Synthesis, Spectroscopic Characterization, DFT Calculations and Preliminary Antifungal Activity of New Piperine Derivatives

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Four new piperine derivatives, PC1-PC4, were synthesized, and their structures were fully characterized by infrared (IR) and 1H and 13C nuclear magnetic resonance (NMR) spectroscopies. Quantum chemical calculations were performed using density functional theory (DFT) with the B3LYP-D3/6-31G(d,p) and 6-311+G(2d,p) basis sets. Electronic properties, such as the energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) and some global chemical reactivity descriptors, were evaluated to study the reactivity and stability of the compounds. 1H and 13C NMR chemical shifts were calculated by using the gauge-invariant atomic orbital (GIAO) method and compared with experimental values. In addition, the compounds were evaluated in an antifungal study against Candida, Trichophyton and Microsporum strains, and only PC4 showed 70% inhibition in ten tested strains, with a minimum inhibitory concentration (MIC) ranging from 1.23-2.46 μmol mL⁻¹ and a minimum fungicide concentration (MFC) ranging from 9.84-19.68 μmol mL⁻¹, and presented a fungistatic effect.

Keywords: piperine, synthesis, NMR, DFT, antifungal activity

Introduction

The rates of fungal resistance to drugs highlight an increasingly serious health problem and make it necessary to develop new therapeutic alternatives to treat these infections, since the options available today are mainly limited to azoles and echinocandins. More than one billion individuals worldwide are affected by fungal infections, and the associated mortality is over 1.5 million deaths each year. In this context, traditional medicine can serve as a guide during the process of discovering antifungal drugs, using the knowledge of plants used historically as anti-infectious agents.1,2

Plants, which are a major source of traditional medicines, are also promising sources for new drugs due to the presence of secondary metabolites with a wide range of biological activity, such as antimicrobial activity. In many cases, these substances play a role in plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some plants used for their odor (terpenoids), pigment (quinones and tannins), and flavor (terpenoid capsaicin from chili peppers) have been found to also possess medicinal properties. There is an enormous chemical diversity of natural products from plants, and these products can be used directly or used as a precursor for developing better molecules.3,4

Piperine (C17H19NO3) is a naturally occurring alkaloid and is one of the main secondary metabolites found in Piper nigrum and P. longum.5 However, piperine can also be found in other species of the genus Piper, such as P. guineense,6 P. interruptum,7 P. sarmentosum8 and P. chaba.9 Amide alkaloids have pharmacological efficacies, such as antifungal,10 antibacterial,11 analgesic,12 antipyretic,12 anti-inflammatory,12 antileishmanial,13 and larvicidal14 activities. Piperine can be isolated by various methods, such as maceration using acetic acid, extraction
with ethanol in a Soxhlet apparatus, microwave-assisted extraction, and extraction using ultrasound. Piperine derivatives represent a wide range of important biological properties, such as antifungal (1), larvicidal (2), trypanocidal (3), antidiabetic (4), and antitumor (5) activities (Figure 1).

Thus, taking into account the potential of piperine in the development of drug candidates, four new compounds were synthesized and characterized by infrared (IR) and 'H and '\(^1\)C nuclear magnetic resonance (NMR) spectroscopy. In addition, the conformational, electronic and NMR spectroscopic properties of the compounds were calculated by using density functional theory (DFT). Using this method, the quantum chemical parameters, such as hardness (\(\eta\)), chemical potential (\(\mu\)) and electrophilic index (\(\omega\)), were calculated to characterize the global chemical reactivity of these compounds. The 'H and '\(^1\)C NMR chemical shifts were calculated and used to evaluate the correlation between the theoretical and experimental data. Furthermore, a preliminary study of the antifungal activity of the compounds against 10 species of fungi (7 yeasts and 3 filamentous) was included.

Experimental

Chemistry

All reagents and solvents used were purchased from commercial sources (Sigma-Aldrich \(^®\), São Paulo, Brazil) and used without further purification. The purification of the compounds was performed by recrystallization in a mixture of \(N,N\)-dimethylformamide (DMF)/water and confirmed by determining the melting range on an MQAPF-302 hotplate (Microquímica). 'H and '\(^1\)C nuclear magnetic resonance (NMR) spectra were obtained on two different machines: a Bruker Avance Ultrashield TM (400 MHz for 'H and 101 MHz for '\(^1\)C) and a Bruker Avance Ultrashield TM (500 MHz for 'H and 126 MHz for '\(^1\)C). Deuterated chloroform (CDCl\(_3\)) and deuterated dimethyl sulfoxide (DMSO-\(d_6\)) were used as solvents, and tetramethylsilane (TMS) was used as the internal standard. Chemical shifts (\(\delta\)) were measured in parts per million (ppm), and the coupling constants (\(J\)) were measured in hertz (Hz). Infrared (IR) spectra were obtained on a Shimadzu model IR Prestige-21 FTIR spectrometer with an attenuated total reflection (ATR) accessory.

Piperine extraction (6)

Two hundred grams of black pepper was ground to a fine powder and extracted with 1000 mL of 95% ethanol in a Soxhlet extractor for 2 h. The solution was filtered and concentrated under vacuum. Then, 200 mL of a 10% alcoholic KOH solution was added to the residue, and the precipitated material was filtered. A small amount of water was added to the alcoholic solution, sufficient for the medium to become cloudy. The alcoholic solution was left overnight, and the precipitate was obtained as yellow needles. The obtained solid was washed with a small amount of ice water, and 7.0 g of piperine was obtained. mp 126-128 °C (lit.\(^2\) 125-126 °C); 'H NMR (500 MHz, CDCl\(_3\));

\[\text{Figure 1. Piperine derivatives have antifungal (1), larvicidal (2), trypanocidal (3), antidiabetic (4) and antitumor (5) activities.}\]
Potassium piperate (7)

In a 100 mL flask, 6.0 g (0.021 mol) of piperine was suspended in 60 mL of 20% KOH alcoholic solution. The reaction mixture was kept under reflux and stirring for 24 h. After the end of the reaction, the reaction mixture was subjected to extraction, washed with ethanol, and dried, and 5.0 g (92.9%) of a brown granular solid was obtained. 1H NMR (400 MHz, CDCl3) δ 7.05 (dd, J 15.5, 9.6 Hz, 1H, =C-H), 6.86 (d, J 1.6 Hz, 1H, C-HAr), 6.82 (dd, J 8.2, 1.6 Hz, 1H, C-HAr), 6.73 (d, J 8.0 Hz, 1H, C-HAr), 6.60 (m, 2H, =C-H), 5.92 (d, J 15.2 Hz, 1H, =C-H), 5.88 (s, 2H, O-CH2-O).

General preparation of arylacyl bromide (9a-9d)22

In a 100 mL flask, 0.004 mol of potassium piperate and 0.004 mol of arylacyl bromide. The reaction mixture was stirred at a temperature of 100 °C for 24 h. After the end of the reaction, the mixture was cooled, ice water was added, and the solid formed was separated by vacuum filtration and washed with 2-Ox0-2-phenethyl-piperate (PC1)

Yield: 60%; pale yellow solid; mp 167-169 °C; IR (ATR) ν / cm−1 3062 (C-HAr), 3037, 3014 (C-HAr), 2933, 2906 (C-H), 1714 (C=O, ester), 1689 (C=O, ketone), 1620 (C=CAr), 1608 and 1490 (C=CAr), 1448 (CH3), 1257 and 1143 (O-CH2-O), 927, 852, 752 (C-HAr); 1H NMR (500 MHz, CDCl3) δ 7.94 (d, J 7.7 Hz, 2H, H-16 and H-16’), 7.61 (t, J 7.4 Hz, 1H, H-18), 7.51 (dt, J 15.0, 9.3 Hz, 3H, H-3, H-17 and H-17’), 7.00 (s, 1H, H-7), 6.92 (d, J 7.9 Hz, 1H, H-10), 6.78 (dd, J 26.3, 22.5, 13.2 Hz, 3H, H-4, H-5 and H-11), 6.10 (d, J 15.2 Hz, 1H, H-2), 5.98 (s, 2H, H-12), 5.42 (s, 2H, H-13); 13C NMR (126 MHz, CDCl3) δ 166.38 (C-1), 119.06 (C-2), 146.94 (C-3), 124.15 (C-4), 140.93 (C-5), 130.50 (C-6), 105.98 (C-7), 143.82 (C-8), 148.70 (C-9), 108.55 (C-10), 123.12 (C-11), 101.42 (C-12), 65.97 (C-13), 192.43 (C-14), 134.40 (C-15), 128.84 (C-16 and C-16’), 127.82 (C-17 and C-17’), 133.80 (C-18).

2-(4-Nitrophenyl)-2-oxoethyl-piperate (PC2)

Yield: 50%; orange solid; mp 209-211 °C; IR (ATR) ν / cm−1 3105 (C-HAr), 3070 (C-HAr), 2910 (C-H), 1716 (C=O, ester), 1703 (C=O, ketone), 1620 (C=CAr), 1483 (C=CAr), 1523 and 1346 (NO2), 1444 (CH3), 1249 and 1139 (O-CH2-O), 875, 858, 844 (C=H); 1H NMR (500 MHz, DMSO-d6) δ 8.35 (d, J 8.8 Hz, 2H, H-17 and H-17’), 8.20 (d, J 8.8 Hz, 2H, H-16 and H-16’), 7.46 (dd, J 15.3, 9.9 Hz, 1H, H-3), 7.20 (d, J 1.6 Hz, 1H, H-7), 7.03 (m, 2H, 3H, H-4, H-5 and H-11), 6.91 (d, J 8.0 Hz, 1H, H-10), 6.14 (d, J 15.2 Hz, 1H, H-2), 6.04 (s, 2H, H-12), 5.56 (s, 2H, H-13); 13C NMR (126 MHz, CDCl3) δ 165.31 (C-1), 118.52 (C-2), 145.87 (C-3), 124.37 (C-4), 140.78 (C-5), 130.18 (C-6), 105.73 (C-7), 147.78 (C-8), 148.11 (C-9), 108.24 (C-10), 122.89 (C-11), 101.11 (C-12), 66.27 (C-13), 192.37 (C-14), 138.64 (C-15), 129.00 (C-16 and C-16’), 123.59 (C-17 and C-17’), 150.16 (C-18).

2-Oxo-2-(p-tolyl)ethyl-piperate (PC3)

Yield: 65%; orange solid; mp 187-189 °C; IR (ATR) ν / cm−1 3039 (C-HAr), 3000 (C-HAr), 2924 (C-H), 1712 (C=O, ester), 1695 (C=O, ketone), 1608 (C=CAr), 1602 and 1485 (C=CAr), 1438 (CH3), 1226 and 1128 (O-CH3-O), 925, 864, 819 (C-HAr); 1H NMR (400 MHz, DMSO-d6) δ 7.86 (d, J 8.0 Hz, 2H, H-17 and H-17’), 7.45 (dd, J 15.3, 9.3 Hz, 1H, H-3), 7.38 (d, J 8.0 Hz, 2H, H-16 and H-16’), 7.20 (s, 1H, H-7), 7.03 (t, J 11.0 Hz, 3H, H-4, H-5 and H-11), 6.91 (d, J 8.0 Hz, 1H, H-10), 6.14 (d, J 15.2 Hz, 1H, H-2), 6.04 (s, 2H, H-12), 5.47 (s, 2H, H-13), 2.40 (s, 3H, CH3); 13C NMR (101 MHz, DMSO-d6) δ 165.37 (C-1), 118.87 (C-2), 145.55 (C-3), 124.42 (C-4), 140.56 (C-5), 130.22 (C-6), 105.72 (C-7), 147.78 (C-8), 148.07 (C-9), 108.23 (C-10), 122.85 (C-11), 101.10 (C-12), 66.88 (C-13), 192.19 (C-14), 131.52 (C-15), 127.51 (C-16 and C-16’), 129.12 (C-17 and C-17’), 144.03 (C-18), 20.87 (CH3).

2-(4-Bromophenyl)-2-oxoethyl-piperate (PC4)

Yield: 58%; pale yellow solid; mp 213-214 °C; IR
The four lowest-energy conformers were optimized in chloroform solution by means of the B3LYP-D3/6-311+G(2d,p) method and using the integral equation formalism polarizable continuum model (IEFPCM) continuum model solvent. The Gibbs free energies of the conformers were computed at the same theoretical level to calculate the Boltzmann populations.

From the most stable PC1 conformer, the structures of the PC2, PC3 and PC4 compounds were built by the addition of the substituents NO₂, CH₃ and Br, respectively, on the phenyl ring. Their geometries were optimized in the gas phase and in DMSO by using the B3LYP-D3/6-311+G(2d,p) method and the IEFPCM solvent model. The vibrational frequency was calculated to ensure that the structures are minima on the potential energy surface.

Electronic properties

The molecular electrostatic potential, frontier molecular orbitals (highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)) and DFT chemical reactivity descriptors of the compounds were calculated from a single point by using the B3LYP/6-311+G(2d,p) method. Molecular electrostatic potential surfaces (MEPS) were obtained with fixed values of electronic density (isosurface) of 0.001 e bohr⁻³. This electronic density value was recommended by Barde et al. and is considered standard in the calculation of the MPES of several organic molecules. The global chemical reactivity descriptors, such as chemical hardness (η), electronic chemical potential (μ) and electrophilicity (ω), were evaluated from the HOMO and LUMO energies using the following equations:

$$\eta = \frac{E_{\text{LUMO}} - E_{\text{HOMO}}}{2}$$  \( (1) \)

$$\mu = \frac{E_{\text{LUMO}} + E_{\text{HOMO}}}{2}$$  \( (2) \)

$$\omega = \frac{\mu^2}{2\eta}$$  \( (3) \)

NMR calculations

The ¹H and ¹³C NMR chemical shifts were obtained from single point calculations by using the B3LYP/6-311+G(2d,p) method, from the optimized geometries at the same theoretical level and using the gauge invariant atomic orbital (GIAO) model. Previous studies show that this functional in combination with the basis set provides good results in calculations of NMR chemical shifts. The formalism of the IEFPCM model for chloroform and DMSO as solvents was used in the NMR calculations. The calculated NMR shielding tensors were converted to chemical shifts by use of empirical scaling factors that are derived from linear regression analysis for a set of molecules. To assess the performance of the DFT method in predicting the ¹H and ¹³C NMR chemical shifts of the investigated compounds,
the mean absolute deviation (MAD) was used, which is defined as follows:

\[
\text{MAD} = \frac{1}{N} \sum_{i}^{N} |\chi_i^{\text{cal}} - \chi_i^{\exp}|
\]  \hspace{1cm} (4)

where \(\chi_i^{\text{cal}}\) and \(\chi_i^{\exp}\) are the chemical shifts calculated by the DFT method and its corresponding experimental value, respectively. \(N\) is the total number of chemical shifts associated with each molecular structure.

**Antifungal activity**

**Test substance**

For microbiological tests, the synthesized compounds were used in emulsion forms, using 5% dimethylsulfoxide (DMSO) and 2% Tween 80 (Sigma-Aldrich®, São Paulo, Brazil) and completing the volume with sterile distilled water until obtaining the necessary concentrations for each test. 32,33

**Culture mediums**

The culture media used to maintain the fungal strains was Sabouraud dextrose agar (SDA) (Difco Laboratories Ltd, Detroit, USA). For the biological activity tests, Roswell Park Memorial Institute (RPMI-1640) medium was used with L-glutamine and without sodium bicarbonate (Difco Laboratories Ltd, Detroit, USA and INLAB, São Paulo, Brazil). The culture media were prepared according to the manufacturer’s instructions.

**Microorganisms**

For the biological activity assays of the test products, the following strains were used: *Candida albicans* ATCC 76645, *C. albicans* LM-111, *C. albicans* LM-122, *C. tropicalis* ATCC-13803, *C. tropicalis* LM-04, *C. krusei* LM-656, *C. krusei* LM-13, *Trichophyton rubrum* LM-49, *Microsporum canis* LM-12, and *M. gypseum* LM-512. The microorganisms belong to MICOTECa of the Mycology Laboratory, Department of Pharmaceutical Sciences (DCF), Health Sciences Center (CCS) of the Federal University of Paraíba (UFPB). The strains were maintained in SDA at 4 °C. For use in the assays, cultures were reactivated in SDA, and colonies were removed for inoculum preparation according to the standard 0.5 McFarland scale, which corresponds to approximately 10⁶ colony-forming unit (CFU) mL⁻¹. 34-36

**Determination of the minimum inhibitory concentration (MIC)**

The MIC of the substances was determined using the microdilution technique in liquid medium in 96-well plates. Initially, 100 μL of double-concentrated RPMI broth was distributed to the microdilution plate wells. Subsequently, 100 μL of the substances were dispensed into the wells of the plates and diluted sequentially to obtain different substances. Finally, previously standardized fungal inoculums were added. At the same time, controls were performed to prove the viability of the strains (RPMI + fungal inoculums), the sterility of the culture medium (RPMI) and the positive control with amphotericin B (0.034 μmol mL⁻¹). The prepared plates were aseptically closed and incubated at a temperature of 35 ± 2 °C for 24-48 h for yeast fungi and 7-14 days at 28 ± 2 °C for filamentous fungi. The MIC for each product was defined as the lowest concentration capable of visually inhibiting microbial growth. The result was expressed as the arithmetic mean of the MIC obtained, performed in triplicate.

**Determination of minimum fungicidal concentration (MFC)**

After reading the MIC, aliquots of 10 μL of the supernatants were withdrawn from the wells of the microdilution plates at concentrations corresponding to the MIC, MIC × 2, MIC × 4 and MIC × 8 of each product for each strain and inoculated into new microdilution plates containing only RPMI medium. The assay was performed in triplicate. The plates were incubated at 35 ± 2 °C for 24-48 h for yeasts and 28 ± 2 °C for 7-14 days for filamentous fungi, and fungal growth was observed. The tests were performed in triplicate and the results expressed as the arithmetic mean of the MFCs obtained. 37

**Results and Discussion**

**Chemistry**

The synthesis of target molecules derived from piperine (PC1-PC4) was performed in three synthetic stages, as described in Scheme 1.

In the first step, the piperine (6) obtained from the black pepper through Soxhlet extraction was hydrolyzed in a basic solution of 20% KOH using ethanol as a solvent under reflux for 20 h, obtaining potassium piperate (7) in 93% yield. In the second step, the preparation of arylacyl bromide (9a-9d) with a yield between 75 and 80% was obtained from the reaction between bromine and different substituted acetophenones (8a-8d) using chloroform as a solvent at room temperature. In the third and last stage, compounds PC1-PC4 were obtained from the S₂N₂ nucleophilic substitution reaction between arylacyl bromide (9a-9d) and potassium piperate (7) using dimethylformamide (DMF) as a solvent at a temperature of
100 °C for 24 h. The products obtained with yields between 50 and 65% were purified using the recrystallization method in a solvent mixture of DMF/water (8:2).

The structures of the compounds derived from piperine (PC1-PC4) were characterized by infrared (IR) and ¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy. In the infrared spectra of the compounds derived from piperine, two stretches attributed to carbonyls are observed, one carbonyl in the ester group that appears at approximately 1716-1712 cm⁻¹ and one carbonyl in the ketone group at approximately 1703-1689 cm⁻¹. The presence of alkene C-H and aromatic C-H stretches can be observed at approximately 3105-3062 cm⁻¹ and 3070-3000 cm⁻¹, respectively. In the 2933-2900 region, aliphatic C-H stretches are observed. Stretches attribute to the C=C of aromatic rings in the range of 1606-1483 cm⁻¹ are observed, while stretching of the of C=C of alkenes can be observed at approximately 1620 cm⁻¹. The asymmetric and symmetrical stretching of the methylenedioxy group varies between 1257-1226 and 1143-1128 cm⁻¹, respectively. In the PC2 compound, asymmetric and symmetrical stretching at approximately 1523 and 1346 cm⁻¹ can be observed, respectively, for the NO₂ group.

In the ¹H NMR spectrum for all compounds (PC1-PC4), two characteristic signals present in the structures were shown in the form of singlets: a singlet for two methylene protons (H-13) in the range of 5.56-5.42 ppm and another singlet for two methylene protons referring to the methylenedioxy group (H-12) in the range of 6.04-5.98 ppm. The protons present in the aromatic ring and the olefinic protons resonated in the region 8.35-6.10 ppm. In the PC3 spectra, the methyl group was characterized by a singlet at 2.40 ppm. For the ¹³C NMR spectrum for all compounds, the carbon from the carbonyl corresponding to the ketone was characterized at approximately 192 ppm, and the carbon corresponding to the ester was characterized at approximately 166 ppm. The compounds showed characteristic signs of methylene carbons: one peak in the region of 66.3-65.9 ppm referring to C-13 and another peak in the range of 148.7-105.72 ppm. In the spectra of compound PC3, an additional signal in the aliphatic region at 28.87 ppm for the carbon of the methyl group was observed.

Conformational analysis

An understanding of molecular conformation has been shown to be fundamental to spectroscopy, material sciences, organic synthesis and biochemistry. Since the experimental structural determination of new piperine derivatives was not possible, a conformation search of the PC1 compound was performed by using quantum mechanical calculations. The potential energy surfaces were calculated by the B3LYP/6-31G(d,p) method with the dispersion contribution obtained from the D3 correction and are shown in Figures S16 and S17 (Supplementary
Information (SI) section). The lowest energy conformers were optimized using the B3LYP-D3/6-311+G(2d,p) method in chloroform solution and are shown in Figure 3. Only the conformations in which piperate moieties are in the same π-plane were considered.

The relative Gibbs energies, Boltzmann population and selected dihedral angles for conformers are listed in Table 1.

| Conformer | $\Delta G$ / (kJ mol$^{-1}$) | Population / % | C–C–O–C angle / degree |
|-----------|-----------------------------|----------------|-------------------------|
| a         | 0.00                        | 45.45          | 78.7                    |
| b         | 0.74                        | 33.68          | $-62.9$                 |
| c         | 3.59                        | 10.70          | 128.4                   |
| d         | 3.71                        | 10.17          | 128.3                   |

Conformer a was predicted by DFT calculations to be approximately 0.74–3.71 kJ mol$^{-1}$ more stable than the other conformers and to contribute approximately 45.5% of the Boltzmann population at 298.15 K (see Table 1). The main factors that determine the stability of the conformers are apparently the C=O···H and C$_2$O···H intramolecular interactions (see Figure 3, conformer a) and steric effects of the 2-phenyl-2-oxoethyl group. Furthermore, pronounced conjugation on the piperate moiety plays an important role in determining the stability of the conformers.

From the most stable conformer a, the structures of PC2, PC3 and PC4 compounds were built by the addition of the substituents NO$_2$, CH$_3$ and Br to the phenyl ring, respectively. The geometries were optimized in the gas phase and in DMSO solvent by using the B3LYP-D3/6-311+G(2d,p) method and used in the calculation of their electronic and spectroscopic properties.

Electronic properties

Electronic properties of the piperine analogs were calculated at the B3LYP/6-311+G(2d,p) theoretical level to obtain information on reactivity, stability and the electrophilic and nucleophilic sites in the molecules.

The molecular electrostatic potential (MEP) provides information about the charge distribution on the molecules, and it is very useful in understanding the sites of electrophilic attacks and nucleophilic reactions for the study of biological recognition processes and hydrogen bonding interactions. To predict the molecular reactive sites, the MEPs for the studied compounds were calculated and are shown in Figure 4.

The negative regions in red are related to electrophilic reactivity and positive regions in blue are related to nucleophilic reactivity. As seen from the MEP maps of the compounds, the higher negative regions include carbonyl groups. The positive regions are over CH$_2$ in the 1,3-benzodioxole group. For the PC2 molecule, the most positive region includes C–C bonds in the 4-nitrophenyl group. These results show that the NO$_2$ substituent in PC2 increases its nucleophilic reactivity in comparison with other piperine derivatives.

Frontier molecular orbitals and their energies are important quantum chemical parameters used for predicting the most reactive regions in molecular systems. In addition, the energy gap between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) reflects the biological activity of the molecule. A molecule with a small frontier orbital gap is more polarizable and commonly has high chemical reactivity and low kinetic stability.

Figure 5 shows the HOMO and LUMO for PC1 and PC2 compounds. In the PC1 molecule, both its HOMO and LUMO frontier orbitals are delocalized over the piperate moiety. Similar frontier orbitals are observed...
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for the PC1, PC3 and PC4 molecules (see Figure S18, SI section).

On the other hand, the LUMO of PC2 is localized over the 2-(4-nitrophenyl)-2-oxoethyl moiety (Figure 5). Hence, the HOMO-LUMO transition on the PC2 compound implies an electron density transfer from the piperate to the 2-(4-nitrophenyl)-2-oxoethyl moiety.

DFT chemical reactivity descriptors of the compounds were calculated by means of the B3LYP/6-311+G(2d,p) method and are listed in Table 2.

Table 2. DFT descriptors calculated at the B3LYP/6-311+G(2d,p) level

| Compound | Quantum descriptor / eV | E_HOMO | E_LUMO | ΔE | η  | μ  | ω  |
|----------|-------------------------|--------|--------|----|----|----|----|
| PC1      |                         | −5.77  | −2.24  | 3.54 | 1.77 | −2.93 | 2.42 |
| PC2      |                         | −5.90  | −3.43  | 2.40 | 1.20 | −2.98 | 3.69 |
| PC3      |                         | −5.74  | −2.21  | 3.54 | 1.77 | −2.91 | 2.40 |
| PC4      |                         | −5.81  | −2.30  | 3.52 | 1.76 | −2.95 | 2.47 |

eV: electron-volts; E_HOMO: HOMO energy; E_LUMO: LUMO energy; ΔE: energy gap HOMO-LUMO; η: hardness; μ: electronic chemical potential; ω: electrophilicity.

HOMO and LUMO energies are associated with the electron donor and acceptor capacities of the molecule, respectively. Overall, the greater the HOMO energy is (smaller negative value), the superior the capacity to donate electrons. The smallest energy gap HOMO-LUMO (ΔE) for the PC2 compound increases its reactive nature and polarizability.

The global electrophilicity index (ω), proposed by Parr et al., is a measure of the energy stabilization of a molecule when it acquires an additional electronic charge from the environment. The electrophilicity ω index encompasses both the tendency of an electrophile to acquire an extra electron density (chemical potential, μ) and the resistance of a molecule to exchange electron density with the environment (hardness, η). It contains information about electron transfer, given by μ, and molecular stability, given by η. Piperine derivatives show similar electrophilicity values (2.40-2.47 eV), except for the PC2 compound, which is the strongest electrophilic among the molecules investigated.

NMR calculation

The 1H and 13C experimental spectra of the piperine analog were recorded in deuterated chloroform and DMSO solution with tetramethysilane (TMS) as the internal standard. The chemical shifts were calculated using the B3LYP functional with the 6-311+G (2d,p) basis set in chloroform for the PC1 compound and DMSO (PC2-PC4).
in the framework of the IEFPCM solvent model. The \( ^{13}\text{C} \) and \( ^{1}\text{H} \) chemical shifts calculated by the DFT method and adjusted by scale factors were compared with experimental values. Table 3 lists the \( ^{13}\text{C} \) chemical shifts for piperine derivatives, and the numbering of each atom used in the investigation is shown in Figure 6.

The carbon chemical shift data for all compounds showed similar results, with a mean absolute deviation (MAD) in the range of 2.1-3.3 ppm. The largest MAD was obtained for the PC4 compound, and it was associated with poor prediction of the C18 carbon signal by DFT calculation (deviation of 19.1 ppm). When the C18 carbon signal for this compound is excluded in the calculation of MAD, the new value is 2.4 ppm. These errors can be attributed to the lack of relativistic effects in the calculation of chemical shifts of carbon atoms linked to halogens resulting in the elimination of these data in the calculation of the \( ^{13}\text{C} \) NMR scaling factors.

For other piperine derivatives, the largest deviation was associated with C2, C4 and C11 carbons. In the PC1 compound, the maximum deviation was for C4 carbon at 6.8 ppm, while for PC2, it was associated with C2 carbon at 5.8 ppm. For the PC3 compound, the maximum deviation was for C11 carbon at 5.8 ppm. These deviations can be mainly assigned to the use of the implicit solvent model (IEFPCM) in the calculation of NMR chemical shifts. This model neglects the solute-solvent interactions present in experimental measurements.

The mean deviation between experimental \( ^{1}\text{H} \) NMR chemical shifts and their corresponding calculated values for piperine derivatives were larger in DMSO solvent than chloroform (Table 4).

The results suggest that the solute-solvent intermolecular interactions are stronger in DMSO. For both compounds, the largest deviations observed in the range 0.33-0.62 ppm.

Table 3. The experimental (Exp.) and calculated (Calcd.) \( ^{13}\text{C} \) NMR chemical shifts of piperine derivatives in chloroform for PC1 and DMSO (PC2-PC4) solvents using the B3LYP/6-311+G(2d,p) method

| Assignment | PC1    | PC2    | PC3    | PC4    |
|------------|--------|--------|--------|--------|
|            | Calcd. / ppm | Exp. / ppm | Calcd. / ppm | Exp. / ppm | Calcd. / ppm | Exp. / ppm | Calcd. / ppm | Exp. / ppm |
| C1         | 166.2  | 166.4  | 165.6  | 165.3  | 165.8  | 165.4  | 165.7  | 165.3  |
| C2         | 115.0  | 119.1  | 112.7  | 118.5  | 113.5  | 118.9  | 113.1  | 118.7  |
| C3         | 148.6  | 146.9  | 147.6  | 145.9  | 147.1  | 145.6  | 147.3  | 145.7  |
| C4         | 117.6  | 124.5  | 120.7  | 124.4  | 120.8  | 124.4  | 120.7  | 124.4  |
| C5         | 143.1  | 140.9  | 143.5  | 140.8  | 143.1  | 140.6  | 143.3  | 140.7  |
| C6         | 127.5  | 130.5  | 127.9  | 130.2  | 128.0  | 130.2  | 128.0  | 130.2  |
| C7         | 106.9  | 106.0  | 109.3  | 105.7  | 109.0  | 105.7  | 109.1  | 105.7  |
| C8         | 147.6  | 148.3  | 146.7  | 147.8  | 146.6  | 147.8  | 146.8  | 147.8  |
| C9         | 147.6  | 148.7  | 148.7  | 148.1  | 148.3  | 148.1  | 148.5  | 148.1  |
| C10        | 109.8  | 108.6  | 106.1  | 108.2  | 106.1  | 108.2  | 106.0  | 108.2  |
| C11        | 126.6  | 123.1  | 117.3  | 122.9  | 117.0  | 122.9  | 117.0  | 122.9  |
| C12        | 103.3  | 101.4  | 103.0  | 101.1  | 102.9  | 101.1  | 102.7  | 101.1  |
| C13        | 66.0   | 66.0   | 66.1   | 66.3   | 65.7   | 65.9   | 65.7   | 65.9   |
| C14        | 191.2  | 192.4  | 191.2  | 192.4  | 190.7  | 192.2  | 190.5  | 192.2  |
| C15        | 132.9  | 134.4  | 135.5  | 138.6  | 127.6  | 131.5  | 129.1  | 133.0  |
| C16        | 124.1  | 128.8  | 127.0  | 129.0  | 125.9  | 127.5  | 127.2  | 129.5  |
| C17        | 128.3  | 127.8  | 123.0  | 123.6  | 126.2  | 129.1  | 129.2  | 131.7  |
| C18        | 131.7  | 133.8  | 148.6  | 150.2  | 146.0  | 144.0  | 146.7  | 127.6  |
| CH₃        |        |        |        |        | 21.1   | 20.9   |        |        |

MAD: mean absolute deviation.
were for the H7 and H11 protons of the 1,3-benzodioxole group. For the PC1 compound, the largest deviation was for the H11 proton at 0.62 ppm, while for PC2, PC3 and PC4, they were associated with the H7 proton at 0.55, 0.57 and 0.57 ppm, respectively.

As can be observed, for all piperine derivatives, there is good agreement between the experimental and calculated $^1$H chemical shifts (MAD < 0.3 ppm). The low MDA for the PC1 compound indicates better DFT calculation performance in chloroform solvent.

### Antifungal activity

The preliminary study on antifungal activity in vitro of compounds derived from piperine PC1-PC4 was evaluated by the microdilution method with 10 strains of pathogenic fungi divided into yeast (Candida albicans ATCC 76645, C. albicans LM-111, C. albicans LM-122, C. tropicalis ATCC-13803, C. tropicalis LM-04, C. kruzei LM-656, C. kruzei LM-13) and filamentous fungi (Trichophyton rubrum LM-49, Microsporum canis LM-12, M. gypseum LM-512) using amphotericin B (0.034 μmol mL$^{-1}$) as the standard drug. Of the four compounds tested, only PC4 showed antifungal activity in seven of the ten strains tested (Table 5).

PC4 showed a minimum inhibitory concentration of 2.46 μmol mL$^{-1}$ for all C. albicans strains (ATCC 76645, LM-111 and LM-122). For the C. kruzei strains (LM-656 and LM-13), the MIC was 1.23 μmol mL$^{-1}$. For filamentous fungi, the compound showed a minimum inhibitory concentration of 1.23 μmol mL$^{-1}$ only for M. canis.

### Table 4. The experimental (Exp.) and calculated (Calcd.) $^1$H NMR chemical shifts of piperine derivatives in chloroform PC1 and DMSO (PC2-PC4) solvents using the B3LYP/6-311+G(2d,p) method

| Assignment | PC1 | PC2 | PC3 | PC4 |
|------------|-----|-----|-----|-----|
|            | Calcd. / ppm | Exp. / ppm | Calcd. / ppm | Exp. / ppm | Calcd. / ppm | Exp. / ppm | Calcd. / ppm | Exp. / ppm |
| H2         | 5.91 | 6.10 | 5.93 | 6.14 | 5.94 | 6.14 | 5.94 | 6.13 |
| H3         | 7.56 | 7.51 | 7.55 | 7.46 | 7.53 | 7.45 | 7.53 | 7.45 |
| H4         | 6.93 | 6.78 | 6.95 | 7.03 | 6.95 | 7.03 | 6.96 | 7.03 |
| H5         | 6.77 | 6.78 | 6.84 | 7.03 | 6.81 | 7.03 | 6.82 | 7.03 |
| H7         | 6.63 | 7.00 | 6.65 | 7.20 | 6.63 | 7.20 | 6.63 | 7.20 |
| H10        | 6.69 | 6.92 | 6.69 | 6.91 | 6.70 | 6.91 | 6.69 | 6.91 |
| H11        | 7.40 | 6.78 | 7.36 | 7.03 | 7.38 | 7.03 | 7.37 | 7.03 |
| H12        | 5.95 | 5.98 | 5.99 | 6.04 | 5.92 | 6.04 | 5.97 | 6.04 |
| H13        | 5.41 | 5.42 | 5.35 | 5.56 | 5.36 | 5.47 | 5.30 | 5.48 |
| H16        | 7.92 | 7.94 | 8.08 | 8.20 | 7.79 | 7.36 | 7.81 | 7.90 |
| H17        | 7.40 | 7.51 | 8.43 | 8.35 | 7.31 | 7.86 | 7.48 | 7.76 |
| H18        | 7.57 | 7.61 |       |       |       |       |       |       |
| CH$_3$     |       |       | 2.26 | 2.40 |       |       |       |       |
| MAD        | 0.15 | 0.19 | 0.27 | 0.21 |       |       |       |       |

MAD: mean absolute deviation.

### Table 5. Antifungal activity (MIC and MFC values) of compounds PC1-PC4

| Microorganism           | PC1 | PC2 | PC3 | PC4 |
|-------------------------|-----|-----|-----|-----|
|                         | MIC / (μmol mL$^{-1}$) | MFC / (μmol mL$^{-1}$) | MIC / (μmol mL$^{-1}$) | MFC / (μmol mL$^{-1}$) | MIC / (μmol mL$^{-1}$) | MFC / (μmol mL$^{-1}$) |
| C. a. ATCC 76645        | +   | +   | +   | +   | +   | +   | 2.46 | 19.68 |
| C. a. LM-111            | +   | +   | +   | +   | +   | +   | 2.46 | 19.68 |
| C. a. LM-122            | +   | +   | +   | +   | +   | +   | 2.46 | 19.68 |
| C. t. ATCC-13803        | +   | +   | +   | +   | +   | +   | 2.46 | 19.68 |
| C. t. LM-04             | +   | +   | +   | +   | +   | +   | 1.23 | 9.84 |
| C. k. LM-656            | +   | +   | +   | +   | +   | +   | 1.23 | 9.84 |
| C. k. LM-13             | +   | +   | +   | +   | +   | +   | 1.23 | 9.84 |
| T. r. LM-49             | +   | +   | +   | +   | +   | +   | 1.23 | 9.84 |
| M. c. LM-12             | +   | +   | +   | +   | +   | +   | 1.23 | 9.84 |
| M. g. LM-512            | +   | +   | +   | +   | +   | +   | 1.23 | 9.84 |

C. a.: Candida albicans; C. t.: Candida tropicalis; C. k.: Candida kruzei; T. r.: Trichophyton rubrum; M. c.: Microsporum canis; M. g.: Microsporum gypseum; MIC: minimum inhibitory concentration; MFC: minimum fungicide concentration. +: indicates growth of the microorganism.
LM-12 and M. gypseum LM-512. However, for all of the aforementioned situations, it is possible to observe the bioactive potential of the PC4 compound, which was also evidenced by revealing an excellent MIC against yeast and filamentous fungi for this work. With the need to further investigate the real dimension of its antifungal potential, a minimum fungicide concentration (MFC) assay was carried out. The MFC for PC4 against the strains under study was found to vary between 9.84 and 19.68 μmol mL⁻¹. This behavior of different sensitivities of the strains is related to individual particularities of each species and strain. A substance with antifungal activity can have a fungistatic or fungicidal effect, being considered fungistatic when it is able to inhibit or delay fungal growth and fungicidal when it promotes cell death. According to Siddiqui et al.,⁴ if the MFC/MIC ratio results in a value ≤ 4, the effect is fungicidal. However, if this ratio is > 4, the substance has a fungistatic profile. When analyzing the nature of the antifungal action of the PC4 compound, it was revealed that it is of the fungistatic type, since the MFC/MIC ratio obtained was 8.

Conclusions

Four new piperine derivatives were synthesized and characterized using IR and ¹H and ¹³C NMR spectroscopic techniques. The geometries of the ground state and the electronic and NMR spectroscopic properties of piperine derivatives were computed by using the B3LYP-D3/6-311+G(2d,p) and B3LYP/6-311+G(2d,p) methods. The ¹H and ¹³C NMR chemical shifts were calculated and compared with the experimental values. The DFT study indicates that the methyl and bromine substituents on the phenyl ring do not have a significant influence on the chemical reactivity and stability of their compounds. On the other hand, the nitro group contributes to the high reactivity of the PC2 compound. The MPE maps suggest that the carbonyl groups are the main regions of electrophilic reactivity of the compounds. In addition, the ¹H and ¹³C NMR chemical shifts calculated by the B3LYP method showed good agreement with the experimental values. In addition, all compounds were evaluated in vitro against different fungi, and only the PC4 compound showed inhibition against seven of the ten strains tested, with MICs ranging from 1.23-2.46 μmol mL⁻¹, and the compound had a fungistatic effect.

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Author Contributions

P. F. A.-F., J. S. S. J., B. F. L. and E. P. S. M. conceived and designed the experiment; J. S. S. J., F. S. A., R. F. O. and E. O. T. performed the experiments; J. S. S. J., E. O. T., R. F. O. and H. D. S. S. analyzed the data; L. V. C. and E. O. L. performed the antifungal study; R. F. O. and H. D. S. S. performed the computational study; J. S. S. J., H. D. S. S., E. P. S. M. and B. F. L. participated in writing-original draft preparation; J. S. S. J., E. P. S. M., G. B. R., J. M. B.-F. and P. F. A.-F. participated in writing-review and editing.

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