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Article

New Diesters Derived from Piperine: *in silico* Study and Evaluation of Their Antimicrobial Potential

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Piperine, previously extracted from black pepper (*Piper nigrum* L.), was used as a precursor for the synthesis of twelve new diester derivatives. The final products were obtained through the bimolecular nucleophilic substitution reaction (S_N2) of the alkyl 2-chloroacetates and the salt of piperic acid, obtained from the basic hydrolysis of piperine. The compounds were synthesized with yields of 55-84% and characterized by infrared spectroscopy and ^1H and ^{13}C nuclear magnetic resonance. The evaluation of the compounds' potential as new drug candidates was done through an *in silico* study of ADME properties (absorption, distribution, metabolization and excretion) and evaluation of antimicrobial activity against bacterial strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*), yeasts (*Candida albicans* and *Candida tropicalis*) and filamentous fungi (*Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*). The *in silico* study showed that the compounds were good drug candidates and antimicrobial evaluation demonstrated that 9 of the 12 compounds exhibited a minimum inhibitory concentration (MIC) ranging 1024-256 μg mL^{-1}.

Keywords: piperine, diesters, antimicrobial activity

Introduction

The number of drug-resistant microorganisms is increasing at alarming rates. The antibiotic resistance crisis has been attributed to the overuse and misuse of these medications, as well as a lack of new drug development by the pharmaceutical industry. When considering the emergence of resistant strains, effective treatment of fungal and bacterial infections has become increasingly challenging for public health systems.[1,2] Microorganisms such as bacteria and fungi have the genetic ability to acquire and transmit resistance to these drugs.[3] Pathogenic agents resist antimicrobial action through mechanisms such as: reduction of the accessibility of the drug to its molecular target, decrease in cellular uptake and increase in drug efflux, resulting in a low and ineffective concentration of the drug, or even mutations that alter their molecular targets, rendering the antibiotic useless.[4] Besides, toxicity and therapy costs are other factors that hinder adequate, successful and safe treatment against infectious agents. Accordingly, the research and discovery of new, safe and effective antibiotics is of utmost importance to tackle the growing threat of infections caused by multidrug resistant microorganisms.[3,5]

Piperine (1-piperoyl-piperidine) is a natural amide with a molecular formula of $C_{17}H_{19}NO_3$. It is a versatile bioactive compound found in almost 2000 species of the genus *Piper*, being also the most abundant alkaloid present in black pepper (*Piper nigrum*) and long pepper (*P. longum*).[6,7] Piperine alone has a broad spectrum of biological activities such as antiinflammatory, analgesic, anticonvulsant, antimicrobial, antioxidant, antitumor, antidepressant, hepatoprotective, antithyroid and immunomodulatory, among others.[8,9] Its abundance in plant material, as well as its ease of extraction and possible synthetic manipulations, make piperine a rich source for the discovery of numerous derived molecules with promising biological potential. The literature reports several activities of piperine derivatives,

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such as antiinflammatory,[10] antimicrobial,[11] antineoplasic,[12] antidiabetic,[13] antichagasic[14] and antivitiligo,[15] among others. Thus, piperine derivatives have become notorious for its promising pharmacological activities, often superior to those of piperine itself. This in turn has led to an increased interest in the research and discovery of new molecules derived from such natural compound.

Considering these aspects, twelve new diesters derived from piperine were designed, synthesized and evaluated as new drug candidates through *in silico* study and evaluation of *in vitro* antimicrobial activity.

Experimental

Chemistry

Piperine (**1**) was obtained by the extraction of black pepper (*P. nigrum* L.) with ethanol as described by Ikan[16] in 1991. The other reagents and solvents were acquired from Sigma-Aldrich (São Paulo, Brazil) and used without further purification. The progress of the reactions was monitored by thin layer chromatography (TLC) on silica gel plates. The compounds were purified by recrystallization in ethanol and the structures of compounds **6a**-**6l** were confirmed by the following: infrared spectroscopy (IR) spectra obtained with a FTIR Shimadzu spectrometer, model IR Prestige-21, with an attenuated total reflection (ATR) accessory; ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra and two-dimensional (2D) NMR (correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC)) obtained with a Varian spectrometer, Mercury model (400 MHz for ^{1}H and 101 MHz for ^{13}C); and melting point (mp) range on a MQAPF-3 heating plate. Deuterated dimethyl sulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$) were used as solvents for dissolving the samples. The chemical shifts (δ) were measured in parts *per* million (ppm) and the coupling constants (J) in hertz (Hz).

Isolation of the amide 1-piperoyl-piperidine (piperine) (**1**)

In a Soxhlet apparatus, 100 g of black pepper and 1000 mL of ethanol (95%) were added. The mixture was refluxed for approximately 8 h. After concentrating the extract on a rotary evaporator, 100 mL of an alcoholic solution of 10% KOH were added, and the precipitated material was then filtered out. A small amount of water was added to the alcoholic solution until the mixture became turbid. After allowing the mixture to stand for 72 h, a yellow precipitate formed,[16] and 3.5 g of piperine (3.5% yield) was obtained with the following characteristics. Molecular weight (MW) 285.34 g mol^{-1}; mp 126-128 °C (lit.:[15] 129-130 °C); IR (ATR) ν / cm^{-1} 3008 (C–H_{Ar}), 1631 (C=O), 1581-1442 (C=C_{Ar}), 1249 (C–O–C); 930 oop (C–H_{Ar}); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (ddd, J 14.7, 8.9, 1.2 Hz, 1H, CH_{olef}), 6.95 (s, J 1.6 Hz, 1H, CH_{Ar}), 6.86 (dd, J 8.1, 1.7 Hz, 1H, CH_{Ar}), 6.76-6.66 (m, 3H, CH_{olef} and CH_{Ar}), 6.41 (d, J 14.6 Hz, 1H, CH_{olef}), 5.94 (s, 2H, OCH_2O), 3.60-3.48 (m, 4H, $CH_{2cycloalk.}$), 1.64 (m, 2H, H-15, $CH_{2cycloalk.}$), 1.59-1.53 (m, 4H, $CH_{2cycloalk.}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.5, 148.2, 148.1, 142.8, 138.4, 130.9, 125.3, 122.5, 119.7, 108.4, 105.6, 101.3, 46.3, 26.1, 24.6.

Preparation of (2*E*,4*E*)-5-(benzo[*d*][1,3]dioxol-5-yl)penta-2,4-dienoic acid (piperic acid) (**2**)

In a 50 mL flask, 2.20 g (7.72 mmol) of piperine and 22 mL of the ethanolic solution of 20% KOH were added. The reaction mixture was refluxed with stirring for 20 h. At the end of the reaction, the mixture was filtered, and the precipitate formed was washed with ethanol and dried. The precipitate was dissolved in water and acidified with 10% HCl solution down to pH 3. The yellowish precipitate formed was filtered out, washed with water, dried and recrystallized in ethanol.[8] Piperic acid was obtained at 1.67 g (94.5% yield) with the following characteristics. MW 218.21 g mol^{-1}; mp 217-218 °C (lit.:[17] 216-217 °C); IR (ATR) ν / cm^{-1} 3448 (O–H), 2922 (C–H_{Aliph}), 1676 (C=O), 1604-1419 (C=C_{Ar}), 1255 (C–O–C), 927 (C–H_{Ar}); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.20 (s, 1H, O–H), 7.36-7.26 (m, 1H, CH_{olef}), 7.23 (s, 1H, CH_{Ar}), 7.03-6.89 (m, 4H, CH_{Ar} and CH_{Olefin}), 6.05 (s, 2H, O–CH_2–O), 5.93 (d, J 15.2 Hz, 1H, CH_{Ar}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 148.5, 148.4, 145.1, 140.2, 130.9, 125.3, 123.5, 121.5, 108.4, 106.1, 101.8.

Preparation of potassium piperate (**3**)

An ethanolic solution of 10 mmol KOH was slowly added to a mixture of ethanol and piperic acid (10 mmol). The reaction mixture was stirred continuously at room temperature for 1 h. The solid obtained was filtered and dried and had a yield of 93% and the following characteristics. MW 256.30 g mol^{-1}; IR (ATR) ν / cm^{-1} 3022 (C–H_{Ar}), 2908 (C–H_{Aliph}), 1550 (C=O), 1500-1448 (C=C_{Ar}), 1255 (C–O).

General procedure for obtaining alkyl 2-chloroacetates via Fisher esterification (**5a**-**5h**)

A mixture of 2-chloroacetic acid (20 mmol), the respective alcohol (**4a**-**4h**) (60 mL) and concentrated

sulfuric acid (1 mL) was refluxed for 6 h. Afterwards, the excess solvent was rotary-evaporated, and the residue poured into cold water. The residue was transferred to a separation funnel containing 250 mL of water, and 100 mL of ethyl ether were then added. The organic phase was separated, washed repeatedly with 10% sodium bicarbonate until neutral pH and then dried with anhydrous $NaSO_4$. Ethyl ether was rotary-evaporated, obtaining the respective esters (**5a**-**5h**).

Methyl 2-chloroacetate (**5a**)

MW 108.52 g mol^{-1}; yield: 89%; IR (ATR) ν / cm^{-1} 1753 (C=O), 1317, 1199 (C–O), 1172, 788 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.05 (s, 2H, C$\underline{H}_{2Aliph}$), 3.78 (s, 3H, OC$\underline{H}_{3Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 53.1, 40.7.

Ethyl 2-chloroacetate (**5b**)

MW 122.55 g mol^{-1}; yield: 93%; IR (ATR) ν / cm^{-1} 1753 (C=O), 1311, 1166 (C–O), 1266, 761 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.22 (q, 2H, OC$\underline{H}_{2Aliph}$), 4.03 (s, 2H, C$\underline{H}_{2Aliph}$), 1.27 (t, 3H, C$\underline{H}_{3Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4, 62.3, 41.0, 14.1.

Propyl 2-chloroacetate (**5c**)

MW 136.58 g mol^{-1}; yield: 92%; IR (ATR) ν / cm^{-1} 1755 (C=O), 1359, 1184 (C–O), 1290, 788 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (t, 2H, OC$\underline{H}_{2Aliph}$), 4.04 (s, 2H, C$\underline{H}_{2Aliph}$), 1.73-1.62 (hex, 2H, C$\underline{H}_{2Aliph}$), 0.93 (t, 3H, C$\underline{H}_{3Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4, 67.8, 41.0, 21.9, 10.2.

Isopropyl 2-chloroacetate (**5d**)

MW 136.58 g mol^{-1}; yield: 85%; IR (ATR) ν / cm^{-1} 1751 (C=O), 1307, 1103 (C–O), 1184, 840 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.07 (hept, 1H, OC$\underline{H}_{Aliph}$), 4.00 (s, 2H, C$\underline{H}_{2Aliph}$), 1.26 [d, 6H, (C$\underline{H}_{3Aliph}$)$_2$]; ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 70.2, 41.3, 21.7.

Butyl 2-chloroacetate (**5e**)

MW 150.60 g mol^{-1}; yield: 81%; IR (ATR) ν / cm^{-1} 1757 (C=O), 1309, 1182 (C–O), 1288, 785 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (t, 2H, OC$\underline{H}_{2Aliph}$), 4.04 (s, 2H, C$\underline{H}_{2Aliph}$), 1.67-1.59 (qt, 2H, C$\underline{H}_{2Aliph}$), 1.37 (sext, 2H, C$\underline{H}_{2Aliph}$), 0.92 (t, 3H, C$\underline{H}_{3Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 66.2, 41.0, 30.5, 19.0, 13.7.

Isobutyl 2-chloroacetate (**5f**)

MW 150.60 g mol^{-1}; yield: 75%; IR (ATR) ν / cm^{-1} 1757 (C=O), 1311, 1188 (C–O), 1290, 766 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.05 (s, 2H, OC$\underline{H}_{2Aliph}$), 3.95 (d, 2H, C$\underline{H}_{2Aliph}$), 1.96 (hept, 1H, C$\underline{H}_{Aliph}$), 0.93 [d, 6H, (C$\underline{H}_{3Aliph}$)$_2$]; ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 72.2, 41.0, 27.7, 19.0.

Pentyl 2-chloroacetate (**5g**)

MW 164.63 g mol^{-1}; yield: 84%; IR (ATR) ν / cm^{-1} 1753 (C=O), 1317, 1199 (C–O), 1172, 788 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (t, 2H, OC$\underline{H}_{2Aliph}$), 4.04 (s, 2H, C$\underline{H}_{2Aliph}$), 1.70-1.59 (qt, 2H, C$\underline{H}_{2Aliph}$), 1.38-1.28 (m, 4H, C$\underline{H}_{2Aliph}$), 0.89 (t, 3H, C$\underline{H}_{3Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 66.5, 41.0, 28.2, 27.9, 22.3, 14.0.

Isopentyl 2-chloroacetate (**5h**)

MW 164.63 g mol^{-1}; yield: 78%; IR (ATR) ν / cm^{-1} 1757 (C=O), 1309, 1184 (C–O), 1290, 758 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (t, 2H, OC$\underline{H}_{2Aliph}$), 4.03 (s, 2H, C$\underline{H}_{2Aliph}$), 1.72-1.60 (hept, 1H, C$\underline{H}_{Aliph}$), 1.54 (q, 2H, C$\underline{H}_{2Aliph}$), 0.91 [d, 6H, (C$\underline{H}_{3Aliph}$)$_2$]; ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4, 65.0, 41.0, 37.1, 25.0, 22.4.

General procedure for obtaining alkyl 2-chloroacetates via acid chloride (**5i**-**5l**)

The respective alcohols (**4i**-**4l**) (10 mmol) were diluted together with triethylamine (11 mmol), in 20 mL of dichloromethane at 0 °C. Next, 2-chloroacetyl chloride (11 mmol) was slowly added and the reaction mixture was vigorously stirred for 20 h at room temperature. Afterwards, the mixture was poured into water, washed with sodium bicarbonate and extracted with ethyl acetate. The organic phase was separated and dried with Na_2SO_4, and the solvent was removed by rotary evaporation to obtain the respective esters (**5i**-**5l**).

Cyclohexyl 2-chloroacetate (**5i**)

MW 176.64 g mol^{-1}; yield: 65%; IR (ATR) ν / cm^{-1} 1751 (C=O), 1303, 1184 (C–O), 1288, 763 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.84 (qt, 1H, OC$\underline{H}$), 4.02 (s, 2H, C$\underline{H}_{2Aliph}$), 1.88-1.82 (m, 2H, C$\underline{H}_{2cycloalk.}$), 1.75-1.68 (m, 2H, C$\underline{H}_{2cycloalk.}$), 1.53-1.25 (m, 6H, C$\underline{H}_{2cycloalk.}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 74.9, 41.3, 31.4, 25.3, 23.6.

Octyl 2-chloroacetate (**5j**)

MW 206.71 g mol^{-1}; yield: 67%; IR (ATR) ν / cm^{-1} 1759 (C=O), 1307, 1174 (C–O), 1288, 788 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (t, 2H, OC$\underline{H}_{2Aliph}$), 4.05 (s, 2H, C$\underline{H}_{2Aliph}$), 1.72-1.61 (m, 2H, C$\underline{H}_{2Aliph}$), 1.30 (m, 10H, C$\underline{H}_{2Aliph}$), 0.87 (t, 3H, C$\underline{H}_{3Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 66.5, 41.0, 31.8, 29.2, 28.5, 25.8, 22.7, 14.1.

Decyl 2-chloroacetate (**5k**)

MW 234.77 g mol^{-1}; yield: 64%; IR (ATR) ν / cm^{-1} 1761 (C=O), 1307, 1174 (C–O), 1288, 790(C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (t, 2H, OC$\underline{H}_{2Aliph}$), 4.04 (s, 2H, C$\underline{H}_{2Aliph}$), 1.64 (m, 2H, C$\underline{H}_{2Aliph}$), 1.25 (m, 14H, C$\underline{H}_{2Aliph}$),

0.85 (t, 3H, C$\underline{H}_{3Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 66.5, 41.0, 31.9, 29.6, 29.5, 29.3, 29.2, 28.5, 25.8, 22.7, 14.2.

Dodecyl 2-chloroacetate (**5l**)

MW 262.82 g mol^{-1}; yield: 63%; IR (ATR) ν / cm^{-1} 1761 (C=O), 1307, 1172 (C–O), 1288, 790 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (t, 2H, OC$\underline{H}_{2Aliph}$), 4.05 (s, 2H, C$\underline{H}_{2Aliph}$), 1.73-1.58 (m, 2H, C$\underline{H}_{2Aliph}$), 1.41-1.19 (m, 18H, C$\underline{H}_{2Aliph}$), 0.88 (t, 3H, C$\underline{H}_{3Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 66.5, 41.0, 32.0, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 28.5, 25.8, 22.8, 14.2.

General procedure for obtaining diesters derived from piperine (**6a**-**6l**)

In a 25 mL flask containing 10 mL of dimethylformamide (DMF), 0.002 mol of the respective alkyl 2-chloroacetate **5a**-**5l** and 0.002 mol potassium iodide were added. Next, 0.022 mol potassium piperate (**3**) was added, and the reaction mixture was heated at 100 °C with stirring for 24 h. Afterwards, the reaction mixture was cooled, and cold distilled water was added. The precipitate formed was vacuum-filtered out, washed with water and recrystallized in ethanol.

2-Methoxy-2-oxoethyl-piperate (**6a**)

Yellow solid; MW 290.27 g mol^{-1}; yield: 79%; mp 85-86 °C; IR (ATR) ν / cm^{-1} 3074, 3024 (C–H_{Ar}), 2943 (C–H), 1761, 1712 (C=O), 1610, 1440 (C=C_{Ar}), 1255 (C–O–C), 1220, 1033 (C–O), 846 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (ddd, *J* 15.2, 8.9, 1.4 Hz, 1H, H-3), 7.25 (d, 1H, *J* 1.6 Hz, H-7), 7.07-7.01 (m, 3H, H-4, H-5, H-11), 6.94 (d, *J* 8.0 Hz, 1H, H-10), 6.09 (d, *J* 16.3 Hz, 3H, H-2, H-12), 4.75 (s, 2H, H-13), 3.69 (s, 3H, H-15); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.8 (C-14), 166.1 (C-1), 148.8 (C-9), 148.5 (C-8), 146.9 (C-3), 141.8 (C-5), 130.8 (C-6), 125.0 (C-4), 123.9 (C-11), 118.9 (C-2), 109.0 (C-10), 106.2 (C-7), 101.9 (C-12), 60.9 (C-13), 52.3 (C-15).

2-Ethoxy-2-oxoethyl-piperate (**6b**)

Yellow solid; MW 304.30 g mol^{-1}; yield: 72%; mp 77-78 °C; IR (ATR) ν / cm^{-1} 3080 (C–H_{Ar}), 2976, 2893, 2787 (C–H), 1759, 1707 (C=O), 1618, 1442 (C=C_{Ar}), 1247 (C–O–C), 1134, 1016 (C–O), 856 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (ddd, *J* 15.2, 8.8, 1.5 Hz, 1H, H-3), 7.25 (d, *J* 1.6 Hz, 1H, H-7), 7.11-7.00 (m, 3H, 3H, H-4, H-5, H-11), 6.95 (d, *J* 8.0 Hz, 1H, H-10), 6.09 (d, *J* 15.1 Hz, 3H, H-2, H-12), 4.73 (s, 2H, H-13), 4.15 (q, 2H, H-15), 1.21 (t, 3H, H-16); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3 (C-14), 166.1 (C-1), 148.8 (C-9), 148.5 (C-8), 146.9 (C-3), 141.7 (C-5), 130.8 (C-6), 125.0 (C-4), 123.9 (C-11), 119.0 (C-2), 109.0 (C-10), 106.2 (C-7), 101.9 (C-12), 61.2 (C-15), 61.0 (C-13), 14.4 (C-16).

2-Propoxy-2-oxoethyl-piperate (**6c**)

Yellow solid; MW 318.32 g mol^{-1}; yield: 74%; mp 78-79 °C; IR (ATR) ν / cm^{-1} 3062 (C–H_{Ar}), 2970, 2899 (C–H), 1745, 1714 (C=O), 1608, 1448 (C=C_{Ar}), 1255 (C–O–C), 1211, 1132, 1035 (C–O), 852 (C–H_{Ar}); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, *J* 15.2, 10.8 Hz, 1H, H-3), 6.99 (s, 1H, H-7), 6.91 (d, *J* 8.1 Hz, 1H, H-11), 6.85-6.65 (m, 3H, H-4, H-5, H-10), 6.02 (d, *J* 15.2 Hz, 1H, H-2), 5.98 (s, 2H, H-12), 4.69 (s, 2H, H-13), 4.14 (t, 2H, H-15), 1.88-1.49 (m, 3H, H-16), 0.94 (t, 3H, H-17); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.12 (C-14), 166.4 (C-1), 148.8 (C-9), 148.5 (C-8), 146.4 (C-3), 141.1 (C-5), 130.8 (C-6), 124.4 (C-4), 123.2 (C-11), 118.9 (C-2), 108.6 (C-10), 106.0 (C-7), 101.5 (C-12), 66.9 (C-15), 60.8 (C-13), 22.0 (C-16), 10.3 (C-17).

2-Isopropoxy-2-oxoethyl-piperate (**6d**)

Yellow solid; MW 318.32 g mol^{-1}; yield: 70%; mp 84-85 °C; IR (ATR) ν / cm^{-1} 3070, 3012 (C–H_{Ar}), 2981, 2910 (C–H), 1737, 1714 (C=O), 1610, 1450 (C=C_{Ar}), 1257 (C–O–C), 1217, 1139, 1035 (C–O), 850 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (ddd, *J* 15.2, 8.6, 1.7 Hz, 1H, H-3), 7.25 (d, *J* 1.6 Hz, 1H, H-7), 7.11-6.99 (m, 3H, H-4, H-5, H-11), 6.94 (d, *J* 8.0 Hz, 1H, H-10), 6.09 (d, *J* 20.8 Hz, 3H, H-2, H-12), 5.07-4.89 (m, 1H, H-15), 4.69 (s, 2H, H-13), 1.21 (d, 6H, H-16, H-16'); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.8 (C-14), 166.1 (C-1), 148.8 (C-9), 148.5 (C-8), 146.8 (C-3), 141.7 (C-5), 130.8 (C-6), 125.0 (C-4), 123.9 (C-11), 119.0 (C-2), 109.0 (C-10), 106.2 (C-7), 101.9 (C-12), 68.9 (C-15), 61.0 (C-13), 21.9 (C-16, C-16').

2-Butoxy-2-oxoethyl-piperate (**6e**)

Yellow solid; MW 332.35 g mol^{-1}; yield: 84%; mp 70-71 °C; IR (ATR) ν / cm^{-1} 3026 (C–H_{Ar}), 2960, 2872 (C–H), 1745, 1718 (C=O), 1618, 1444 (C=C_{Ar}), 1256 (C–O–C), 1211, 1128, 1035 (C–O), 848 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (ddd, *J* 15.2, 8.6, 1.8 Hz, 1H, H-3), 7.25 (d, *J* 1.6 Hz, 1H, H-7), 7.10-6.98 (m, 3H, H-4, H-5, H-11), 6.94 (d, *J* 8.0 Hz, 1H, H-10), 6.09 (d, *J* 15.2 Hz, 3H, H-2, H-12), 4.74 (s, 2H, H-13), 4.11 (t, 2H, H-15), 1.64-1.46 (m, 2H, H-16), 1.33 (m, 2H, H-17), 0.89 (t, 3H, H-18); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.4 (C-14), 166.1 (C-1), 148.8 (C-9), 148.5 (C-8), 146.8 (C-3), 141.7 (C-5), 130.8 (C-6), 125.0 (C-4), 123.9 (C-11), 118.9 (C-2), 109.2 (C-10), 106.2 (C-7), 101.9 (C-12), 64.8 (C-15), 61.0 (C-13), 30.5 (C-16), 18.9 (C-17), 13.9 (C-18).

2-Isobutoxy-2-oxoethyl-piperate (**6f**)

Yellow solid; MW 332.35 g mol^{-1}; yield: 65%; mp 67-68 °C; IR (ATR) ν / cm^{-1} 2980 (C-H_{Ar}), 2924, 2972 (C–H), 1753, 1697 (C=O), 1612, 1435 (C=C_{Ar}), 1251 (C–O–C), 1203, 1124, 1033 (C–O), 867 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (ddd, *J* 15.2, 8.6, 1.8 Hz, 1H, H-3), 7.25 (d, *J* 1.6 Hz, 1H, H-7), 7.12-6.98 (m, 3H, H-4, H-5, H-11), 6.95 (d, *J* 8.0 Hz, 1H, H-10), 6.09 (d, *J* 16.0 Hz, 3H, H-2, H-12), 4.76 (s, 2H, H-13), 3.91 (d, 2H, H-15), 1.88 (m, 1H, H-16), 0.89 (d, 6H, H-17, H-17'); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.4 (C-14), 166.2 (C-1), 148.8 (C-9), 148.5 (C-8), 146.9 (C-3), 141.7 (C-5), 130.8 (C-6), 125.0 (C-4), 123.9 (C-11), 118.9 (C-2), 109.0 (C-10), 106.2 (C-7), 101.9 (C-12), 70.7 (C-15), 61.0 (C-13), 27.7 (C-16), 19.1 (C-17, C-17').

2-Oxo-2-(pentyloxy)ethyl-piperate (**6g**)

Yellow solid; MW 346.38 g mol^{-1}; yield: 60%; mp 57-58 °C; IR (ATR) ν / cm^{-1} 3026 (C–H_{Ar}), 2953, 2866 (C–H), 1747, 1712 (C=O), 1612, 1448 (C=C_{Ar}), 1257 (C–O–C), 1217, 1130, 1041 (C–O), 835 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (ddd, *J* 15.2, 8.3, 2.0 Hz, 1H, H-3), 7.25 (d, *J* 1.6 Hz, 1H, H-7), 7.10-6.98 (m, 3H, H-4, H-5, H-11), 6.94 (d, *J* 8.0 Hz, 1H, H-10), 6.09 (d, *J* 15.8 Hz, 3H, H-2, H-12), 4.74 (s, 2H, H-13), 4.10 (t, 2H, H-15), 1.58 (q, 2H, H-16), 1.34-1.17 (m, 4H, H-17, H-18), 0.86 (t, 3H, H-19); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.4 (C-14), 166.1 (C-1), 148.8 (C-9), 148.5 (C-8), 146.8 (C-3), 141.7 (C-5), 130.8 (C-6), 125.0 (C-4), 123.9 (C-11), 118.9 (C-2), 109.0 (C-10), 106.2 (C-7), 101.9 (C-12), 65.1 (C-15), 61.0 (C-13), 28.1 (C-16), 27.8 (C-17), 22.1 (C-18), 14.2 (C-19).

2-Isopentyloxy-2-oxoethyl-piperate (**6h**)

Yellow solid; MW 346.38 g mol^{-1}; yield: 55%; mp 83-84 °C; IR (ATR) ν / cm^{-1} 3025 (C–H_{Ar}), 2951, 2904 (C–H), 1753, 1714 (C=O), 1604, 1446 (C=C_{Ar}), 1257 (C–O–C), 1217, 1128, 1010 (C–O), 835 (C–H_{Ar}); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (dd, *J* 15.1, 11.0 Hz, 1H, H-3), 6.99 (s, 1H, H-7), 6.92 (d, *J* 7.9 Hz, 1H, H-11), 6.86-6.68 (m, 3H, H-4, H-5, H-10), 6.02 (d, *J* 15.3 Hz, 1H, H-2), 5.98 (s, 2H, H-12), 4.68 (s, 2H, H-13), 4.21 (t, 2H, H-15), 1.69 (m, 1H, H-17), 1.55 (q, 2H, H-16), 0.92 (d, 6H, H-18, H-18'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.2 (C-14), 166.4 (C-1), 148.8 (C-9), 148.5 (C-8), 146.4 (C-3), 141.1 (C-5), 130.5 (C-6), 124.4 (C-4), 123.2 (C-11), 118.9 (C-2), 108.6 (C-10), 106.0 (C-7), 101.5 (C-12), 64.1 (C-15), 60.8 (C-13), 37.3 (C-16), 25.1 (C-17), 22.5 (C-18, C-18').

2-Cyclohexyloxy-2-oxoethyl-piperate (**6i**)

Pale yellow solid; MW 358.39 g mol^{-1}; yield: 80%; mp 112-114 °C; IR (ATR) ν / cm^{-1} 3022 (C–H_{Ar}), 2929, 2858 (C–H), 1753, 1716 (C=O), 1606, 1446 (C=C_{Ar}), 1257 (C–O–C), 1215, 1132, 1043 (C–O), 806 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (ddd, *J* 15.2, 8.5, 1.8 Hz, 1H, H-3), 7.25 (d, *J* 1.6 Hz, 1H, H-7), 7.11-6.97 (m, 3H, H-4, H-5, H-11), 6.94 (d, 1H, *J* 8.0 Hz, H-10), 6.09 (d, 3H, *J* 16.1 Hz, H-2, H-12), 4.84-4.65 (m, 3H, H-15, H-13), 1.85-1.72 (m, 2H, H-16), 1.71-1.57 (m, 2H, H-16'), 1.56-1.16 (m, 6H, H-17, H-17', H-18); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7 (C-14), 166.1 (C-1), 148.8 (C-9), 148.5 (C-8), 146.8 (C-3), 141.7 (C-5), 130.8 (C-6), 125.0 (C-4), 123.9 (C-11), 119.0 (C-2), 109.0 (C-10), 106.2 (C-7), 101.9 (C-12), 73.3 (C-15), 61.2 (C-13), 31.5 (C-16, C-16'), 25.2 (C-17, C-17'), 23.3 (C-18, C-18').

2-Octyloxy-2-oxoethyl-piperate (**6j**)

Yellow solid; MW 388.46 g mol^{-1}; yield: 73%; mp 69-70 °C; IR (ATR) ν / cm^{-1} 3014 (C–H_{Ar}), 2951, 2927, 2858 (C–H), 1749, 1716 (C=O), 1608, 1452 (C=C_{Ar}), 1257 (C–O–C), 1134, 1035 (C–O), 852 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (ddd, *J* 15.2, 7.9, 2.4 Hz, 1H, H-3), 7.24 (d, *J* 1.5 Hz, 1H, H-7), 7.10-6.98 (m, 3H, H-4, H-5, H-11), 6.94 (d, *J* 8.0 Hz, 1H, H-10), 6.08 (d, *J* 15.2 Hz, 3H, H-2, H-12), 4.73 (s, 2H, H-13), 4.09 (t, 2H, H-15), 1.56 (m, 2H, H-16), 1.25 (m, 10H, H-17, H-18, H-19, H-20, H-21), 0.84 (t, 3H, H-22); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3 (C-14), 166.1 (C-1), 148.8 (C-9), 148.5 (C-8), 146.8 (C-3), 141.7 (C-5), 130.8 (C-6), 124.9 (C-4), 123.9 (C-11), 118.9 (C-2), 109.0 (C-10), 106.2 (C-7), 101.9 (C-12), 65.1 (C-15), 61.0 (C-13), 31.6 (C-16), 29.1 (C-17), 29.0 (C-18), 28.5 (C-19), 25.7 (C-20), 22.5 (C-21), 14.3 (C-22).

2-Decyloxy-2-oxoethyl-piperate (**6k**)

Pale yellow solid; MW 416.51 g mol^{-1}; yield: 64%; mp 73-74 °C; IR (ATR) ν / cm^{-1} 3018 (C–H_{Ar}), 2918, 2846 (C–H), 1739, 1722 (C=O), 1602, 1450 (C=C_{Ar}), 1253 (C–O–C), 1201, 1085, 1031 (C–O), 852 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (ddd, *J* 15.2, 7.8, 2.5 Hz, 1H, H-3), 7.25 (d, *J* 1.5 Hz, 1H, H-7), 7.10-6.98 (m, 3H, H-4, H-5, H-11), 6.94 (d, *J* 8.0 Hz, 1H, H-10), 6.08 (d, *J* 15.6 Hz, 3H, H-2, H-12), 4.73 (s, 2H, H-13), 4.09 (t, 2H, H-15), 1.66-1.50 (m, 2H, H-16), 1.22 (m, 14H, H-17, H-18, H-19, H-20, H-21, H-22, H-23), 0.84 (t, 4H, H-24); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3 (C-14), 166.1 (C-1), 148.8 (C-9), 148.4 (C-8), 146.8 (C-3), 141.7 (C-5), 130.8 (C-6), 124.9 (C-4), 123.9 (C-11), 118.9 (C-2), 109.0 (C-10), 106.2 (C-7), 101.9 (C-12), 65.1 (C-15), 61.0 (C-13), 31.7 (C-16), 29.4 (C-17), 29.4 (C-18), 29.2 (C-19), 29.1 (C-20), 28.5 (C-21), 25.7 (C-22), 22.5 (C-23), 14.3 (C-24).

2-Dodecyloxy-2-oxoethyl-piperate (**6l**)

Pale yellow solid; MW 444.57 g mol^{-1}; yield: 63%;

mp 65-66 °C; IR (ATR) ν / cm^{-1} 3024 ($C–H_{Ar}$), 2953, 2920, 2852 (C–H), 1749, 1712 (C=O), 1606, 1446 ($C=C_{Ar}$), 1251 (C–O–C), 1134, 1035 (C–O), 850 ($C–H_{Ar}$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.44 (ddd, *J* 15.2, 7.7, 2.6 Hz, 1H, H-3), 7.24 (d, *J* 1.5 Hz, 1H, H-7), 7.09-6.98 (m, 3H, H-4, H-5, H-11), 6.93 (d, *J* 8.0 Hz, 1H, H-10), 6.08 (d, *J* 15.4 Hz, 3H, H-2, H-12), 4.73 (s, 2H, H-13), 4.09 (t, 2H, H-15), 1.67-1.48 (m, 2H, H-16), 1.41-1.13 (m, 18H, H-17, H-18, H-19, H-20, H-21, H-22, H-23, H-24, H-25), 0.84 (t, 3H, H-26); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.3 (C-14), 166.1 (C-1), 148.8 (C-9), 148.4 (C-8), 146.8 (C-3), 141.7 (C-5), 130.8 (C-6), 124.9 (C-4), 123.9 (C-11), 118.9 (C-2), 108.9 (C-10), 106.2 (C-7), 101.9 (C-12), 65.0 (C-15), 61.0 (C-13), 31.7 (C-16), 29.5 (C-17), 29.5 (C-18), 29.4 (C-19, C-20), 29.2 (C-21), 29.1 (C-22), 28.5 (C-23), 25.7 (C-24), 22.5 (C-25), 14.3 (C-26).

In silico study

The parameters of Lipinski's rule of five: lipophilicity (clogP), molecular weight (MW), hydrogen bond acceptors (HBA), hydrogen bonding donors (HBD) and topological polar surface area (TPSA) were calculated using the online program Molinspiration.[18] The aqueous solubility (LogS), drug-likeness and drug-score parameters were calculated using the OSIRIS Property Explorer software.[19] The percentage of theoretical absorption (ABS) of the compounds was calculated using the equation: ABS(%) = 109 − 0.345 TPSA.[20]

Antimicrobial activity

Culture media

The culture media used for maintenance of bacterial and fungal strains were brain heart infusion (BHI) and Sabouraud dextrose agar (SDA) (acquired from Difco Laboratories Ltd., Detroit, USA), respectively. For the pharmacological activity assays, BHI liquid nutrient medium for bacteria and Roswell Park Memorial Institute (RPMI) 1640 with L-glutamine and without bicarbonate for fungi (Difco Laboratories Ltd., Detroit, USA, and INLAB, São Paulo, Brazil) were used. The culture media were prepared according to the manufacturers' instructions.

Microorganisms

For the antimicrobial activity assays of the compounds, the following strains were used: *Staphylococcus aureus* (American Type Culture Collection (ATCC)-25923), *Pseudomonas aeruginosa* (ATCC-25853), *Candida albicans* (ATCC-60193 and LM-92), *Candida tropicalis* (ATCC-13803 and LM-18), *Aspergillus fumigatus* (ATCC-40640 and IPP-210), *Aspergillus flavus* (LM-714) and *Aspergillus niger* (LM-108). The microorganisms belong to the collection of the Mycology Laboratory, Department of Pharmaceutical Sciences (DCF), Center of Health Sciences (CCS) of the Federal University of Paraíba (UFPB). The strains were stored in BHI (bacteria) and in SDA (fungi) at 4 °C. Samples of bacterial and fungal (yeasts) colonies incubated at 35 ± 2 °C for 24-48 h and filamentous fungi colonies incubated at 28 ± 2 °C for 7-14 days were used for the assays. To prepare the inoculum, the colonies obtained from cultures of bacterial strains in BHI medium and fungi in SDA medium were suspended in sterile saline solution (0.9% NaCl) according to the 0.5 McFarland standard, adjusted using a spectrophotometer (Leitz-Photometer 340-800) to 90% T (530 nm), corresponding to approximately 10^6 colony forming unit (CFU) mL^{-1} for fungi and 10^8 CFU mL^{-1} for bacteria.[21,22]

Determination of minimum inhibitory concentration (MIC)

The determination of the MIC of the products against the bacterial and fungal strains was performed using the broth microdilution technique with cell-culture microplates (TPP, Trasadingen, Switzerland, Europe) with 96 round-bottom wells. Initially, 100 µL of RPMI/BHI broth were distributed in the wells of the microdilution plates. Next, 100 µL of the substances were dispensed in the wells of the first row of the plate, and 2-fold serial dilution was performed, giving concentrations of 1024 up to 64 µg mL^{-1}. Finally, 10 µL of the bacterial and fungal suspensions were added to the wells. In parallel, the controls were performed: microorganisms (BHI + bacteria and RPMI + fungi) and culture medium (RPMI/BHI), to assure the strains viability and sterility of the medium, respectively; and negative control with antimicrobials: gentamicin (100 µg mL^{-1}) for bacteria and amphotericin B (1 µg mL^{-1}) for fungi. The prepared plates were aseptically closed and incubated at 35 ± 2 °C for 24-48 h for bacteria and yeasts at 28 ± 2 °C for 7-14 days for filamentous fungi. MIC was defined as the lowest concentration capable of visually inhibiting complete microbial growth. The results were expressed as the mean. In the biological assay with bacteria, after 24 h of incubation, 20 µL of 0.01% resazurin dye solution (INLAB) were added; this dye is recognized as a colorimetric redox indicator.[23] A change in dye color (blue to red) indicated microbial growth; and, if the color remained blue, there was no microbial growth. The MIC for each product was defined as the lowest concentration capable of visually inhibiting microbial growth and/or verified by no change in color of the indicator dye.

Results and Discussion

Chemistry

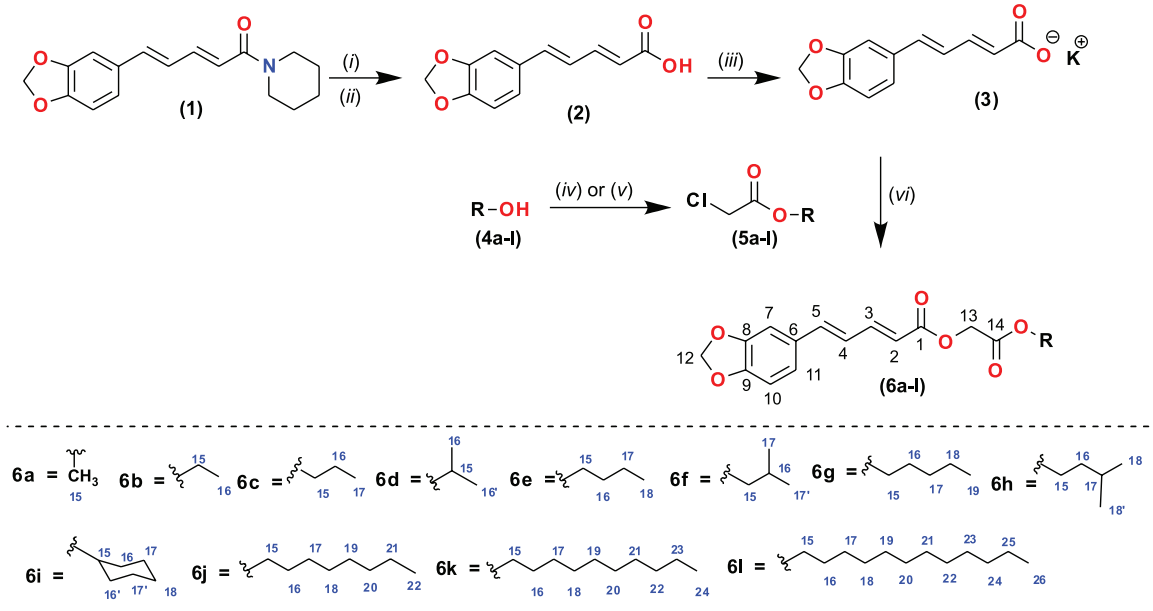
The synthesis of the diesters derived from piperine (**6a**-**6l**) was performed in five stages, which are described in Scheme 1.

Initially, piperine (**1**), extracted from black pepper (*Piper nigrum* L.), was subjected to basic hydrolysis (*i*) followed by acidification (*ii*) to obtain piperic acid (**2**).[16] We decided to use a salt of piperic acid (**3**), as a nucleophile, to easily remove the reaction medium by the addition of water, which was obtained by the neutralization reaction of piperic acid with an ethanolic KOH solution (1:1) (*iii*). The alkyl 2-chloroacetate intermediates (**5a**-**5l**) were obtained via two methods: (*iv*) Fisher esterification,[24] readily and suitable for small liquid alcohols molecules, where the excess alcohol can be removed by rotary evaporation, and (*v*) esterification via acid chloride,[25] an efficient method for larger chain alcohols. The final compounds were obtained through the bimolecular nucleophilic substitution reaction (S_N2) between alkyl 2-chloroacetates (**5a**-**5l**) and the piperate (**3**) in slight excess to ensure that there was no residual chloroester, where this excess salt could be easily removed by the addition of water. Thus, it was possible to obtain 12 novel piperine diester derivatives (**6a**-**6l**).

Characterization of final products

The structures of the piperine derivatives were confirmed using IR and ^{1}H and ^{13}C NMR, including the 2D techniques ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HSQC and HMBC. In the IR spectrum of the piperine derivatives (**6a**-**6l**), the presence of the aromatic and aliphatic groups was evidenced by the axial deformation of the C–H bonds in the region from 3080 to 2850 cm^{-1}. Axial deformation of the C=C connections between 1612 and 1450 cm^{-1} was also observed in the spectra. The absorptions of the carbonyl groups (C=O) appeared between 1761 and 1697 cm^{-1}. The axial deformation bands of the C–O linkage of the esters appeared around 1300 and 1100 cm^{-1}, and in the region of 1250 cm^{-1} there was a band referring to the (C–O–C) portion of the methylenedioxy ring, an important signal in identifying compounds derived from piperine.

In the NMR spectrum of compound **6c**, signals were observed in the aromatic and olefinic regions at δ_H 7.52-6.00, referring to the seven hydrogens. At δ_H 5.98, there was a singlet for two hydrogens (H-12), referring to the hydrogens of the methylenedioxy ring. The 2D studies (^{1}H,^{1}H-COSY) showed the following correlations: the triplet [δ_H 4.13 (t, *J* 6.7 Hz)] and the multiplet [δ_H 1.67 (m)] for the hydrogens of H-15 with H-16; between the multiplet H-16 [δ_H 1.67 (m)] and triplet H-15 [δ_H 4.14 (t, *J* 6.7 Hz)] and the multiplet H-16 [δ_H 1.67 (m)] with the triplet of H-17 [δ_H 0.94 (t, *J* 7.4 Hz)]; and the multiplet [δ_H 1.67 (m)] and triplet [δ_H 0.94 (t, *J* 7.4 Hz)] for the hydrogens of H-16 with H-17. The 2D direct correlation spectrum (^{1}H,^{13}C-HSQC) showed correlations between the signal at δ_H 4.69, referring to the methylene hydrogens (H-13), and the carbon signal at δ_C 60.8 (C-13), and between the signal at δ_H 5.98, referring to the hydrogens (H-12), and the carbon signal at δ_C 101.5 (C-12).



Scheme 1. Synthetic route for the target molecules. Reagents and conditions: (*i*) KOH 20%, EtOH, reflux, 20 h; (*ii*) HCl (94%); (*iii*) KOH, EtOH, room temperature, 1 h, 93%; (*iv*) for **4a**-**4h**; $ClCH_2COOH$, $H_2SO_{4(Cat)}$, 6 h, (75-93%); (*v*) for **4i**-**4l**; $ClCH_2COCl$, Et_3N, DCM, 20 h, 63-67%; (*vi*) DMF, KI, 100 °C, 24 h, 55-84%.

Concerning the ^{1}H NMR analysis of compound **6c**, all other diesters showed a characteristic singlet for methylene hydrogens, referring to the methylenedioxy ring (H-12) with a shift at δ_H 6.07-5.98 and a singlet of methylene hydrogens (H-13) at δ_H 4.76-4.69.

In the ^{13}C NMR spectrum, all piperine derivatives (**6a**-**6l**) showed two characteristic signals attributed to C-1 and C-14 carbonyls varying in the range of δ_C 168.8-166.1. Analyzing the 2D HMBC spectrum of compound **6c**, it was possible to unequivocally attribute the chemical shifts of both carbonyl moieties present in the compound from the correlations between ^{13}C and ^{1}H separated by 2 and 3 bonds. The H-15 methylene hydrogens at δ_H 4.13 couples with the carbon C-16 at δ_C 22.0, with carbon C-17 at δ_C 10.3 and carbonyl C-14 at δ_C 168.1. Olefinic hydrogen H-2 at δ_H 6.02 correlated with carbonyl C-1 at δ_C 166.4.

Based on compound **6c** analyses, the C-1 and C-14 carbons of the **6a**-**6l** compounds were recorded in the range of δ_C 166.4-166.1 and δ_C 168.8-167.7, respectively. The compounds showed two more characteristic signals of methylene carbons referring to C-12 and C-13, in the range of δ_C 101.9-101.4 and δ_C 61.2-60.7, respectively. In all compounds, the signals attributed to the aromatic carbons were in the range of δ_C 148.8-105.9.

In the ^{13}C NMR spectrum for compound **6a**, a characteristic signal is observed for the methyl group in the aliphatic region at δ_C 52.3. For compound **6b** it shows two signals of the ethyl group, at δ_C 61.0 and 14.4. For compound **6d**, two characteristic signals of the isopropyl group appear in δ_C 68.9 and 21.9. For compound **6e**, four signals were found for the butyl group, a signal at δ_C 64.8 and three in the δ_C range of 30.5-13.9. For the **6f** compound, the three signals for isobutyl group appear in δ_C 70.7, 27.7 and 19.1. For the **6g** compound, it shows five signals that characterizes the pentyl group, being a chemical displacement at δ_C 65.1 and four signals in the δ_C range of 28.1-14.2. For compound **6h**, four characteristic signals of the isopentyl group are observed, a signal around δ_C 63.6 and three signals in δ_C range of 37.1-22.6. For compound **6i**, four signals were observed, one in δ_C 73.3 and three signals in the range of δ_C 31.5-23.3, regarding the cyclohexyl group. For compound **6j**, eight signals belonging to the octyl group were recorded, one in δ_C 65.1 and the other seven signals are in the range of δ_C 31.6-14.3. For compound **6k**, ten chemical shift signals were attributed to the decyl group, one in δ_C 65.1 and nine in the range of δ_C 31.7-14.3. Compound **6l** showed eleven signals representing the dodecyl group, with ten chemical shift signals in the range of δ_C 31.7-14.3 and one around δ_C 65.0.

In silico study

In the present study, the theoretical potential of the synthesized compounds was investigated by the *in silico* approach of the Lipinski's rule of five,[26-28] where they identified that, for good absorption and permeation, the drug must comply with at least three of the following four criteria: HBA ≤ 10; HBD ≤ 5; MW ≤ 500; clogP ≤ 5. The parameters as percentage of theoretical absorption (ABS), aqueous solubility (LogS), drug-likeness and drug-score were also calculated. The results of the *in silico* study for the diesters derived from piperine are presented in Table 1.

According to the results of the *in silico* study presented in Table 1, all compounds satisfy the Lipinski's rule with no violation, with the exception of compounds **6j**, **6k** and

Table 1. *In silico* study of piperine derivatives (**6a**-**6l**)

Compound	Lipinski's parameter					TPSA / Å^2	ABS / %	LogS	Drug-likeness	Drug score
	HBA	HBD	MW	clogP	nViol					
6a	6	0	290.27	2.23	0	71.06	84.484	−3.05	−2.82	0.28
6b	6	0	304.30	2.63	0	71.06	84.484	−3.35	−4.39	0.26
6c	6	0	318.32	3.09	0	71.06	84.484	−3.62	−0.82	0.31
6d	6	0	318.32	2.99	0	71.06	84.484	−3.73	−3.16	0.25
6e	6	0	332.35	3.54	0	71.06	84.484	−3.89	−5.58	0.13
6f	6	0	332.35	3.31	0	71.06	84.484	−3.78	−0.72	0.18
6g	6	0	346.38	4.00	0	71.06	84.484	−4.16	−9.82	0.13
6h	6	0	346.38	3.76	0	71.06	84.484	−4.05	−0.31	0.24
6i	6	0	358.39	3.78	0	71.06	84.484	−4.63	−5.40	0.20
6j	6	0	388.46	5.36	1	71.06	84.484	−4.97	−22.25	0.14
6k	6	0	416.51	6.27	1	71.06	84.484	−5.51	−22.25	0.11
6l	6	0	444.57	7.18	1	71.06	84.484	−6.05	−22.25	0.09

HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; MW: molecular weight; clogP: octanol/water partition coefficient based on Molinspiration; nViol: number of violations; TPSA: topological surface area; ABS: absorption percentage; LogS: solubility.

6l, which violated the lipophilicity parameter clogP > 5, suggesting that the great majority of the compounds should demonstrate good oral bioavailability. In the TPSA parameter, which indicates that molecules with TPSA ≤ 140 Å^2 have better oral bioavailability and a higher permeation rate,[27] the results showed that the synthesized compounds showed TPSA values equal to 71.06 Å^2, which indicates good permeability through the cell membrane, reflecting a high percentage of absorption (84.48%). Most commercial medications have LogS (mol L^{-1}) > −4.00 (OSIRIS Property Explorer),[19] while in the results presented, we found that only compounds **6a**-**6f** had LogS > −4.00. The compounds displayed values of drug-likeness in the range of −22.25 to −0.31, with **6h** having the highest value. The drug-score values combine the parameters of lipophilicity, aqueous solubility, molecular weight, similarity of the drug and risk of toxicity, and their values are often used to predict the potential of the test compounds to be new medications. The drug-score values of the diesters derived from piperine ranged between 0.09 and 0.31, with the lowest value for **6l** and the highest value for **6c**.

Antimicrobial study

The *in vitro* antimicrobial activity of compounds **6a**-**6l** was evaluated by the microdilution method on bacterial strains (*Staphylococcus aureus* ATCC-25923; *Pseudomonas aeruginosa* ATCC-25853), yeasts (*Candida albicans* ATCC-60193 and LM-92; *C. tropicalis* ATCC-13803 and LM-18), and filamentous fungi (*Aspergillus fumigatus* ATCC-40640 and IPP-210; *A. flavus* LM-714; *A. niger* LM-108). The products were weighed and dissolved in 5% DMSO-2% Tween 80 completing the final volume with sterile distilled water, obtaining an emulsion of the products at the initial concentration of 1024 μg mL^{-1}.[29-31] The results of the antimicrobial activity of compounds **6a**-**6l** are shown in Table 2.

As shown in Table 2, no substance was able to inhibit microbial growth of the bacterial strains. Substances **6j**, **6k** and **6l** were inactive for all microorganisms tested. Substances **6a**-**6i** were active against all *Candida* yeasts, displaying an MIC of 1024-256 μg mL^{-1}. Compounds **6a**-**6e** had an MIC of 256 μg mL^{-1} against the filamentous fungus *A. niger* LM-108. Of the test substances, only **6e** was active against *A. flavus* LM-714 with MIC of 1024 μg mL^{-1}. Compound **6g** was effective with an MIC of 1024 μg mL^{-1} against 40% of the microorganisms used, and this percentage was composed only of yeasts. For 50% of the microbial strains used, substance **6d** showed an MIC of 512 μg mL^{-1}; product **6c** had an MIC of 256 μg mL^{-1}, while **6f** and **6i** had an MIC of 1024 μg mL^{-1}. **6a**, **6b** and **6e** were active against 70% of the microorganisms used, with an MIC of 1024 μg mL^{-1} for compound **6e** and an MIC of 512 μg mL^{-1} for **6a** and **6b**.

The variation in antimicrobial capacity of the final compounds **6a**-**6l** may probably be related to differences in lipophilicity and solubility. The lack of activity displayed

Table 2. Minimum inhibitory concentration of the piperine-derived diesters **6a**-**6l** against bacterial and fungal strains

Compound	MIC / (μg mL^{-1})									
	Bacteria		Yeast				Filamentous fungi			
	S. aureus ATCC-25923	*P. aeruginosa* ATCC-25853	*C. albicans* ATCC-60193	*C. albicans* LM-92	*C. tropicalis* ATCC-13803	*C. tropicalis* LM-18	*A. flavus* LM-714	*A. niger* LM-108	*A. fumigatus* ATCC-40640	*A. fumigatus* IPP-210
6a	+	+	256	1024	1024	1024	+	256	256	256
6b	+	+	256	1024	1024	1024	+	256	256	256
6c	+	+	1024	256	1024	256	+	256	+	+
6d	+	+	1024	1024	512	512	+	256	+	+
6e	+	+	1024	1024	1024	1024	1024	256	+	1024
6f	+	+	1024	1024	1024	1024	+	+	+	512
6g	+	+	1024	512	1024	1024	+	+	+	+
6h	+	+	256	[a]	1024	1024	+	+	256	+
6i	+	+	1024	1024	1024	1024	+	+	+	1024
6j	+	+	+	+	+	+	+	+	+	+
6k	+	+	+	+	+	+	+	+	+	+
6l	+	+	+	+	+	+	+	+	+	+
Culture media	−	−	−	−	−	−	−	−	−	−
Microorganism	+	+	+	+	+	+	+	+	+	+
Amphotericin B	[a]	[a]	−	−	−	−	−	−	−	−
Gentamicin	−	−	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]

[a]Not tested. MIC: minimum inhibitory concentration; ATCC: American Type Culture Collection; +: presence of microbial growth; −: no microbial growth.

by compounds **6j**, **6k** and **6l** may be due to their high lipophilicity values. As seen in the *in silico* study, only these diesters showed lipophilicity values (clogP) higher than 5. If a drug is very lipophilic, it can very strongly bind to plasma proteins, being unable to reach the intracellular space, and thus, the plasma concentration of free drug decreases and drug's potency/efficacy may be reduced.[32]

Conclusions

In this work, twelve new diesters derived from piperine were synthesized and their structures were confirmed by IR, ^{1}H and ^{13}C NMR, COSY, HMBC and HSQC. The *in silico* study showed that compounds **6a**-**6i** did not violate the Lipinski's rule of five, so they should have good oral bioavailability. The *in vitro* antimicrobial activity assay showed that compounds **6a**, **6b** and **6e** were active against 70% of the strains used with an MIC of 1024-256 μg mL^{-1}, while compounds **6j**, **6k** and **6l** were inactive against all strains at the concentrations used. The antimicrobial activity of these compounds may be related to lipophilic factors and the hydrophobic character of these molecules. To fully understand the relationship between the physicochemical properties and the biological activity observed in the *in vitro* study, further structure-activity relationship studies are warranted.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Emmely O. Trindade and Petrônio F. de Athayde-Filho conceived and designed the study; Emmely O. Trindade, Maria Cláudia R. Brandão, Helivaldo D. S. Souza and Bruno F. Lira performed the experiments; Emmely O. Trindade, Helivaldo D. S. Souza, Maria Cláudia R. Brandão carried out the *in silico* study and analyzed the data; Hermes D. Neto and Edeltrudes O. Lima performed the antimicrobial study; and Emmely O. Trindade, Helivaldo D. S. Souza, Petrônio F. de Athayde-Filho and José M. Barbosa-Filho wrote the paper.

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