Phebalosin and its Structural Modifications are Active against the Pathogenic Fungal Causing Paracoccidioidomycosis

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Abstract

The phebalosin was isolated from hexane extract of the Polygala paniculata (Polygalaceae). The structural modifications of phebalosin were performed on the epoxy group with different nucleophiles such as H2O, ethoxy, methoxy, isopropoxy and n-butoxy for the corresponding derivatives and with acetic anhydride and aceton to give the diacetyl and cyclic acetal derivatives. The phebalosin and their derivatives were tested against four isolates of the pathogenic fungus Paracoccidioides brasiliensis. In this work phebalosin showed promising antifungal activity against isolates of P. brasiliensis with MIC value of 31.2 and 62.5 µg/ml. The compound 3 presented the better activity with MIC value of 1.9 µg/ml against the isolate P. brasiliensis Pb03. Besides, it was used methods of theoretical Chemistry, and chemometrics analysis techniques to perform a SAR study. The compounds activity is due to both types of properties – electronic and structural ones. Overall, these data open new possibilities for the potential use of phebalosin and its structural modifications as antifungal.

Keywords: Phebalosin; Paracoccidioidomycosis; antifungal; Polygala paniculata; Molecular modeling; Chemometrics

Introduction

Coumarins are a vast 1,2-benzopyrone group of natural compounds essentially found in green plants. The substitutions into their basic skeleton 1,2-benzopyrone can occur at any of the six available sites providing them extremely variable structures [1]. Although no longer used as food flavouring, coumarin is present in certain tobaccos and alcoholic beverages and is used in various soup, detergent and cosmetic preparations [2].

Hydroxy derivatives of 4-methyl coumarin are important group of coumarin derivatives showing medicinal as well as other biotechnological applications. For example, 5,7-dihydroxy 4-methyl coumarin and 7,8-dihydroxy 4-methyl coumarins are useful precursors to synthesize respective diacetoxy and hydroxyl-amino derivatives of 4-methyl coumarin, which are known to be good antioxidants having excellent radical scavenging properties [3].

Among the various coumarin derivatives, 7-substituted coumarins are important groups showing various kinds of bioactivities and also other applications. For example, 7-hydroxy 4-methyl coumarin is used in fluorometric determination of enzymatic activity, as a starting material for the preparation of insecticides and furano coumarins [4]. Due to their inherent photochemical characteristics, reasonable solubility and relative ease of synthesis, coumarin derivatives have been extensively investigated for electronic and photonic applications [5].

In this present work we screened the coumarin phebalosin and seven phebalosin-derivatives against four clinical isolates of the pathogenic dimorphic fungus Paracoccidioides brasiliensis, the causing agent of Paracoccidioidomycosis (PCM). PCM is the most prevalent systemic endemic mycosis in South America with most reported cases in Brazil [6]. In the absence of drug therapy the disease is usually fatal. The treatment of PCM is usually long, with many patients receiving therapy for one to two years or even more. It appears that the number of drugs active against PCM is scarce [7]. Although azoles and other drugs can arrest the progression of PCM, the fibrosis sequelae persist, probably constituting a source of fungi that could lead to a relapse in the disease following termination of treatment [8,9]. The strong toxicity of amphotericin B makes the effective management of this severe disease difficult [10]. For this reason the discovery of new drugs for the treatment of PMC is very important.

Materials and Methods

Chemistry

1H and 13C NMR spectra were measured using a Varian 400 MHz spectrometer. A solvent used for NMR measurements was TMS-CDCl3. IR spectra were recorded with a Perkin-Elmer FT 16PC spectrometer (KBr pellets). Mass spectra were taken with a Shimadzu QP 5050A at 70 eV. TLC: Merck Kieselgel 60 F254, spots were visualized under UV at 254 and 360 nm.

Plant material

Polygala paniculata was collected in March 2004 on Daniela beach, Florianópolis, Santa Catarina State, Brazil. The specimen was identified by a botanist, Prof. Dr. Olavo Araújo Guimarães, and the voucher specimen number 26027 was deposited at the Herbarium of the Botany Department of Universidade Federal do Paraná (Curitiba, PR, Brazil).

Extraction and isolation

The dried whole plant (3500 g) was extracted with hexane twice, each for 24 hours at room temperature. The extract was partially concentrated in vacuum with controlled temperature observing the

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precipitation of a white amorphous solid. The solid was separated from the supernatant solution followed by successive washing with hexane, yielding 2 g of epoxy coumarin, phebalosin (1).

**Structural modification**

**Epoxy Ring Hydrolysis of the phebalosin (2):** For ring epoxy hydrolysis of phebalosin (1), 100 mg was dissolved in dichloromethane (CH₂Cl₂), 10 ml distilled water, 1 ml chloric acid (HCl) and 5 g of silica gel (catalyst). The mixture was subjected to magnetic agitation for 72 h and temperature control (50°C). After this period, the reaction mixture was extracted with CH₂Cl₂, dried with anhydrous sodium sulfate (Na₂SO₄), filtered and the organic solvent was concentrated in a vacuum desiccator. The crude product was purified by chromatography on a flash column (230-400 mesh) with hexane/ethyl acetate 10:90 to yield 19% as a white solid.

**Epoxy Ring Hydrolysis of the phebalosin (2):** The crude product was purified by chromatography on a flash column (230-400 mesh) with hexane/ethyl acetate 40:60 to yield 26% as a white solid.

**Epoxy Ring Ethanolysis of phebalosin (3):** The same method of (2) was used for ethanolysis with the substitution of the solvent (water by ethanol). The crude product was purified by chromatography on a flash column (230-400 mesh) with hexane/ethyl acetate 40:60 to yield 26% as a white solid.

**Epoxy Ring Hydrolysis of the phebalosin (2):** The reaction mixture was subjected to magnetic agitation for 72 h and temperature control (50°C). After this period, the reaction mixture was extracted with (CH₂Cl₂), dried with anhydrous sodium sulfate (Na₂SO₄), filtered and the organic solvent was concentrated in a vacuum desiccator. The crude product was purified by chromatography on a flash column (230-400 mesh) with hexane/ethyl acetate 40:60 to yield 70% as white solid.

**Obtaining cyclic acetal of phebalosin with acetone (7):** The acetalation reaction of phebalosin (1) was performed in dry acetone (100 mg) by dissolution of the 100 mg of phebalosin with addition 1 ml of chloric acid (HCl) and 5 g of silica gel (catalyst). The mixture was subjected to magnetic agitation for 72 h and temperature control (50°C). After this period, the reaction mixture was extracted with (CH₂Cl₂), dried with anhydrous sodium sulfate (Na₂SO₄), filtered and the organic solvent was concentrated. The crude product was purified by chromatography on a flash column (230-400 mesh) with hexane/ethyl acetate 40:60 to yield 70% as white solid.

**Acetylation of phebalosin (8):** The acetylation of phebalosin (1) (100 mg) with acetic anhydride (2 ml), acetic acid (1 ml), catalyzed by 4-(dimethylamino)pyridine (DMAP) was dissolved in ethyl acetate (3 ml). The mixture was submitted to magnetic agitation for 72 h. After the reaction the solvent is evaporated in vacuum desiccator. The crude product was purified by chromatography on a flash column (230-400 mesh) with hexane/ethyl acetate 50:50 to yield 65% as white solid.

**Paracoccidioides brasiliensis strains:** Four clinical isolates of *P. brasiliensis*, Pb18 (from the fungal collection of the Faculty of Medicine of the Universidade de São Paulo, São Paulo, SP, Brazil), Pb03 (clinical isolates from chronic PCM, São Paulo, Brazil– MHH Forjaz / TIE Svidzinski), Pb01 (ATCC: MYA-826) and Pb339 (ATCC 32069), were used in the biological assays.

**Inoculum preparation:** The strains were maintained by weekly passage in solid Yeast-
Peptone-Dextrose medium, at 37°C and were used after 7-10 days of fungus growth. Yeast cells in the exponential phase were collected aseptically with a platinum loop and re-suspended in a tube containing 5 ml of sterile saline. If large aggregates existed, they were allowed to settle for several minutes, and the supernatants were collected. The suspensions were then diluted in synthetic RPMI medium (Sigma, St. Louis, MO, USA) with L-glutamine buffered to pH 7.0 with 0.165 morpholine propanesulfonic acid (MOPS, Sigma), and prepared according to the CLSI document M 27-A, [11] to obtain a final inoculum size suitable for the strains [12]. After homogenization by vortexing, transmittance was measured at 520 nm and adjusted to 69 to 70% [13].

Susceptibility test

The compounds were dissolved in dimethylsulfoxide (DMSO). Serial dilutions were then performed, using RPMI as a diluent, maintaining a constant volume of 1 ml per tube. The extracts were tested at eight different concentrations that ranged from 500-1.9 µg/ml. Volumes of 100 µl of each dilution were distributed in sterile flat-bottom 96-well microplates (Difco Laboratories, Detroit, MI, USA).

Susceptibility was determined by the broth microdilution method. Broth microdilution testing was performed in accordance with the guidelines in the CLSI M27-A, document [11] and Nakai et al. [12] RPMI medium was used without compounds or solvents as a control for growth and sterility. Solvent DMSO at the same volumes used in the assay was used as control for toxicity. Amphotericin B (Sigma, St Louis, USA) (25 to 0.03 µg/ml) and (25 to 0.03 µg/ml) in DMSO was included as positive antifungal control with the stock solutions prepared in DMSO and water, respectively. After inoculation of fungal strains the plates were incubated at 37°C for 14 days. The tests were performed in triplicate in at least two independent experiments. The endpoints were determined visually by comparison with the drug-free growth control well. MICs were expressed in µg/ml and defined as the lowest compound concentration for which the well was optically clear. All assays were performed in triplicate and repeated at least once.

Theoretical Chemistry methodology and chemometrics

Structural and electronic properties were obtained from classical mechanics [14] calculations (AMBER force field, at 298 K, in a vacuum) implemented in HYPERCHEM 7.5 molecular modelling program [15] and HF/6-31G** electronic structure calculations (ab initio method of Quantum Chemistry [16], 298 K, in a vacuum), implemented in GAUSSIAN program [17]. The obtained data were compared and correlated with antifungal activity by using chemometrics analysis [18]: Hierarchical Clustering Analysis (HCA), Partial Least Squares (PLS) and Principal Component Analysis (PCA) methods by using MINITAB 15® program [19].

Results

Whole P. paniculata plants were extracted with hexane to afford the coumarin phebalosin as a white amorphous solid, m.p. 124°C (Lit. 121-123°C); Rf=0.39 (eluent 60:40 hexane/ethyl acetate) and was identified by spectroscopic analysis and comparison with literature data [20]. A study of the reactivity of phebalosin (1) was planned, preparing a series of derivatives involving the opening epoxide ring by nucleophilic substitution reactions using different reagents, acetylation and formation of the cyclic acetal as shown in Figure 1.

The phebalosin (1) was treated with water and silica gel under
agitation being partially converted to a dihydroxy derivative 3 (19%) which presented itself in the form of a white solid with MP 135-136°C (Lit. 135-136°C; [18]) Rf=0.38 (eluent ethyl acetate 100%). The spectrum in region IR in KBr pellets showed the appearance of intense hydroxyl absorption at υ 3384 cm⁻¹, showing the opening of the epoxide ring, while it maintained the other absorption characteristics of coumarin. The 'H NMR spectrum of the reaction product kept the absorption characteristics of the coumarin nucleus: Halfway prenyl, the displacement of hydrogen atoms H-1' and H-2' of 3.92 ppm and 3.99 ppm (epoxide) to 5.31 ppm (1H, d) and 4.53 ppm (1H, d, J = 8.4 Hz) respectively was observed due to the formation of a diol derivative.

In the treatment with ethanol and silica gel under agitation, phebalosin was converted to a 1'-hydroxy-2'-ethoxy derivative (26%) which was presented in the form of white solid, with MP 130-131°C, with Rf=0.32 (eluent hexane/ethyl acetate 50:50). The spectrum in region IR in KBr pellets showed the appearance of intense hydroxyl absorption at υ 3473 cm⁻¹, showing the opening of the epoxide ring, and the other absorption characteristics of coumarin. The 'H NMR spectrum of the reaction product kept the absorption characteristics of the coumarin nucleus. Halfway prenyl, we observed the displacement of hydrogen atoms H-1' and H-2' of 3.92 and 3.99 ppm (epoxide) to 5.14 ppm (1H, d, J = 7.20 Hz) and 4.91 ppm (1H, d, J = 5.14 ppm (1H, d, J = 8.4 Hz) respectively was observed due to the formation of the ethoxy derivative; furthermore was observed an additional spin system to ethoxy group at 1.18 ppm (3H, t) and 3.44 ppm (2H, m).

The phebalosin react quickly with sodium methoxide to give 1'-hydroxy-2'-methoxy derivative 4 (71%) as a white solid, with MP 140-142°C, and Rf=0.24 (eluent hexane/ethyl acetate 50:50). The spectrum in region IR in KBr pellets showed the appearance of intense hydroxyl absorption at υ 3476 cm⁻¹, showing the opening of the epoxide ring, keeping the other absorption characteristics of coumarin. The 'H NMR spectrum of the reaction product kept the absorption characteristics of the coumarin nucleus. Halfway prenyl, the displacement of hydrogen atoms H-1' and H-2' of 3.92 and 3.99 ppm (epoxide) to 4.65 ppm (1H, d, J = 2.0 Hz) and 4.51 ppm (1H, d, J = 2.0 Hz) respectively was observed, due to the formation of the methoxy derivative. Additionally, the 'H NMR spectrum recorded the appearance of a absorption at 3.25 ppm (3H, s) to additional methoxy group.

The reaction with sodium isopropoxide afforded 1'-hydroxy-2'-isopropoxy derivative 5 (4%) presented in the form of a white solid, with MP 101-103°C, and Rf=0.48 (eluent hexane/ethyl acetate 50:50). The spectrum in region IR in KBr pellets showed the appearance of hydroxyl absorption at υ 3557 cm⁻¹, showing the opening of the epoxide ring, keeping the other absorption characteristics of coumarin. The 'H NMR spectrum of the reaction product kept the absorption characteristics of the coumarin nucleus. Halfway prenyl, we observed the displacement of hydrogen atoms H-1' and H-2' of 3.92 and 3.99 ppm (epoxide) to 4.83 ppm (1H, d, J = 8.4 Hz) and 4.65 ppm (1H, d, J = 8.4 Hz) respectively, due to the opening of the ring and the appearance of an additional spin system at 1.01 ppm (3H, t), 1.24 ppm (3H, d) and 3.54 ppm (1H, m) to isopropyloxy group inserted.

The reaction with sodium n-butoxy gives the 1'-hydroxy-2'-n-Butoxy derivative 6 (38%). The compound was presented in the form of yellow solid, with MP 97-99°C, and Rf=0.52 (eluent hexane/ethyl acetate 50:50). The spectrum in region IR in KBr pellets showed the appearance of hydroxyl absorption at υ 3435 cm⁻¹, showing the opening of the epoxide ring, keeping other absorption characteristics of coumarin. The 'H NMR spectrum of the reaction product kept the absorption characteristics of the coumarin nucleus. Halfway prenyl, the displacement of hydrogen atoms H-1' and H-2' of 3.92 and 3.99 ppm (epoxide) to 5.14 ppm (1H, d, J = 8.8 Hz) and 4.92 ppm (1H, d, J = 8.8 Hz) was observed respectively. We also observed the appearance of the signals 0.82 ppm (3H, t), 1.31 ppm (2H, m), 1.53 ppm (2H, m) and 3.33 ppm (2H, t) of the n-butoxy group inserted.

The treatment of phebalosin with acetone and silica gel under agitation lead to formation of the respective cyclic derivative (20%) as a colorless crystal, with MP 111-112°C, with Rf=0.58 (eluent hexane/ethyl acetate 50:50). The spectrum in the IR region in KBr pellets showed the appearance of intense hydroxyl absorption at υ 2980 cm⁻¹, showing the opening of the epoxide ring, while it maintained the other absorption characteristics of coumarin. The NMR spectrum of the reaction product kept the absorption characteristics of the coumarin nucleus. Halfway prenyl, a pair of doublet absorbing at δH 5.57 and δH 5.02 ppm (1H each, d, J = 9.60 Hz) was displaced in relation to epoxy moiety. The singlets that absorb at 1.70 ppm and 1.74 ppm, with integration for three hydrogen atoms each, were assigned to the two methyl H-8' and H-7' of the acetal group.

Under acetylation with acetic acid and acetic anhydride under agitation, the phebalosin was partially converted to a diacetyl derivative 8 (65%) which presented itself in the form of solid white, with MP 120-121°C, with Rf=0.40 (eluent hexane/ethyl acetate 50:50). The spectrum in the IR region in KBr pellets showed no absorption of hydroxyl and retained all other characteristic absorptions of coumarin. The 'H NMR spectrum of the reaction product kept the absorption characteristics of the coumarin nucleus. Halfway prenyl, we observed the displacement of hydrogen atoms H-1' and H-2' of 3.92 and 3.99 ppm (epoxide) to 6.08 ppm (1H, d, J = 12.0 Hz) and 5.99 ppm (1H, d, J = 12.0 Hz) respectively, due to the formation of acetyl derivative. The acetylation is confirmed by the presence of signals 1.87 ppm (3H, s) and 1.92 ppm (3H, s) relative to the acetyl groups.

Antifungal activity

In this work, the phebalosin was active against all isolates of *P. brasiliensis* tested (Table 1). The MIC values ranged from 31.2 to 500 µg/ml In relation to the phebalosin-derivative compounds, an increase of antifungal activity was observed for compounds 3, 5 and 6 against isolate P803 of *P. brasiliensis*. However, P818 did not present significant susceptibility against the compounds 7, 8 and 4. Isolate P803 was more susceptible to drugs tested but not for trimeprilum-sulfamethoxazole (300 µg/ml) in the tested conditions.

Molecular modelling and chemometrics

The coumarin phebalosin compound and its seven phebalosin derivatives had the optimization of their geometries realized through Classical Mechanics (AMBER force field, vacuum, 298 K) aimed at obtaining the structure with a minimum of energy. Then used the PM3 level of theory [15] (semiempirical quantum mechanics) at 298 K, vacuum, in order to determine the physico-chemical properties of interest (descriptors). All protocol for obtaining descriptors was held in the programmes HyperChem Professional 7 5 and GAUSSIAN 09. In this work, the following descriptors were determined through the protocol described in the previous section (Table 2): enthalpy of formation (ΔHf°), variation of energy between the frontier orbitals (the highest occupied energy - HOMO and lowest unoccupied energy - LUMO), ΔE_HOMO-LUMO, lipophilicity parameter (log P), dipole moment (µ), polarizability, molecular volume, solvent accessible area (SA) and system energy (Hartree Fock energy, at 6-31G** level of theory). The values of activity were converted into log(1/α) from each antifungal analysis, where α = experimental activity.

Initially it was applied a hierarchical method agglomerative (HCA)
with all submissions, each forming its own grouping and by similarity, the final grouping of the clusters (also called final partition) which must, as expected, identify groups whose comments or variables have characteristics in common was determined. PCA and PLS techniques were performed in order to separate the compound as function of their antifungal activity.

Despite a few numbers of compounds and a low range of activity values observed, a chemometric analysis was performed: in a general sense, it seems that polarizability is the most important descriptor for the properties which describe adequately the antifungal activity (Cumulative eigenvalues 73.8%; 98.0%):

\[ PC1 = -0.152 \log(1/c_{Pb18}) + 0.579 \text{volume} + 0.570 \text{polarizability} + 0.558 \Delta E_{\text{HOMO-LUMO}}; \]

\[ PC2 = 0.981 \log(1/c_{Pb03}) + 0.022 \text{volume} + 0.056 \text{polarizability} + 0.185 \text{SA}. \]

The PC2 component is responsible for the compounds separation. When those three values increases, the activity increases, either.

Finally, for the Pb03 fungus, whose compounds revealed the best activities, the second component (PC2) showed a good separation as function of activity (Figure 5):

\[ \text{For Pb18 the results are not conclusive (Figure 4), but they pointed to three descriptors: volume, surface area (SA) and polarizability (Cumulative eigenvalues 73.8%; 98.0%):} \]

\[ PC1 = -0.152 \log(1/c_{Pb18}) + 0.579 \text{volume} + 0.570 \text{polarizability} + 0.558 \Delta E_{\text{HOMO-LUMO}}; \]

\[ PC2 = 0.981 \log(1/c_{Pb03}) + 0.022 \text{volume} + 0.056 \text{polarizability} + 0.185 \text{SA}. \]

The PC2 component is responsible for the compounds separation. When those three values increases, the activity increases, either.

\[ \text{For Pb03 the results are not conclusive (Figure 4), but they pointed to three descriptors: volume, surface area (SA) and polarizability (Cumulative eigenvalues 73.8%; 98.0%):} \]

\[ PC1 = -0.152 \log(1/c_{Pb18}) + 0.579 \text{volume} + 0.570 \text{polarizability} + 0.558 \Delta E_{\text{HOMO-LUMO}}; \]

\[ PC2 = 0.981 \log(1/c_{Pb03}) + 0.022 \text{volume} + 0.056 \text{polarizability} + 0.185 \text{SA}. \]

The PC2 component is responsible for the compounds separation. When those three values increases, the activity increases, either.

\[ \text{Finally, for the Pb03 fungus, whose compounds revealed the best activities, the second component (PC2) showed a good separation as function of activity (Figure 5):} \]

\[ \text{The properties which describe adequately the antifungal activity are polarizability, volume and the variation of energy between the frontier orbitals (} \Delta E_{\text{HOMO-LUMO}} \text{). Cumulative eigenvalues 66.4%; 93.0%):} \]

\[ PC1 = 0.040 \log(1/c_{Pb03}) - 0.538 \Delta E_{\text{HOMO-LUMO}} + 0.590 \text{polarizability} + 0.600 \text{volume}; \]

\[ PC2 = 0.959 \log(1/c_{Pb03}) + 0.252 \Delta E_{\text{HOMO-LUMO}} + 0.122 \text{polarizability} + 0.042 \text{volume}. \]

In this case, the antifungal activity should be increased by increasing the values of the cited properties.

\[ \text{Discussion}\]

Currently, there are few works on the search for new drugs against \emph{P. brasiliensis}. San-Blas et al. [21] was one of the prime works with a natural product against this fungus. Recently, Thomaz et al. [22]
showed that alone from garlic therapy was effective in Balb/c mice infected intra tracheally with the virulent isolate *P. brasiliensis* Pb18. Pelegrini et al. [23] have tested peptides from *Passiflora edulis* against this fungus, but they did not find any effective activity. Marques et al. [24] studied a peptide vaccine with P10, derived from gp43 (major diagnostic antigen), as an adjuvant to chemotherapy, which reduced treatment time, and prevented relapsing disease. In another work, Martins et al. [25], studied the antifungal activity of curcumin, against 23 fungal strains and observed that *P. brasiliensis* were the most susceptible to curcumin. In present work, the phebalosin was active against all isolates of *P. brasiliensis* tested. Due to the great relationship between the coumarins and the biological activities mentioned above, a series of modifications were made to the majority (phebalosin) of *Polygala paniculata* for the evaluation of a possible improvement in antifungal activity displayed by these compounds. The compound of reaction of phebalosin with ethanol (3) presented strong activity against isolate Pb03 of *P. brasiliensis* with a MIC values of the 1.9 µg/ml (6.5 µmol/L), sixteen times more active than phebalosine. The isopropoxy (5) and n-butoxy (6) derivatives were respectively four times and twice more active than phebalosine against Pb03 (Table 1). These experimental results suggest important hydrophobic and steric interaction simultaneously with alkyl groups in the moiety isoprenyl of the phebalosin. This result indicates an activity intermediate to the two drugs used in the treatment of PCM, and the most used in Brazil by the National Health Care System (SUS) is trimethoprim-sulfamethoxazole [25]. Other authors also tested the antifungal activity of amphotericin B and trimethoprim-sulfamethoxazole against several isolates of

Figure 2: PCA technique showing the compounds separation as function of antifungal activity (Score plot) and the mainly descriptors (Biplot) for Pb339 fungus.

Figure 3: PCA technique showing the compounds separation as function of antifungal activity (Score plot) and the mainly descriptors (Biplot) for Pb01 fungus.

Figure 4: PCA technique showing the compounds separation as function of antifungal activity (Score plot) and the mainly descriptors (Biplot) for Pb18 fungus.

Figure 5: PCA technique showing the compounds separation as function of antifungal activity (Score plot) and the mainly descriptors (Biplot) for Pb03 fungus.
Paracoccisides, with MIC values close to those found in present work, using RPMI medium, range 0.25-2 µg/ml and 300-75 µg/ml for amphotericin B and trimethoprim-sulfamethoxazole, respectively [25].

The chemometric results, derived from quantum chemistry calculations, revealed the same tendency observed from experimental data: structural properties are responsible for the antifungal activity. Polarizability, surface area and volume showed a good correlation with activity. In spite of all the observations, it’s important to remark that the variation of energy between the frontier orbitals (\(\Delta E_{\text{HOMO-LUMO}}\)) is an important property related to activity, either.

Despite the chemometric analysis is not completely conclusive, PCA technique was able to separate the compounds as function of antifungal activity. In order to increase the antifungal activity, it can be suggested that structural modifications of phebalosin should have the following properties: greater values for polarizability, volume and the variation of energy between the frontier orbitals (\(\Delta E_{\text{HOMO-LUMO}}\)). It pointed to steric influence (volume), lipophilicity character (polarizability and volume) and compound stability during the interaction (electronic influence, \(\Delta E_{\text{HOMO-LUMO}}\)) for the substituents alkyl groups.

In conclusion, phebalosin showed promised antifungal activity against isolates of *P. brasiliensis* tested. The structural modifications of phebalosin with different nucleophiles yielded seven compounds, four being new compounds (8, 4, 5, 6). Overall, these data open new possibilities for the potential use of phebalosin and its structural modifications as possible antifungal drugs against *P. brasiliensis*. However, new studies are necessary to characterize the mechanism of action of these compounds and produce more conclusive chemometric data.

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