\ r^2 = 0.99,\ p < 0.0001$$

This is a parabolic correlation in terms of K′, which suggests that the inhibitory activities of the phenolic compounds first increased with a rise in hydrophobicity up to an optimum K′ (6.1), and then decreased after a further rise in hydrophobicity. The inhibitory activity of the phenolic compounds increased with a decrease in saturated area and with a rise in the molar refractivity.

4. Discussion

Although essential oils rich in phenolic compounds have been found to be active in inhibiting fumonisin production (Dambolena et al., 2010; López et al., 2004; Velluti et al., 2004), the antifumonisin activity of natural phenolic compounds is still poorly understood. To our knowledge, only the inhibitory effects of benzoic acid, caffeic acid, vanillic acid and thymol on the FB_1 production by *F. verticillioides* have been reported (Beekrum et al., 2003, Dambolena et al., 2008; Samapundo et al., 2007). This is in agreement with our finding that revealed thymol, carvacrol, and isoeugenol followed by eugenol to be the most active antifumonisin compounds (higher FB_1/biomass inhibition). These phenolic compounds also had the highest antifungal activity. On the other hand, m-cresol, creosol and guaiacol did not show any significant effects on FB_1 production or on biomass (Table 1). The results reported in this work demonstrate the potential of some natural phenolic compounds to prevent fumonisin production. The present findings also highlight that, in addition to the functional group, other properties of the compounds should be taken into account when investigating the antifumonisin activity of the phenolic compounds. For example, slight structural differences may be sufficient to affect the physical or chemical properties of the compounds, such as lipophilicity, and hence alter their bioactivity. Accordingly, a structure–activity relationship analysis was performed in order to determine the molecular properties related to the antitoxigenic activity.

Concerning QSAR models, a major step in their construction is to find a set of molecular descriptors that represents variation in the structural properties of the molecules (Ghasemi et al., 2010). The two principal types of descriptors used in QSAR studies are the physicochemical properties (empirical) and the topological indices that encode the chemical structure and are generated from the molecular structure by counting its fragments, paths, bonds, atoms, etc. (non-empirical) (Padrón et al., 2002). The structure–activity relationship analysis (SAR) revealed lipophilicity (determined as phospholipid interaction capacity − K′), molar refractivity and the saturated area to be the descriptors that best explained the antifumonisin activity of the natural phenolic compounds, with lipophilicity being the descriptor with the strongest influence on this activity. On the other hand, the quantitative structure-activity relationship analysis (QSAR) revealed a parabolic correlation in terms of K′, which suggests that an optimal lipophilicity of the phenolic

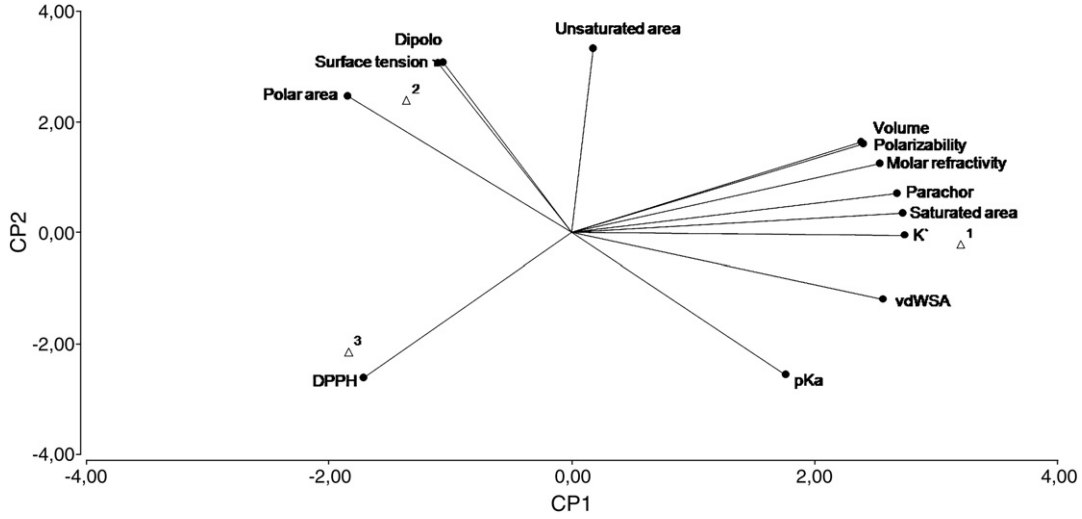

Fig. 3. Principal component analysis based on experimental and theoretical properties of 10 natural phenolic compounds. Score plots of molecular parameters and Groups of phenolic compounds derived from the hierarchical cluster analysis into the plane defined by the first two principal components (PC1 against PC2). The molecular parameters were standardized.

compounds occurred at $K' = 6.1$. Furthermore, the inhibitory activity of the phenolic compounds increased with a decrease in saturated area and with a rise in the molar refractivity, with the statistical parameters used for the evaluation of the regression equations demonstrating that the obtained model was statistically significant ($p<0.0001$). The dependent variable variance of an MLR model is expressed by r^2 (measure of the goodness of fit between predicted and experimental values). Therefore, the high value of r^2 (0.99) confirmed the validity of the model. The agreement among the descriptors obtained in both analyses (QSAR and SAR) reaffirms the influence of these in the marked antifumonisin activity of carvacrol, thymol and isoeugenol, with respect to the rest of the used phenolic compounds.

Although many QSAR studies have reported lipophilicity and molar refractivity to be molecular descriptors of the biological action of phenolic compounds (Hansch et al., 2003a; O'Brien et al., 2003; Voda et al., 2004), the present study is the first work that has performed SAR and QSAR studies on mycotoxin production. The biological activity of a chemical compound depends on the ability of these molecules to reach the specific target molecule and its subsequent reactivity (Voda et al., 2004, and references therein). Hence, there are three major types of interactions that the modeller must deal with: hydrophobic, electronic and steric (Hansch et al., 2003b). The fact that the lipophilicity was the most influential descriptor of the antifumonisin activity of the phenolic compounds suggests that the key step was to reach the inside of the fungal cells. Molar refractivity has been used to explain the electronic effects in chemical–biological interactions (Hansch et al., 2003b) and has been reported to be very valuable in the correlation of the allosteric effects in enzyme-ligand interactions (Hansch et al., 2003b). The presence of molar refractivity as a descriptor that explains the antifumonisin activity of phenolic compounds, suggests the involvement of a specific interaction with a target molecule.

To date, the mechanisms of the inhibition of fumonisin production by phenolic compounds have not been elucidated. However, studies on another mycotoxin (aflatoxin) have suggested that compounds that inhibit mycotoxin production can act by altering the environmental and physiological modulators of mycotoxin biosynthesis or by altering the signal transduction pathways upstream of the biosynthetic pathway (Holmes et al., 2008). The fact that the phenolic compounds with the greatest antifumonisin activity also had the highest antifungal activity allows us to suggest two hypotheses: (1) the antifumonisin activity of the phenolic compounds may be due to an increase in the lag phase prior to fungal growth and/or result from an effect of the growth rate. Hence, a delay can arise in the onset of the stationary growth phase. This is in agreement with Torres et al. (2003), who reported an effect of the synthetic phenolic antioxidant BHA on fungal growth and fumonisin production, and concurs with Samapundo et al. (2007), who suggested that the mechanisms for the inhibition of fumonisin production by phenolic compounds are related to those described for growth inhibition of the Fusarium isolates. The antifungal activity of the phenolic compounds has been attributed to their lipophilic properties, which determine their ability to penetrate into the plasma membrane and induce changes in the physico-chemical properties of the cell wall, cell membrane, and cellular organelles (Rasooli and Owlia, 2005; Knobloch et al., 1987). Consequently, the fact that the QSAR study revealed lipophilicity to be the most influential descriptor of the antifumonisin activity could indicate that this activity was related to an antifungal effect. However, further studies still need to be carried out in order to determine the antifungal effect of phenolic compounds on the antifumonisin.

Another hypothesis (2) is that during stress, the fungi respond by controlling secondary metabolite production (Picot et al., 2010). In agreement, a strong correlation between the oxidative stress with aflatoxin and deoxynivalenol biosynthesis has been previously reported (Holmes et al., 2008, and references therein; Ponts et al., 2006). Many authors have also linked the antioxidant activity of phenolic compounds with their activity on aflatoxin biosynthesis. Jayashree and Subramanyam (1999) reported that the antiaflatoxigenic activity of eugenol is due to the inhibition of lipid peroxidation. To our knowledge, however, there is no information available linking oxidative stress with fumonisin biosynthesis. Nevertheless, the antifumonisin activity of synthetic antioxidants (Torres et al., 2003) and natural phenolics (Dambolena et al., 2008, Samapundo et al., 2007) would seem to indicate the existence of this relationship. The results reported in the current work revealed that some of the natural phenolic compounds with antiradical activity (Table 2) such as m-cresol, p-cresol, creosol and guaiacol, did not show any significant effects on FB_1 production. Consequently, the radical scavenger activity of an antioxidant compound makes it difficult to predict its effect, if any, on fumonisin biosynthesis. Hence, while antioxidant activity may be important in the action mode of phenolic compounds, it seems likely that additional molecular properties are involved in inhibition (e.g. lipophilicity.).

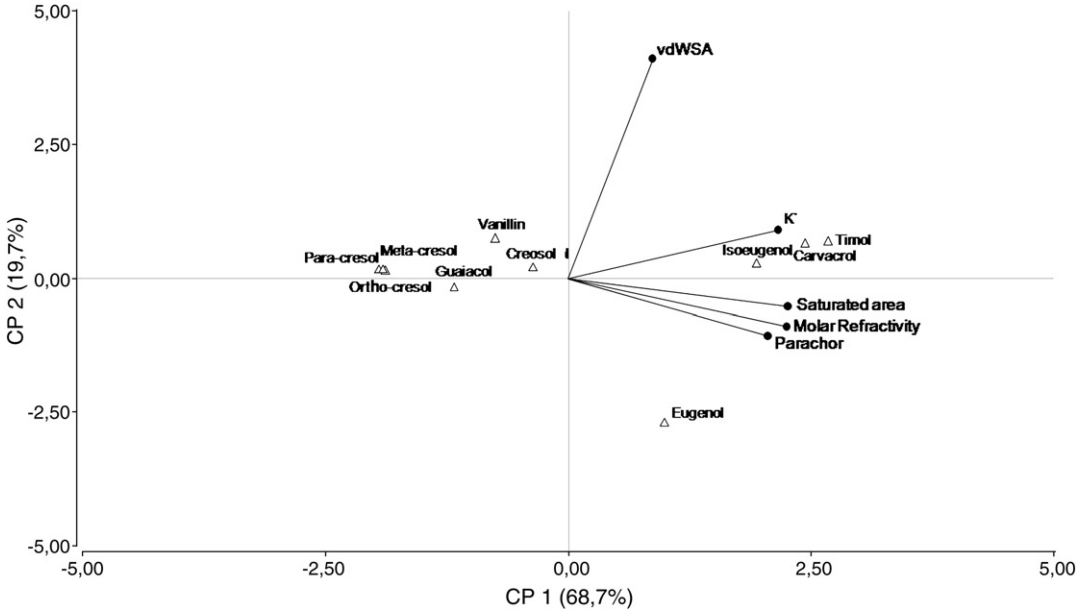

Fig. 4. Principal component analysis based on selected experimental and theoretical properties of 10 natural phenolic compounds. Score plot of the selected molecular parameters (molar refractivity, parachor, saturated area, phospholipids interaction capacity (K′) and vdWAS) and phenolic compounds into the plane defined by the first two principal components (PC1 against PC2). The molecular parameters were standardized.

In the present work, we demonstrated that the molecular properties or descriptors (lipophilicity, molar refractivity and saturated area) of phenolic compounds helped to explain their antifumonisin activities, with the implications of our results indicating that lipophilicity is the key step for the target molecule to be reached inside the fungal cells. Furthermore, the molar refractivity and saturated area descriptors demonstrated the importance of the interactions with specific enzymes, metabolite pools, or signaling pathways. Finally, the mathematical expression obtained by the QSAR analysis can be used to predict the antifumonisin activity of other structurally related molecules. These findings could provide an important contribution in the search for new compounds with antifumonisin activity.

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Table 2
Antiradical activity (DPPH activity) and lipophilicity (partition coefficients by IAM–HPLC method) of phenolic compounds.

Phenolic compounds	DPPH activity IC50[a] (mM)	Partition coefficients K′[b]
Thymol	1.60	5.14
Carvacrol	2.82	5.64
Ortho-cresol	0.57	0.51
Meta-cresol	0.05	0.49
Para-cresol	0.9	0.51
Eugenol	38.6	1.61
Isoeugenol	1.94	3.03
Creosol	0.02	0.41
Guaiacol	0.03	0.1
Vanillin	9.89	0

[a] IC50 — concentration of the solution required to give a 50% decrease in absorbance compared to that of a blank solution.

[b] K` — the capacity factors (k) were calculated from the retention times as follows: $k = (tr - t0)/t0$, where tr is the retention time of the compound and t0 is the dead time of the column, which is equal to the retention time of a substance which is not retained.

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