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ORIGINAL ARTICLE

In vitro activity of natural phenolic compounds against fluconazole-resistant *Candida* species: a quantitative structure–activity relationship analysis

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Abstract

Aims: To evaluate the antifungal activity and to analyse the structure–activity relationship of eleven natural phenolic compounds against four *Candida* species which are resistant to fluconazole.
Methods and Results: Four different species of *Candida* isolates were used: *Candida albicans*, *Candida krusei*, *Candida tropicalis* and *Candida dubliniensis*. The phenolic compound carvacrol showed the highest anti-*Candida* bioactivity, followed by thymol and isoeugenol. The obtained minimum inhibitory concentration (MIC) values obtained were used in a quantitative structure–activity relationship (QSAR) analysis where the electronic, steric, thermodynamic and topological descriptors served as dependent variables. According to the descriptors obtained in this QSAR study, the antifungal activity of phenols has a first action specific character which is based on their interaction with plasma or mitochondrial membranes. The second action is based on a steric descriptor—the maximal and minimal projection of the area—which could explain the inability of some phenolic compounds to be biotransformed to quinones methylene by *Candida* species.
Conclusions: According to the descriptors obtained in this QSAR study, the anti-*Candida* activity of ortho-substituted phenols is due to more than one action mechanism. The anti-*Candida* activity of phenolic compounds can be predicted by their molecular properties and structural characteristics.
Significance and Impact of the Study: These results could be employed to predict the anti-*Candida* activity of new phenolic compounds in the search for new alternatives or complementary therapies to combat against candidiasis.

Introduction

Candida is known to be an opportunistic pathogenic yeast. It can develop into fungal infections, which have been linked to hospital infections (Barros *et al.* 2013). Candidiasis is the most common human fungal infection in the world, where it can especially affect immunocompromised patients (Biswas *et al.* 2007; Zhang *et al.* 2009). The genus *Candida* is made up by more than 200 species, with *Candida albicans* representing the most important causative agent of life-threatening infection. However, other members of this genus such as *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis, Candida krusei*, *Candida dubliniensis* and *Candida guilliermondii* have also been increasingly recognized as significant opportunistic human pathogens (Dorrell 2002; Sullivan *et al.* 2004; Panda *et al.* 2010; Ahmad *et al.* 2011; Bertholom 2012). The emergence of *Candida* species, which are resistant to different conventional antifungal agent, has led to an increase in mortality associated with candidiasis

(Galgóczy *et al.* 2011; Rambach *et al.* 2011; Zore *et al.* 2011; Fortún *et al.* 2012; Tobudic *et al.* 2012). These infections are difficult to treat with conventional antifungal agents because they have multiple side effects, are highly toxic to the host and develop resistance to antifungal chemotherapies. Thus, in recent years, the search for alternative antifungal compounds has been a major concern (Brito Gamboa *et al.* 2006; Neppelenbroek *et al.* 2006).

Natural phenols such as carvacrol, thymol, eugenol and isoeugenol have been shown to possess a wide range of bioactivities. These include the antioxidant properties (Dambolena *et al.* 2010), radical scavengers (Bortolomeazzi *et al.* 2010), antimicrobial (Miñambres *et al.* 2010), including antifungal (Voda *et al.* 2004; Dambolena *et al.* 2011) or antitoxicogenic activity, have been reported (Dambolena *et al.* 2012). Several reports attribute the antimicrobial activity of essential oils from aromatic plants to the terpenoid and phenolic compounds present in them (Oliva *et al.* 2013). Besides, the antifungal effects of natural phenolic compounds such as carvacrol, cinnamic acid, benzoic acid, salicylic acid, thymol, 2,3- and 2,5-dihydroxybenzaldehyde have been previously reported against *C. albicans* and *C. neoformans;* however, this bioactivity was dependent on the strain and the compound tested (Faria *et al.* 2011). The bioactivity of the phenols can be explained by the hydroxyl group attached to a benzene ring. The degree of bioactivity, however, is determined by the substituent present (Miñambres *et al.* 2010; Dambolena *et al.* 2011). Substituents can affect ionizable character (Kapur *et al.* 2000; Zhao *et al.* 2009), the ability to generate radicals (Loader *et al.* 2006; Wright and Shadnia 2008) or the possibility of becoming in methylene quinones, during metabolic activation (Shadnia and Wright 2008).

The quantitative structure–activity relationship (QSAR) is a mathematical equation by which the chemical structure is quantitatively correlated with well-defined processes, such as antifungal activity. This mathematical equation uses descriptors such as steric, topological, electronic and/or thermodynamic properties obtained from the studied molecules to understand their relative importance in promoting antifungal activity. Previous studies of QSAR have shown that the Log P descriptor has the ability to explain the toxic activity of phenols substituted with electron-withdrawing groups (Moridani *et al.* 2003; Lim *et al.* 2005; Wright and Shadnia 2008). The toxicity of phenols with electron-donating substituents is explained by two descriptors: Log P (partition coefficient) and σ^+. The latter descriptor, σ^+, is a measure of the ability of a substituent to donate electrons, at variation of Hammett Brown (Selassie *et al.* 2002, 2005). Steric and/or electronic interactions between neighbouring groups and the phenolic hydroxyl affect the precision of descriptor σ^+. For this reason, the descriptor σ^+ in the ortho-substituted phenols is not used to interpret the ability of these toxic compounds, as it is limited to phenol para- or meta-substituted (Selassie *et al.* 1998; Loader *et al.* 2006; Rincón and Almeida 2012). It is therefore necessary to know which descriptors of ortho-substituted phenols are related to the anti-*Candida* activity. In the ortho-substituted phenols, alkyl or methoxy substituents could generate a difference in anti-*Candida* activity. This work is an extension of our earlier studies on the structure/antifungal activity relationship of natural phenolic compounds (Dambolena *et al.* 2011). Here, we evaluate the antifungal activity and analysed the structure–activity relationship of eleven natural phenolic compounds—eight ortho-substituted, two ethers, a hydrocarbon and three related compounds—against four *Candida* spp.: *C. albicans, C. dubliniensis, C. krusei* and *C. tropicalis* resistant to fluconazole. The aim of this research was to evaluate structural characteristics of natural phenolic compounds, emphasizing their anti-*Candida* bioactivity for potential use as a therapeutic alternative or complement to candidiasis.

Materials and methods

Phenols and related compounds

The compounds used were as follows: phenol, 2-methyl-5-propan-2-ylphenol (carvacrol; ≥97% purity), 5-methyl-2-propan-2-ylphenol (thymol, ≥99%), 2-methylphenol (ortho-cresol; ≥99%), 3-methylphenol (meta-cresol; ≥99%), 4-methylphenol (para-cresol; ≥98%), 2-methoxy-4-prop-2-enylphenol (eugenol; ≥99%), 2-methoxy-4-[(E)-prop-1-enyl]phenol (isoeugenol; ≥98%), 2-methoxy-4-methylphenol (creosol; ≥99%), 2-methoxyphenol (guaiacol; ≥98%) and 4-hydroxy-3-methoxybenzaldehyde (vanillin; ≥99%), 1,2-dimethoxy-4-prop-2-en-1-ylbenzene (eugenol methyl ether; ≥96%), 1-allyl-4-methoxybenzene (estragole; ≥98%) and 1-methyl-4-(1-methylethyl) benzene (p-cymene; ≥97%). The phenols and related compounds were purchased from Sigma-Aldrich (Fig. 1).

Micro-organisms

The following yeasts were used to test the antifungal activity: *Candida albicans, Candida dubliniensis, Candida krusei* and *Candida tropicalis.* These strains were resistant to fluconazole. They were obtained from the Central Hospital of Río Cuarto and identified in the Mycology Area of the Department of Microbiology and Immunology of the National University of Rio Cuarto, Argentina,

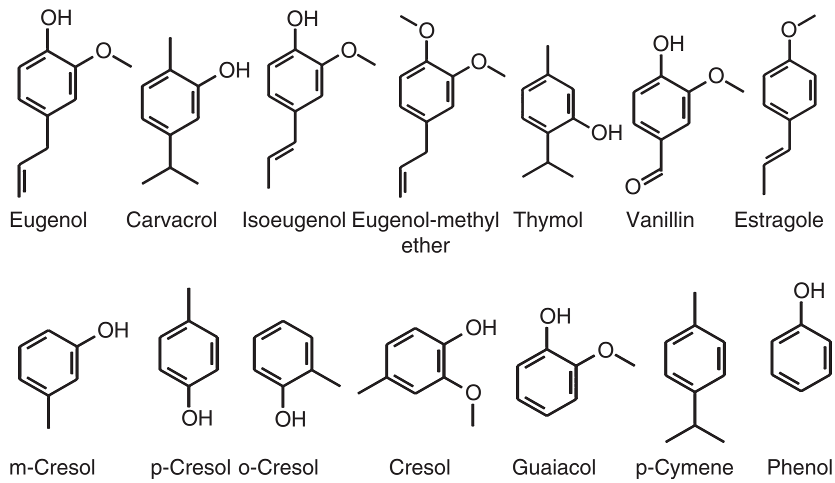

Figure 1 Chemical structures of natural phenolic compounds and relatives compounds studied in the present work.

by conventional biochemical and morphological analysis. The resistance of the four *Candida* isolated against fluconazole and amphotericine B was tested the by resazurin method, in the concentrations range of 0·103–105·25 μg ml^{-1} and 0·020–10·58 μg ml^{-1}, respectively. At the evaluated concentrations, fluconazole and amphotericine B did not show effects on *Candida* growth (not inhibited). According to Collin *et al.* (1999), could be considered resistant to fluconazole the *Candida* strain with MIC values ≥64 μg ml^{-1}, and resistant to amphotericine B with MIC values ≥1 μg ml^{-1}.

Antimicrobial activity

The minimum inhibitory concentration (MIC) of the natural phenolic compounds was evaluated against yeast species by the broth microdilution method (resazurin method) described by Mann and Markham (1998), and the minimum fungicidal concentration (MFC) was performed according to the methodology proposed by Finelgold *et al.* (1992) and Oliva *et al.* (2011). The resazurin method is a rapid, simple and reproducible method that allows us to interpret the results visually. In this method, microbial growth causes the change of state of the indicator (blue in its oxidized form to pink in its reduced form), due to the microbial metabolism. This method has been used by Palomino *et al.* (2002) to determine drug resistance of *Mycobacterium tuberculosis* strain microbial. The technique for determining the MIC employed in this study represents a modification of the microdilution techniques (M27-A2), described by the National Committee for Clinical Laboratory Standards (NCCLS 2002). This modified method was used in several works to determine the antimicrobial activity of essential oils (Gallucci *et al.* 2006; Oliva *et al.* 2011).

Inoculum densities

Tubes containing Sabouraud broth (SB) with 0·1% (w/v) Sabouraud broth agar (SBA) were prepared at pH 7, inoculated with each micro-organism and incubated overnight (18 h) at 37°C. Optical densities were measured at 620 nm in a spectrometer, and number of cells was confirmed by the viable plate count on Sabouraud agar (SA). The cell concentration necessary to cause reduction in resazurin was determined for each yeast species. Briefly, serial 10-fold dilutions of the overnight culture were prepared in SBA, and aliquots (170 μl) from these dilutions were dispensed into microplates containing 20 μl of diluent (dimethyl sulphoxide and distilled water, 1 : 1). Ten microlitres of the resazurin solution (0·01%) was added to each well. They were incubated for 3·50 h at 37°C, and the appropriate dilution to work was considered as the last one unable to reduce resazurin (blue). The CFU per mL of this dilution was confirmed by the plate-counting method on Sabouraud agar (SA).

Determination of the minimum inhibitory concentration

The minimun inhibitory concnetration (MIC) was determined according to Mann and Markham (1998). Briefly, serial twofold dilutions of each phenolic compound were prepared by vortexing them in the diluent at room temperature. The resazurin assay medium, SBA, was inoculated with the test organism to yield a final cell density ≈ 1 log cycle lower than the cell density required to reduce resazurin (usually 10^6 CFU ml^{-1}). A sterile 96-well microtitre tray was set up with each of the tested *Candida* isolate as follows: column 1–10, 170 μl inoculum plus 20 μl of the natural phenols dilution; column 11, 170 μl inoculum plus 20 μl natural phenols diluent (positive control); column 12, sterile resazurin assay medium plus 20 μl of natural phenols diluents (negative control). Well contents were thoroughly mixed and were incubated for 18 h at 37°C. After incubation, 10 μl of resazurin solution was added to all wells. After a second incubation of 3 h at 37°C, wells were assessed visually for colour change, considering the MIC as the highest dilution (lower concentrations of each compound) remaining blue. Each experience was made by triplicate and was repeated twice. The final MIC was calculated as the mean value of all the MICs obtained.

Determination of the minimum fungicidal concentration

Hundred microlitres of the dilution belonging to the MIC and the previous dilutions was inoculated in SA and incubated at 37°C for 24 h. The MFC was considered as the last dilution that did not show cell growth (Finelgold *et al.* 1992; Oliva *et al.* 2011).

Quantitative structure–activity relationship

Statistical analysis

Multiple linear regression analyses (MLR) were calculated to examine the quantitative relationships between linear combinations of the dependent variable (log 1/MIC) and the predictor variables (structure and molecular properties). Molar concentrations of the MIC values were used for the MLR analyses. In the MLR equations, N is the number of data points, r is the correlation coefficient between 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. The QSAR model was validated with the root mean square prediction error (RMSPE) obtained by the cross-validation leave-one-out procedure. Results with p values <0·05 were considered significant. All statistical analyses were calculated using the InfoStat software Professional 2010p (Dambolena *et al.* 2012). For the development of QSAR models, descriptors of potential antifungal compounds were calculated using two software packages of ACD and Chemaxon and the ChemSpider database (Table 1). Descriptor values E (Homo) and E (Lumo) were taken from Voda *et al.* (2004).

Results

Minimum inhibitory concentration (MIC) values of eleven phenolic components, two ethers and a hydrocarbon, and the relative compounds against four investigated *Candida* isolates are shown in Table 2. With the exception of

Table 1 Calculated molecular descriptors for the natural phenols and related compounds used in this work

Descriptors	Guaiacol	Creosol	Vanillin	Isoeugenol	Eugenol	Phenol	O-cresol	M-cresol	P-cresol	Carvacrol	Thymol
Molecular weight (Da)	138	138	152	164	164	94	108	108	108	150	150
Molar refractivity (cm^3)	34·81	39·63	41·56	50·7	48·72	28·134	32·959	32·959	32·95	47·14	47·14
Index of refraction	1·535	1·53	1·587	1·577	1·535	1·553	1·546	1·546	1·545	1·523	1·523
Surface tension (dyne cm^{-1})	38·669	37·2	47·3	38·9	36·5	40·967	38·815	38·815	38·8	34·9	34·9
Density (g cm^{-3})	1·11	1·078	1·231	1·074	1·05	1·071	1·038	1·038	1·038	0·974	0·974
Polarizability 1×10^{-24} (cm^3)	13·801	15·71	16·47	20·1	19·31	11·153	13·066	13·066	13·06	18·68	18·68
Polar surface area (Å^2)	29·46	29·46	46·53	29·46	29·46	20·23	20·23	20·23	20·23	20·23	20·23
Enthalpy of vaporization(kJ mol^{-1})	45·925	47·5	54·25	52·5	51·214	43·524	44·469	45·641	45·61	49·32	48·88
Log P	1·35	1·65	1·19	2·45	2·2	1·54	1·96	2·04	1·94	3·28	3·28
Log D pH 7·35	1·35	1·65	1·19	2·45	2·2	1·54	1·96	2·04	1·94	3·28	3·28
pKa	9·93	10·34	7·81	10·01	9·94	10·02	10·37	10·13	10·36	10·42	10·59
Dipole	1·544	2·261	2·296	1·778	2·199	1·24	0·957	1·103	1·368	1·301	1·374
Charge oxygen phenolic	−0·23	−0·249	−0·242	−0·225	−0·248	−0·252	−0·253	−0·253	−0·252	−0·253	−0·257
Volume (Å^2)	116·5	133·43	135·7	159·85	159·94	90·52	107·38	107·31	107·32	158·3	158·42
Solvent accessible surface area	194·7	227·1	220·7	258·9	257·8	147·2	179	179·3	179·3	271	271·1
Minimal projection area (Å^2)	25·59	27·95	27·57	29·7	32·58	21·02	23·19	22·77	22·29	30·33	30·97
Maximal projection area (Å^2)	43·27	48·45	50·34	59·61	54·62	36·45	41·27	41·5	41·24	53·61	54·27
Hydrophobicity constant (π)	−0·19	0·11	−0·35	0·91	0·66	0	0·42	0·5	0·4	1·74	1·74
Electronegativity (eV)	4·35	4·11	4·8	4·21	4·13	4·36	4·31	4·32	4·23	4·26	4·29

Table 2 Antifungal activity of natural phenolic compounds and related compounds against investigated *Candida* isolates

Phenols (evaluated concentrations)	*C. albicans*		*C. dubliniensis*		*C. krusei*		*C. tropicalis*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Phenol (0·044–45·26)	>45·26	–	>45·26	–	>45·26	–	>45·26	–
Guaiacol (0. 0005–59·42)	0·01	–	0·01	–	0·01	–	0·01	–
Creosol (0·056–57·47)	1·3	28·7	0·67	5·39	1·79	7·18	2·24	9
Vanillin (0·0005–5·26)	0·13	–	0·13	–	0·26	–	0·13	–
Isoeugenol (0·055–56·68)	0·17	2·19	0·11	1·33	0·22	1·32	0·55	2·64
Eugenol (0·054–56·16)	0·66	1·75	0·44	0·88	0·88	1·09	0·44	7·88
O-cresol (0·002–2·105)	1·05	–	0·40	–	1·05	–	1·05	–
M-cresol (0·053–108·84)	0·85	0·85	0·21	0·85	0·85	0·85	0·85	0·85
P-cresol (0·001–1·053)	0·88	–	0·88	–	0·88	–	0·88	–
Carvacrol (0·05–51·36)	0·1	6·5	0·25	0·4	0·2	0·8	0·25	0·6
Thymol (0·0005–1·05)	0·13	6·5	0·26	0·4	0·13	0·8	0·26	0·6
P-cymene (0·044–45·26)	22·6	–	22·6	–	22·6	–	11·3	–
Eugenol methyl ether (0·053–54·52)	1·7	–	3·83	–	1·28	–	1·28	–
Estragole (0·049–50·79)	4·74	–	1·15	–	0·59	–	1·15	–

The MIC and MFC values are expressed as mg ml^{-1} units.
The methodology of Mann and Markham (1998) is qualitative for these reason we cannot determine the standard deviations.
(–) For the evaluated concentrations, these compounds not showed fungicidal activity.

phenol, the fourteen compounds showed inhibitory activity against the four *Candida* isolates. In a decreasing scale of activity, they can be ordered as following: guaiacol, carvacrol, thymol, isoeugenol, eugenol, m-cresol, p-cresol, o-cresol, creosol, vanillin, estragole, eugenol methyl ether, p-cymene and phenol. Guaiacol, carvacrol, isoeugenol and thymol were the most active substances in inhibiting *Candida* growth with MIC values ranging between 0·01 mg ml^{-1} to 0·55 mg ml^{-1}, while cresol isomers, eugenol and creosol had MIC values that ranged from 0·21 to 2·24 mg ml^{-1}. Eugenol methyl ether, estragole and p-cymene were the least active compounds against the yeast strains. Eugenol, isoeugenol, carvacrol, thymol, m-cresol and creosol were able to cause cell death, with MFC values ranging from 0·4 to 28·7 mg ml^{-1} (Table 2).

Quantitative structure–activity relationship (QSAR) studies were performed to understand the relative importance of the substituents in the anti-Candidal activity of phenolic compounds. The obtained QSAR models of phenols are expressed in equations (Eqns) 1, 2, 3 and 4 and are shown in Figs 2–5, respectively. The results obtained show a statistically significant model ($P < 0{\cdot}0001$), which predicted the antifungal activity in lineal equations. The equations obtained represent more than 94% of the variability ($r^2 = 0{\cdot}94$–$0{\cdot}99$), thus demonstrating a strong correlation between antifungal activity and molecular parameters. The statistical parameters used for the evaluation of the regression equations revealed the validity of the obtained models (RMSPE = 5·1–7·1%). The correlation between observed and predicted activity of studied phenols is shown in Figs 2–5. The obtained QSAR models suggest that the inhibitory activities of phenolic compounds against *C. albicans* (Eqn 1), *C. dubliniensis* (Eqn 2) and *C. krusei* (Eqn 1) increased with the lipophilicity (Log P) and maximal projection area (Eqns 1 and 3), and/or decreased with the minimal projection area (Eqns 2 and 3). Furthermore, the antifungal activity on *C. dubliniensis* (Eqn 2) decreases with a rise in 'charge oxygen phenolic'. On the other hand, the obtained QSAR model for *C. tropicalis* shows that the antifungal activities of phenolic compounds increase with lipophilicity (Log P) and molar refractivity (MR) and decrease with maximal projection area (Eqn 4).

Discussion

The results obtained in the present work demonstrate the antifungal activity of natural phenolic compounds against resistant *Candida* strains. Previous studies have reported the antifungal activity of phenolic and related compounds against resistent *C. albicans;* however, those obtained MIC values were slightly different than the results obtained in this study (estragole: MIC_{90} 0·2 mg ml^{-1} methyl eugenol: MIC_{90} 0·35 mg ml^{-1}; and eugenol: MIC_{90} 0·5 mg ml^{-1}) (Khan *et al.* 2011; Zore *et al.* 2011). These compounds cause fungal cell death by disrupting membrane integrity at MIC values, while at sub-MIC doses, significantly impair the defence system in *C. albicans* (Schmidt *et al.* 2007; Khan *et al.* 2011). The results reported by Rao *et al.* (2010) showed that the antifungal activity depends on the presence of a free hydroxyl group on the aromatic ring. However, in our study, the phenol

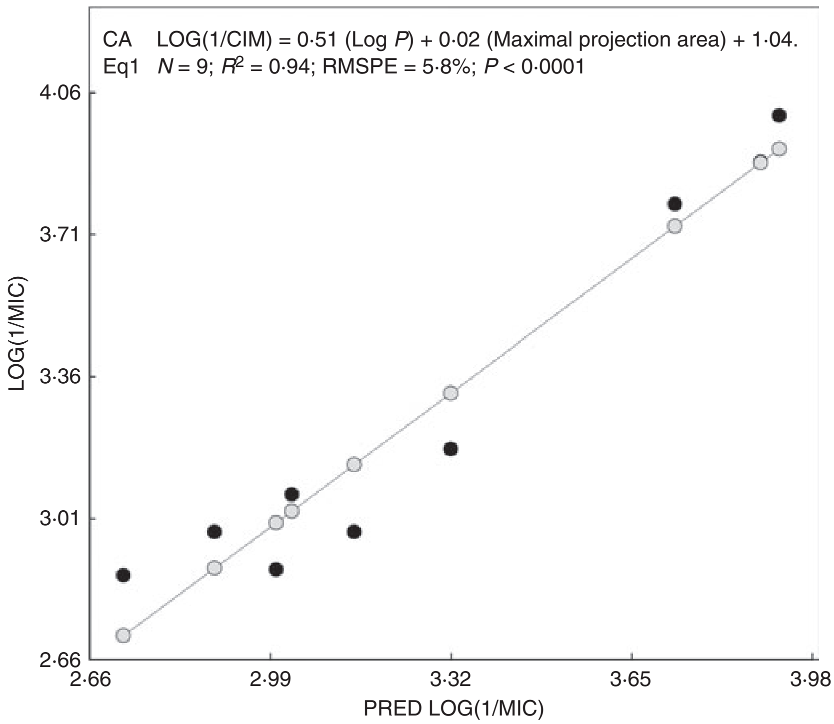

Figure 2 Plot of calculated versus experimental log 1/MIC of the nine phenolic compounds on *Candida albicans* growth. Multiple linear regression analyses (MLR) calculated to examine quantitative relationships between linear combinations of the dependent variable (log 1/MIC) and the predictor variables (structure and molecular properties). Guaiacol was assigned to be outliers on the basis of their deviation between observed activity and calculated activity from the equation. *N* is the number of data points, *r* is the correlation coefficient between 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. The obtained quantitative structure–activity relationship (QSAR) model was validated with the root mean square prediction error (RMSPE) obtained by across validation leave-one-out procedure outlier guaiacol.

compound did not produce any inhibition of the tested *Candida* species, indicating that by itself, the presence of phenolic hydroxyl is not enough to provide antifungal properties to phenols (Leal *et al.* 2009). Accordingly, a QSAR analysis was performed to predict the antifungal activity of the compounds based on their molecular properties. The obtained results showed four statistically significant QSAR models, which predicted the antifungal activity in lineal equations. These analysed phenolic compounds have electron-donating substituents, and according to previous results performed in other biological systems, the Log P descriptor could partially explain their bioactivity (Selassie *et al.* 1998; Moridani *et al.* 2003; Lim *et al.* 2005; Wright and Shadnia 2008). This is in agreement with the QSAR models obtained in the present work, which showed a positive relationship between the antifungal activity and log P for alkyl and methoxy ortho-substituted phenols (Log P is present in all equations). Substituent groups created a differential effect in the hydrophobicity of the molecule, and thereby in the descriptor Log P. The presence of the Log P descriptor in the mathematical models suggested that part of the antifungal activity of the phenolic compounds can be explained by their interaction with the plasma membrane (Turina *et al.* 2006; Cristani *et al.* 2007; Khan *et al.* 2011; Zunino *et al.* 2011). The phenolic compounds used in this work include alkyl and methoxyl substituents. Alkyl groups act as electron-donating groups, while methoxyl groups may act in two distinct ways (i) acting as electron scavengers by inductive effect, or (ii) acting as electron donors by mesomeric effect, which would influence the bond dissociation energy of the hydroxyl phenolic (pka values) that linearly depend on the partial charge of phenoxy oxygen (Denisov and Denisova 2011). QSAR equations show the oxygen phenolic charge as a descriptor for antifungal activity only in *Candida dubliniensis*. This yeast strain was the most sensitive *Candida* isolate analysed in this study (Fig. 3). Previous reports have indicated that the natural phenolic compounds with low pKa were the most cytotoxic. In contrast, Zhao *et al.* (2009) showed that toxicity significantly decreases with an increase in ionization, especially for extremely ionizable compounds. At this point, the literature is contradictory. The equations obtained in this study revealed that pKa is not a descriptor of antifungal activity in phenolic compounds.

Some phenolic compounds may be oxidized to quinones methylene (QM) during the metabolic processes. These QM are structures that are highly toxic in varying biological systems (Thompson *et al.* 1995; Krol and Judy 1997; Moridani *et al.* 2003). The rate of formation and stability of these QM are related to the substituents of the aromatic ring. The increased length of the alkyl group or volume allows for greater QM stability and thus enough time to reach the place where QM will interact, generating a toxic phenomenon (Thompson *et al.* 1995).

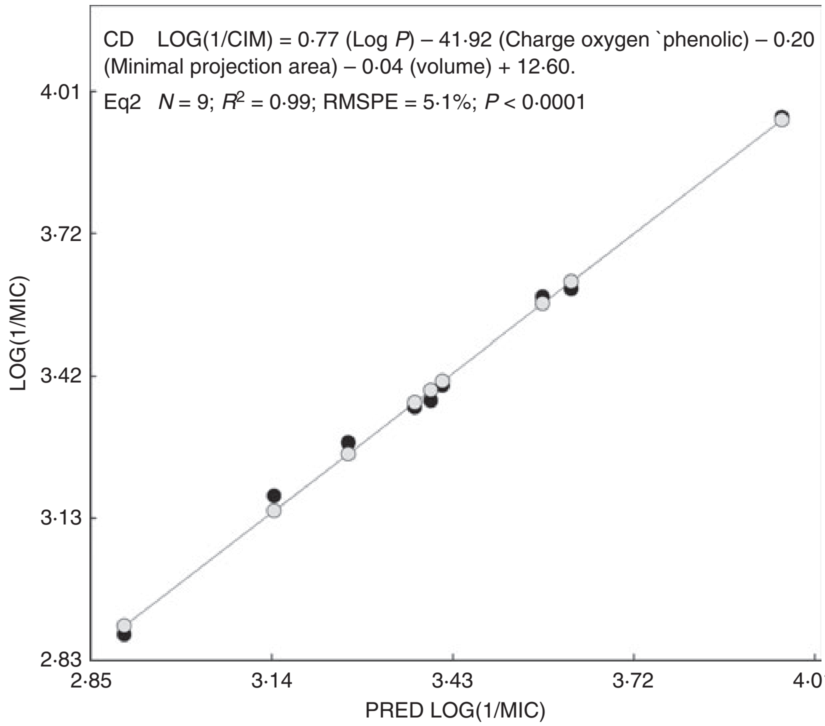

Figure 3 Plot of calculated versus experimental log 1/MIC of the nine phenolic compounds on *Candida dubliniensis* growth. Multiple linear regression analyses (MLR) calculated to examine quantitative relationships between linear combinations of the dependent variable (log 1/MIC) and the predictor variables (structure and molecular properties). Guaiacol was assigned to be outliers on the basis of their deviation between observed activity and calculated activity from the equation. *N* is the number of data points, *r* is the correlation coefficient between 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. The obtained quantitative structure–activity relationship (QSAR) model was validated with the root mean square prediction error (RMSPE) obtained by across validation leave-one-out procedure Outlier guaiacol.

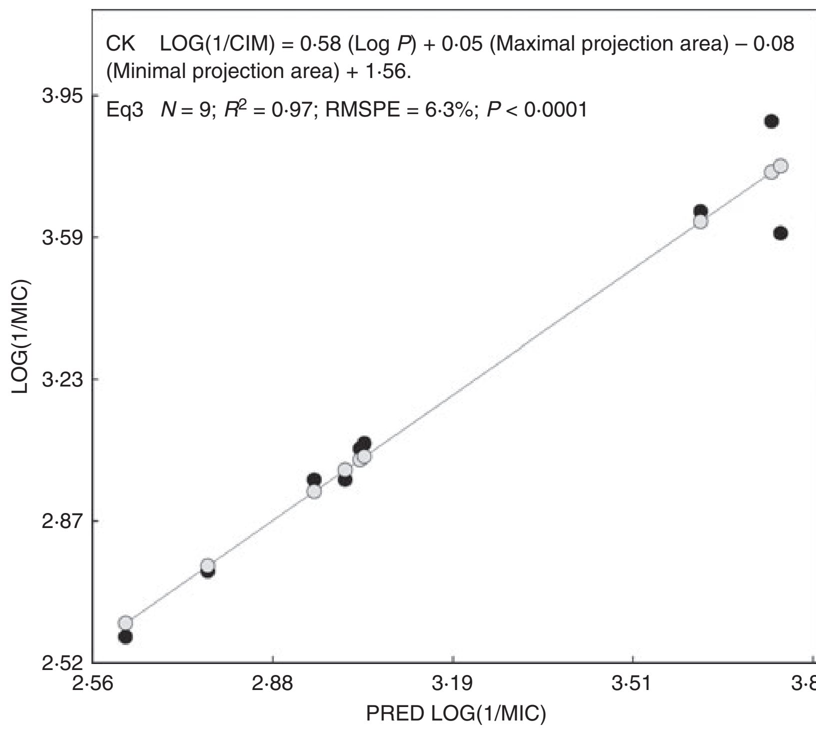

Figure 4 Plot of calculated versus experimental log 1/MIC of the nine phenolic compounds on *Candida krusei* growth. Multiple linear regression analyses (MLR) calculated to examine quantitative relationships between linear combinations of the dependent variable (log 1/MIC) and the predictor variables (structure and molecular properties). Guaiacol was assigned to be outliers on the basis of their deviation between observed activity and calculated activity from the equation. *N* is the number of data points, *r* is the correlation coefficient between 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. The obtained quantitative structure–activity relationship (QSAR) model was validated with the root mean square prediction error (RMSPE) obtained by across validation leave-one-out procedure Outlier guaiacol.

The alkylphenols with isopropyl substituents give rise to a QM with a higher cytotoxic activity than those with methyl groups (Desjardins *et al.* 1998; Zhao *et al.* 2009). This would explain the highest anti-*Candida* activity of carvacrol and thymol (Table 2) and the low activity of phenol. Cresol isomers, which only differ in the different

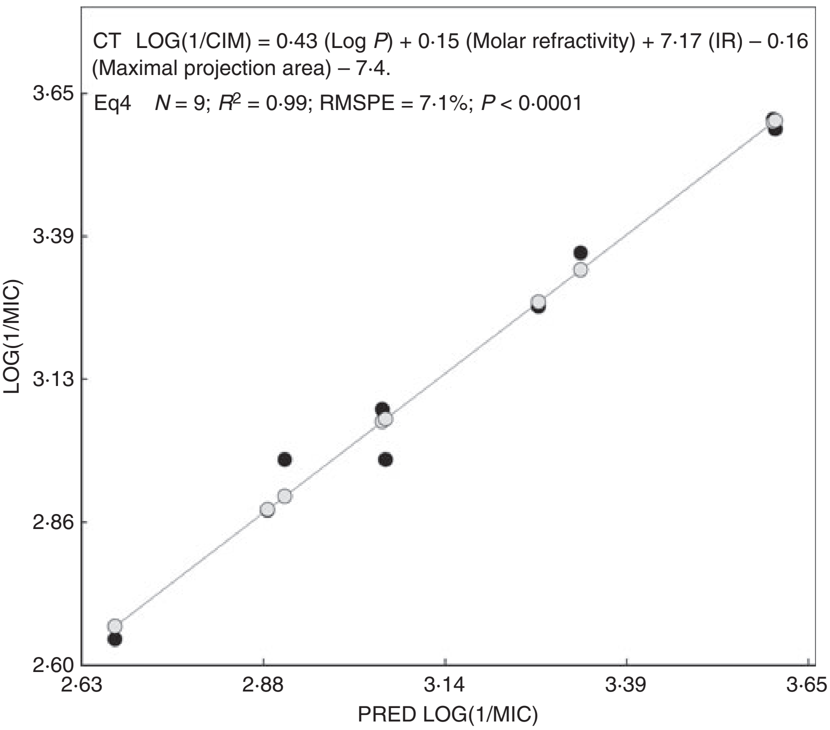

Figure 5 Plot of calculated versus experimental log 1/MIC of the nine phenolic compounds on *Candida tropicalis* growth. Multiple linear regression analyses (MLR) calculated to examine quantitative relationships between linear combinations of the dependent variable (log 1/MIC) and the predictor variables (structure and molecular properties). Guaiacol was assigned to be outliers on the basis of their deviation between observed activity and calculated activity from the equation. N is the number of data points, r is the correlation coefficient between 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. The obtained quantitative structure–activity relationship (QSAR) model was validated with the root mean square prediction error (RMSPE) obtained by across validation leave-one-out procedure Outlier guaiacol.

positions of a methyl substituent, have the following order of antifungal activity, m-cresol>p-cresol>o-cresol (Table 2). These differential anti-*Candida* activities could be attributed to the formation and stability of QM, because the ortho methoxy phenols show steric difficulty when being oxidized to QM by enzymes, which results in lower bioactivity (Krol and Judy 1997). This interaction could be explained in the obtained QSAR models by the presence of the maximal projection area and minimal projection area as descriptors in the equations (Fig 2–5). Importantly, the maximal projection area descriptor was positive in Eqns 1 and 3, while this descriptor showed a negative sign in Eqn 4. An increase in the size of the phenol compounds could be associated with greater antifungal activity against *C. dubliniensis* and *C. krusei*. However, opposite results were obtained with *C. tropicalis*. Therefore, these differences suggest that steric interactions could be different for each *Candida* species. Molar refractivity is related to the London dispersive forces, which that act in the drug–receptor interaction. The positive presence of the molar refractivity descriptor in the QSAR study on *C. tropicalis* also suggested that bulky substituents in the phenols rings—namely alkyl and methoxyl—will increase the binding affinity towards a specific target.

In summary, to our knowledge, this is the first contribution concerning anti-*Candida* activity by ortho-substituted phenolic compounds. According to the descriptors obtained in this QSAR study, the anti-*Candida* activity of ortho-substituted phenols is due to multiple action mechanisms. The first action is a nonspecific interaction with the mitochondrial or plasma membrane indicated by descriptor Log P. The second is based on a steric descriptor, maximal and minimal projection of the area, which could explain the inability of some phenolic compounds to be biotransformed into quinones methylene by *Candida* species. These steric descriptors have a different relationship with each of the evaluated *Candida* species. These results could be employed to predict anti-*Candida* activity of new phenolic compounds in the search for new alternatives or complementary therapies to combat candidiasis.

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Conflict of interest

The authors declare that there is no conflict of interest.

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