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Article

Synthesis, *in silico* Study and Antimicrobial Activity of New Piperine Derivatives Containing Substituted δ-Esters

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A series of fifteen new piperine-derived diesters was synthesized through the substitution reaction between the salt of piperic acid, obtained through piperine basic hydrolysis, with the δ-chloro-esters, obtained through the cleavage of tetrahydrofuran (THF) with acyl chlorides in the presence of $ZnCl_2$. The final compounds were obtained with yields ranging from 50 to 84% and were characterized by infrared (IR) and ^1H and ^{13}C nuclear magnetic resonance spectroscopy (NMR). The new compounds were evaluated *in silico* in regard to their ADME (absorption, distribution, metabolism, and excretion) properties, and *in vitro* for their antimicrobial activity against bacteria strains (*Staphylococcus aureus* and *Pseudomonas aeruginosas*), yeast fungi (*Candida albicans* and *C. tropicalis*) and filamentous fungi (*Aspergillus fumigatus*, *A. flavus* and *A. niger*). The results from the *in silico* studies of Lipinski's rule of five showed that most compounds present good pharmacological possibilities, and the results from *in vitro* antimicrobial activity showed that 8 of the 15 synthesized compounds displayed antimicrobial activity, inhibiting the growth of 40-80% of tested strains, with a minimum inhibitory concentration (MIC) interval ranging from 1024 to 256 μg mL^{-1}.

Keywords: piperine derivatives, diesters, antimicrobial activity, *in silico* study

Introduction

The black pepper (*Piper nigrum* L.) is one of the most consumed spices in the world, as well as the main spice in the food, medical, perfumery and cosmetic industries.[1,2] Its seeds have been used for centuries as a condiment in food preparation, and in folk medicine, in preparations, such as ointments and creams for the treatment of various diseases, given their therapeutic actions.[3] Many of the activities associated with the black pepper fruits are due to its main active principle, piperine, a natural alkaloid with molecular formula $C_{17}H_{19}NO_3$ that is extractable with 3-7% yield from the fruits of several species of the *Piper* genus.[4,5]

Piperine has a wide spectrum of biological activities, such as antioxidant, antitumor, antimicrobial, anti-inflammatory, immunomodulatory, hepatoprotective, antiasthmatic, anticonvulsant, antimutagenic, antidepressant, anti-thyroid, and still others.[6,7] Piperine has received substantive attention in the last two decades, and has been considered an extremely versatile bioactive molecule.[8] Its structure allows several chemical changes, and this has been playing an important role in the synthesis of new derived compounds with therapeutic application in multiple human diseases. Piperine derivatives displayed anti-inflammatory activity,[9] analgesic,[9] antimicrobial,[10] antitumor,[11] antidiabetic,[12] antichagasic,[13] anti-vitiligo[14] and yet other pharmacological properties. Many derivatives showed biological activities superior to that of piperine itself, and thus emerged as a new approach in the research and discovery of new drugs.

Among the various activities presented by piperine-derived compounds, antimicrobial activity deserves emphasis. That is largely because the number of microorganisms resistant to available drugs increases more and more, reaching alarming levels. Hence, effective

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treatment of infections caused by resistant fungi and bacteria is becoming increasingly challenging for public health systems.[15,16] Therefore, there is an increasing need of research, identification and development of new biologically active molecules capable of containing these emerging microbial infections.[17]

In view of these aspects, the use of piperine extracted from black pepper is present in this work, as a good source for the discovery of new molecules with potential antimicrobial activities. For this, a series of δ-chloro-esters was planned and synthesized, which were coupled to the piperinic segment in order to originate fifteen new piperine-derived diesters, with their biological potential evaluated through *in silico* study and *in vitro* antimicrobial activity.

Results and Discussion

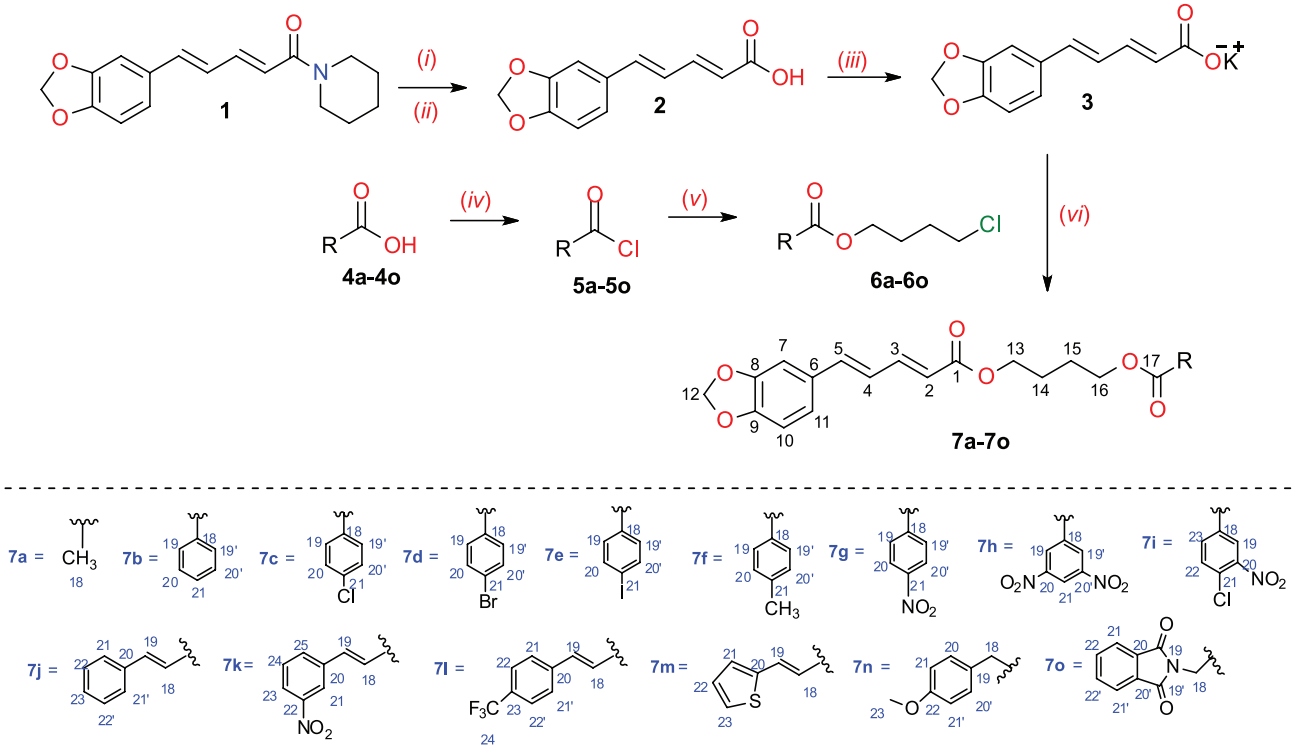
Chemistry

The new piperine-derived diesters (**7a**-**7o**) were efficiently planned and synthesized through six steps, which are described in Scheme 1.

Piperine (**1**), previously extracted from black pepper, was hydrolyzed with KOH (*i*) and then acidified (*ii*) to provide piperic acid (**2**).[18] It was decided to employ potassium piperate (**3**) as reaction nucleophile, due to its easy removal from the reaction medium by the addition of water, and it was obtained through the neutralization reaction of piperic acid with KOH ethanolic solution (1:1) (*iii*). For the synthesis of intermediate δ-chloro-esters compounds (**6a**-**6o**), acid chlorides (**5a**-**5o**) were initially obtained through the acyl nucleophilic substitution reaction (S_NAc) between carboxylic acids (**4a**-**4o**) and thionyl chloride ($SOCl_2$), followed by the *in situ* reaction with tetrahydrofuran (THF) in the presence of zinc chloride ($ZnCl_2$).[19] The final compounds (**7a**-**7o**) were obtained through the S_N2 reaction between the δ-chloro-esters (**6a**-**6o**) and the potassium piperate (**3**) in slight excess to ensure that there was no residual δ-chloro-ester, where this excess salt could be easily removed by the addition of water. Thus, it was possible to obtain fifteen δ-chloro-esters, of which seven are new compounds (**6e**, **6i**, **6k**, **6l**, **6m**, **6n** and **6o**), and fifteen new piperine-derived diesters (**7a**-**7o**).

Characterization of final products

The structures of the final compounds were confirmed using infrared (IR) techniques, ^1H and ^{13}C nuclear magnetic resonance (NMR), including the two-dimensional (2D) NMR techniques ^1H,^1H-COSY (correlation spectroscopy), ^1H,^{13}C-HSQC (heteronuclear single quantum correlation) and HMBC (heteronuclear multiple bond correlation). The infrared spectra from the



Scheme 1. Synthetic route for the synthesis of target molecules. Reagents and conditions: (*i*) 20% KOH, EtOH, reflux, 20 h; (*ii*) HCl (94%); (*iii*) KOH, EtOH, room temperature, 1 h (93%); (*iv*) $SOCl_2$, $DMF_{(cat.)}$, reflux; (*v*) THF, $ZnCl_2$, 75 °C (55-92%); (*vi*) DMF, KI, 100 °C (50-84%).

diesters (**7a**-**7o**) showed the presence of the aromatic and aliphatic groups, indicated by the axial deformation of the C–H bonds between 3103 and 3014 cm^{-1} alongside the angular deformation outside the plane in range or 862 and 702 cm^{-1} to the aromatic groups, and by the axial deformation in region between 2964 and 2858 cm^{-1} for the aliphatic groups, alongside the angular deformation between 2964 and 2858 cm^{-1} for the aliphatic groups. The axial deformations of the C=C bonds were observed in the region of 1620-1442 cm^{-1}. The absorptions of the carbonyl groups (C=O) appeared between 1745 and 1695 cm^{-1}. The axial deformation band of the C–O bond appeared around 1236 and 1016 cm^{-1}. Around 1250 cm^{-1}, the band referring to the methylenedioxy ring (O–CH_2–O) was observed, a characteristic signal of piperine-derived compounds.

Due to their similar structures, it is possible to observe that the signals referring to compounds **7a**-**7i** and **7n**-**7o** are similar to the signals referring to compound **7c**, and the signals referring to compounds **7j**-**7m** are similar to the signals referring to the **7k** compound. Thus, these compounds spectra of ^{1}H NMR, ^{13}C NMR, COSY, HMQC and HMBC will be detailed in this section.

In the ^{1}H NMR spectrum of the compound **7c**, signals are observed for eleven hydrogens in the aromatic and olefinic regions between δ_H 7.97-5.97. Inside the olefinic region in δ_H 6.05, the presence of a singlet for two hydrogen atoms referring to the methylenedioxy ring is observed (H-12); in the region of δ_H 5.99, a doublet (*J* 15.2 Hz, 1H) referring to the hydrogen H-2; and in the region of δ_H 4.33-4.15, two triplets for hydrogens H-13 and H-16.

In the ^{13}C NMR spectra of the final compounds the presence of two carbonyls is also observed. Therefore, in order to unequivocally attribute the chemical displacements present in the compounds, the two-dimensional spectrum of heteronuclear correlations at long distance (HMBC) was used, which made it possible to mark the carbonyl of each ester subunit and the hydrogens H-13 and H-16, unequivocally attributing their couplings.

In the HMBC experiment for compound **7c**, the carbon signal in δ_C 166.2 couples with the signals of H-2 (δ_H 5.99) and H-13 (δ_H 4.16), showing that this signal corresponds to the carbonyl of the piperinic segment (C-1), which is, therefore, the least displaced triplet for hydrogens H-13. The signal of the second carbonyl in δ_C 164.9 couples with the signals of the hydrogens H-19 and H-19' (δ_H 7.96) and H-16 (δ_H 4.31), corresponding to the ester carbonyl bonded to the aromatic segment (C-17).

In the two-dimensional spectrum (^{1}H,^{1}H-COSY) of compound **7c**, it is possible to observe correlations such as: the hydrogen H-3 (δ_H 7.35) with the hydrogen H-2 (δ_H 5.99); the hydrogens H-13 (δ_H 4.16) and H-16 (δ_H 4.31) with the overlap of the quintets referring to the hydrogens H-14 and H-15 in δ_H 1.78. Still in this spectrum, it is possible to observe the correlations between the aromatic hydrogens in *para* position in relation to the substituting chloro in the ring, between H-19 and H-19' in δ_H 7.96 (d, *J* 8.5 Hz) with H-20 and H-20' in δ_H 7.59 (d, *J* 8.4 Hz) of the described system AA'BB'.

In the compound **7c** two-dimensional spectrum of direct correlation (^{1}H,^{13}C-HSQC), it was possible to unequivocally attribute the couplings between the signals of methylenic hydrogens of H-13 (δ_H 4.16) with the carbon C-13 (δ_H 63.4) and between the signal δ_H 4.31 referring the hydrogens H-16 with the carbon C-16 (δ_H 64.6), as well as the following couplings: olefinics (δ_H/δ_C) 5.99 (H-2)/119.7 (C-2), 7.35 (H-3)/145.1 (C-3), 6.98 (H-4)/124.6 (C-4), 6.98 (H-5)/140.4 (C-5); aliphatics (δ_H/δ_C) 6.05 (H-12)/101.3 (C-12), 1.78 (H-14)/24.8 (C-14), 1.78 (H-15)/24.9 (C-15); and aromatics (δ_H/δ_C) 7.21 (H-7)/105.7 (C-7), 6.92 (H-10)/108.7 (C-10), 6.98 (H-11)/123.2 (C-11), 7.96 (H-19, H-19')/130.9 (C-19, C-19'), 7.59 (H-20, H-20')/128.9 (C-19, C-19').

In the ^{1}H NMR spectrum of compound **7k**, signals are observed in the regions of δ_H 8.54-5.98, referring to the thirteen aromatic and olefinic hydrogens, and in δ_H 6.04, the singlet for the hydrogens of the methylenedioxy ring (H-12). In the region of δ_H 4.22-4.16, the presence of two triplets referring to the hydrogens H-13 and H-16 is seen, and in δ_H 1.77-1.70 the overlap of the signals referring to the hydrogens H-14 and H-15 is noted as well.

In the ^{13}C NMR spectra of the final compounds **7j**-**7m**, the presence of two very close carbonyls is noted, which is due to both being conjugated with an olefinic and aromatic segment. For unequivocally attributing the chemical displacements present in these compounds, the HMBC bidimensional spectrum was utilized.

In the HMBC experiment of compound **7k** the carbon signal in δ_H 166.2 couples with the signals of H-2 (δ_H 5.99), H-3 (δ_H 7.36) and H-13 (δ_H 4.16), showing that this signal corresponds to the carbonyl group of the piperinic segment (C-1). The signal in δ_H 165.8 couples with the signals of the hydrogens H-19 (δ_H 7.78), H-18 (δ_H 6.89) and H-16 (δ_H 4.21), corresponding to the carbonyl (C-17).

In the bidimensional spectrum (^{1}H,^{1}H-COSY) of compound **7k**, correlations (^3J) between the aliphatic hydrogens H-13 (δ_H 4.16) with H-14 (δ_H 1.75) are verified. The hydrogens H-14 couple with H-15 (δ_H 1.75), H-15 with H-16 (δ_H 4.21), and *vice versa*. Also in this spectrum, there can be verified important correlations between the aromatic hydrogens of the piperinic segment in the *ortho* position (^3J) referring to the H-10 (δ_H 6.91) with H-11 (δ_H 6.98) and in the *meta* position (^4J) of H-11 (δ_H 6.98)

with H-7 (δ_H 7.19). Similarly, correlations were noted between aromatic hydrogens of the other ester subunit aromatic ring in the *meta* position (^4J) referring to the H-21 (δ_H 8.54) with H-23 (δ_H 8.23), and the H-21 (δ_H 8.54) with H-25 (δ_H 8.19), the remainder in *ortho* position (^3J) of H-23 (δ_H 8.23) with H-24 (δ_H 7.69), of H-24 (δ_H 7.69) with H-25 (δ_H 8.19), and *vice versa*.

The bidimensional spectrum of direct correlation (^{1}H,^{13}C-HSQC) made it possible to establish the following correlations: olefinics (δ_H/δ_C): 5.99 (H-2)/119.7 (C-2), 7.36 (H-3)/145.1 (C-3), 6.98 (H-4)/124.6 (C-4), 6.98 (H-5)/140.5 (C-5), 6.84 (H-18)/121.0 (C-18), 7.78 (H-19)/142.0 (C-19); aliphatics (δ_H/δ_C): 6.04 (H-12)/101.4 (C-12), 4.16 (H-13)/63.9 (C-13), 1.75 (H-14)/24.9 (C-14), 1.75 (H-15)/24.9 (C-15), 4.21 (H-16)/63.4 (C-16) and aromatics (δ_H/δ_C): 7.19 (H-21)/105.7 (C-7), 6.91 (H-10)/108.5 (C-10), 6.98 (H-11)/123.2 (C-11), 8.54 (H-21)/123.0 (C-21), 8.22 (H-23)/124.5 (C-23), 7.69 (H-24)/130.3 (C-24), 8.19 (H-25)/134.1 (C-25).

In silico study

One of the most common theoretical approaches of pharmacokinetic parameters in the *in silico* study is the Lipinski's rule of five.[20] Lipinski's rule applies a set of parameters capable of identifying compounds with absorption and permeability issues, aiming to evaluate the theoretical potential that a molecule has to be absorbed orally. It was identified that, for good absorption and permeation, the drug must meet at least three of the following four criteria: hydrogen bond acceptors (HBA) ≤ 10, hydrogen bond donors (HBD) ≤ 5, molecular weight (MW) ≤ 500 and lipophilicity (clogP) ≤ 5.

In this work, it was decided to investigate the theoretical potential of the new piperine derivatives (**7a**-**7o**) through the *in silico* approach of the parameters from Lipinski's rule of five,[20,21] together with the following parameters: topological polar surface area (TPSA), theoretical absorption percentage (ABS), aqueous solubility (log S), drug-likeness and drug-score.

Results of the *in silico* study for piperine-derived diesters are presented in Table 1.

According to the results shown in Table 1, only compounds **7a**, **7b**, **7g**, **7k**, **7n** and **7o** satisfy the rule of Lipinski with no violation, whereas compounds **7c**, **7d**, **7f**, **7i**, **7j**, **7l**, **7m** violated only one of the parameters. As Lipinski's rule admits one violation, it is posited that these compounds should present good permeability in the cell membrane and good absorption after oral administration. The compounds **7e** and **7h** violated two parameters, thus suggesting insufficient oral bioavailability according to the rule. Molecules with TPSA ≤ 140 Å^2 tend to have better oral bioavailability and higher permeation velocity.[22] The results showed that all compounds, with the exception of **7h**, presented TPSA values between 71.06 and 116.08 Å^2, resulting in a high percentage of absorption (68.70-84.48%). The compound **7h** (TPSA = 162.7 Å^2) was classified with an average absorption (52.87%).

Table 1. *In silico* study of the new piperine derivatives (**7a**-**7o**)

Compound	Lipinski's parameter					TPSA / Å^2	ABS / %	log S	Drug likeness	Drug score
	HBA	HBD	MW	clogP	nViol					
7a	6	0	332.35	3.54	0	71.06	84.484	−3.89	−6.49	0.18
7b	6	0	394.42	4.99	0	71.06	84.484	−5.06	−5.59	0.09
7c	6	0	428.87	5.59	1	71.06	84.484	−5.80	−3.29	0.09
7d	6	0	473.32	5.71	1	71.06	84.484	−5.71	−8.95	0.08
7e	6	0	520.32	5.42	2	71.06	84.484	−6.08	−5.25	0.08
7f	6	0	408.45	5.33	1	71.06	84.484	−5.41	−6.78	0.10
7g	9	0	439.42	4.06	0	116.80	68.704	−5.52	−20.54	0.12
7h	12	0	484.42	3.14	2	162.70	52.869	−5.98	−20.64	0.14
7i	9	0	473.87	4.67	1	116.80	68.704	−6.26	−14.25	0.11
7j	6	0	420.46	5.32	1	71.06	84.484	−5.43	−7.86	0.08
7k	9	0	465.46	4.39	0	116.80	68.704	−5.89	−11.72	0.13
7l	6	0	488.46	6.16	1	71.06	84.484	−6.21	−13.84	0.09
7m	6	0	426.46	5.18	1	99.30	74.742	−5.44	−4.94	0.13
7n	7	0	438.48	4.91	0	80.29	81.300	−5.05	−7.20	0.14
7o	9	0	477.47	3.97	0	108.40	71.602	−4.83	−15.80	0.16

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: adsorption percentage; log S: solubility.

Most commercial medicines have log S > −4.00 (OSIRIS Property Explorer).[23] In the results, however, only compound **7a** had log S > −4.00. For drug-likeness, the compounds presented values in the range of −20.64 to −3.29. Regarding the results for the drug-score study (which combines parameters of lipophilicity, aqueous solubility, molecular mass, drug-likeness and toxicity risk), the values ranged from 0.08 to 0.18, with compound **7a** having the highest value.

Antimicrobial study

The results of the compounds **7a**-**7o** *in vitro* antimicrobial activity assays are shown in Table 2.

As shown in Table 2, among the fifteen tested compounds, only the substances **7b**, **7d**, **7e**, **7g**, **7h**, **7i** and **7l** did not show activity against the selected microorganisms at the evaluated concentrations. The substance **7j** showed activity only over *Candida* yeasts with a minimum inhibitory concentration (MIC) of 1024 μg mL^{-1} against all tested yeasts. For 50% of the species, the substance **7o** presented an MIC of 512 μg mL^{-1}. The compounds **7a**, **7c** and **7m** showed inhibitory activity against 60% of the microorganisms with an MIC of 256 μg mL^{-1}. Substances **7m** and **7n** presented an MIC of 1024 μg mL^{-1} for 70% of the microorganisms (yeasts and filamentous fungi). The substance **7f** presented an MIC of 256 μg mL^{-1} against 80% of the microorganisms. It is noteworthy that this substance inhibited 100% of the fungal species, attesting great antifungal potential due to its broad action.

No substance was able to inhibit the microbial growth of the tested bacterial species. However, the fact that these substances did not present antibacterial activity can still be considered a positive aspect, since a possible antifungal treatment with these molecules would most likely not put at risk the integrity of native microbiota, which is beneficial to the host.[24]

The results of antimicrobial activity of the final compounds (**7a**-**7o**) showed that the compounds that had electron-donating groups (**7c**, **7f**, **7n**) were active against most microorganisms. The compounds **7d** and **7e** that presented donor groups, albeit voluminous (Br and I, respectively), were inactive.

The compounds which possessed substituting electron-removing groups (**7g**, **7h** and **7i**) were inactive against the tested microorganisms. As to the compounds **7j**-**7m**, which

Table 2. Minimum inhibitory concentration (MIC) of piperine derivatives **7a**-**7o** 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
7a	+	+	256	256	1024	1024	+	256	256	+
7b	+	+	+	+	+	+	+	+	+	+
7c	+	+	256	1024	1024	256	+	256	1024	+
7d	+	+	+	+	+	+	+	+	+	+
7e	+	+	+	+	+	+	+	+	+	+
7f	+	+	256	1024	1024	512	512	256	256	256
7g	+	+	+	+	+	+	+	+	+	+
7h	+	+	+	+	+	+	+	+	+	+
7i	+	+	+	+	+	+	+	+	+	+
7j	+	+	1024	1024	1024	1024	+	[a]	+	+
7k	+	+	1024	256	1024	1024	1024	512	512	+
7l	+	+	+	+	+	+	+	[a]	+	+
7m	+	+	1024	1024	256	256	+	256	256	+
7n	+	+	1024	1024	1024	1024	1024	256	256	+
7o	+	+	256	512	512	1024	+	+	1024	+
Culture media	−	−	−	−	−	−	−	−	−	−
Microorganisms	+	+	+	+	+	+	+	+	+	+
Amphotericin B	[a]	[a]	−	−	−	−	−	−	−	−
Gentamicin	−	−	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[*]

[a]Not employed. +: presence of microbial growth; −: absence of microbial growth.

have in their structure an additional α,β-unsaturated bond, all but compound **7l** were active against most of the tested strains.

It is noteworthy that to better understand the relationship between the results observed in the *in vitro* study and the physicochemical properties of synthesized compounds, a structure-activity relationship (SAR) study is necessary.

Conclusions

In this work, twenty-two novel compounds were synthesized: seven new δ-chloro-esters and fifteen new piperine-derived diesters. Their structures were elucidated by infrared spectroscopic techniques, ^{1}H and ^{13}C NMR, COSY, HMBC and HSQC. In the *in silico* study, only compounds **7e** and **7h** violated two of Lipinski's parameters, hence it is inferred that these do not possess good oral bioavailability. The *in vitro* antimicrobial assays showed that the synthesized compounds were not active against the bacterial species tested, displaying only antifungal activity, especially against yeast fungi. The compounds **7a**, **7c** and **7m** were active against 60% of the strains, with an MIC of 256 μg mL^{-1}. The compounds **7m** and **7n** presented an MIC of 1024 μg mL^{-1} against 70% of the tested microorganisms. The compound **7f** showed broad antimicrobial activity, inhibiting 80% of the microorganisms with an MIC of 256 μg mL^{-1}. It is then inferred that these findings are of significant relevance in presenting new molecules with antifungal activity, thus encouraging further research involving these substances against infections of difficult treatment.

Experimental

Chemistry

The piperine (**1**) was extracted from black pepper (*Piper nigrum* L.) according to the methods described by Ikan.[18] The other reagents and solvents used were acquired from Sigma-Aldrich (São Paulo, Brazil) and used without further purification. The structures of the compounds were confirmed by infrared spectra obtained in a FTIR Shimadzu spectrometer, IR Prestige-21 model, with an accessory ATR (attenuated total reflection). The ^{1}H, ^{13}C NMR, and 2D NMR (COSY, HSQC and HMBC) spectra were obtained in a Varian spectrometer, Mercury model (400 and 500 MHz for ^{1}H, and 101 and 126 MHz for ^{13}C), and the fusion range on a heating plate MQAPF-3. For samples solubilization, deuterated dimethyl sulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$) were used. Chemical deviations (δ) were measured in parts *per* million (ppm) and coupling constants (J) in hertz (Hz).

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

In a Soxhlet apparatus, 100 g of black pepper were added with 1000 mL of ethanol 95%. The system was kept in reflux for approximately 10 h. After concentrating the extract in the rotary-evaporator, 100 mL of an alcoholic solution of 10% KOH was added, and then the precipitated material was filtered. A small amount of water was added to the alcoholic solution until the medium became turbid. After 72 h at rest, the formed precipitate was filtered,[18] 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.:[25] 129-130 °C); IR (ATR) ν / cm^{-1} 2939 (C–H_{Ar}), 1631 (C=O), 1581-1442 (C=C_{Ar}), 1249 (C–O–C), 930 (C–H_{Ar}); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (ddd, J 14.7, 8.9, 1.2 Hz, 1H), 6.95 (s, J 1.6 Hz, 1H), 6.86 (dd, J 8.1, 1.7 Hz, 1H), 6.76-6.66 (m, 3H), 6.41 (d, J 14.6 Hz, 1H), 5.94 (s, 2H), 3.60-3.48 (m, 4H), 1.64 (m, 2H, H-15), 1.59-1.53 (m, 4H); ^{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**)

The mixture of 2.20 g (7.72 mmol) of piperine and 22 mL of 20% KOH (ethanolic solution) was subjected to reflux and agitation for 20 h. After the end of reaction, the mixture was filtered, washed with ethanol and dried. The precipitate formed was solubilized in water and acidified with HCl solution 10% down to pH 3. The precipitate formed with a yellowish coloration was filtered, washed with water, dried and recrystallized in ethanol.[18] Yield: 94.5% (1.67 g); MW 218.21 g mol^{-1}; mp 217-218 °C (lit.:[18] 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), 7.23 (s, 1H), 7.03-6.89 (m, 4H), 6.05 (s, 2H), 5.93 (d, J 15.2 Hz, 1H); ^{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 piperate of potassium (**3**)

An ethanolic solution of KOH (10 mmol) was added to a mixture of ethanol and piperic acid (10 mmol). The reaction mixture was constantly agitated at room temperature for 1 h. The solid obtained was filtered and dried. Yield: 93%; 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 acid chlorides (**5a**-**5o**)

In a 50 mL flask coupled with a condenser and drying tube ($CaCl_2$), 2.0 mL of thionyl chloride ($SOCl_2$) were quickly added to 15 mmol of the corresponding carboxylic acid (**4a**-**4o**) and catalytic two drops of dimethylformamide (DMF). The mixture was maintained in reflux and agitation; the reaction time ranged 2-6 h. The excess $SOCl_2$ was then rotary-evaporated and washed with dichloromethane. Thus, it was obtained the acid chlorides (**5a**-**5o**) used *in situ* in the next stage of synthesis.

General procedure for the δ-chloro-esters (**6a**-**6o**)

In a 25 mL flask, 10 mL of THF were added, together with 0.0136 g of $ZnCl_2$ (0.1 mmol) and 10 mmol of the respective acid chlorides (**5a**-**5o**). The mixture was stirred and heated to a temperature of 75 °C and accompanied by thin layer chromatography (TLC). The mixture was subsequently cooled and the solvent rotary-evaporated. The residue was dissolved in dichloromethane (30 mL), transferred to a separation funnel and washed with distilled water (3 × 30 mL). The organic phase was separated and washed with saturated sodium bicarbonate solution (3 × 30 mL), distilled water (3 × 30 mL) and sodium chloride solution (3 × 30 mL), alternating them during the process, until a pH close to 7 was verified, in order to certify the absence of acid or base. The organic phase was treated with anhydrous Na_2SO_4 to eliminate water remnants, and the solvent then rotoevaporated. The product was purified in a chromatographic column using hexane/dichloromethane as eluent.[19]

4-Chlorobutyl acetate (**6a**)

Colorless liquid; yield: 90%; MW 150.60 g mol^{-1}; IR (ATR) ν / cm^{-1} 1735 (C=O), 1234, 1041 (C–O), 1365, 650 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.54 (t, 2H, C$\underline{H}_2$Cl), 2.02 (s, 3H, C$\underline{H}_3$C=O), 2.02 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.1, 63.6, 44.5, 29.2, 26.1, 20.9.

4-Chlorobutyl benzoate (**6b**)

Colorless liquid; yield: 92%; MW 212.68 g mol^{-1}; IR (ATR) ν / cm^{-1} 1716 (C=O), 1271, 1070 (C–O), 1112, 709 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, *J* 8.4, 1.4 Hz, 2H, C$\underline{H}_{Ar}$), 7.55 (t, *J* 7.4 Hz, 1H, C$\underline{H}_{Ar}$), 7.43 (d, 2H, C$\underline{H}_{Ar}$), 4.35 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.60 (t, 2H, C$\underline{H}_2$Cl), 2.00-1.87 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5, 133.0, 130.2, 129.5, 128.4, 64.1, 44.5, 29.3, 26.2.

4-Chlorobutyl 4-chlorobenzoate (**6c**)

Colorless liquid; yield: 85%; MW 247.12 g mol^{-1}; IR (ATR) ν / cm^{-1} 1718 (C=O), 1269, 1014 (C–O), 1116, 759 (C–Cl), 1089 (C_{Ar}–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, *J* 8.8 Hz, 2H, C$\underline{H}_{Ar}$), 7.38 (dd, *J* 8.8 Hz, 2H, C$\underline{H}_{Ar}$), 4.33 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.58 (t, 2H, C$\underline{H}_2$Cl), 2.00-1.78 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 139.4, 130.9, 128.7, 128.7, 64.4, 44.4, 29.2, 26.1.

4-Chlorobutyl 4-bromobenzoate (**6d**)

Colorless liquid; yield: 80%; MW 291.57 g mol^{-1}; IR (ATR) ν / cm^{-1} 1718 (C=O), 1267, 1010 (C–O), 1114, 756 (C–Cl), 1010 (C_{Ar}–Br); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, *J* 8.7 Hz, 2H, C$\underline{H}_{Ar}$), 7.59 (dd, *J* 10.9 Hz, 2H, C$\underline{H}_{Ar}$), 4.34 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.59 (t, 2H, C$\underline{H}_2$Cl), 1.96-1.89 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8, 131.8, 131.1, 129.2, 128.1, 64.4, 44.5, 29.3, 26.2.

4-Chlorobutyl 4-iodobenzoate (**6e**)

Colorless liquid; yield: 74%; MW 338.57 g mol^{-1}; IR (ATR) ν / cm^{-1} 1716 (C=O), 1265, 1101 (C–O), 1114, 752 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* 8.6 Hz, 2H, C$\underline{H}_{Ar}$), 7.73 (d, *J* 8.6 Hz, 2H, C$\underline{H}_{Ar}$), 4.34 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.60 (t, 2H, C$\underline{H}_2$Cl), 2.00-1.84 (m, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 137.8, 131.1, 129.7, 100.8, 64.5, 44.5, 29.3, 26.2.

4-Chlorobutyl 4-methylbenzoate (**6f**)

Colorless liquid; yield: 83%; MW 226.70 g mol^{-1}; IR (ATR) ν / cm^{-1} 1712 (C=O), 1271, 1107 (C–O), 1176, 752 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, *J* 8.1 Hz, 2H, C$\underline{H}_{Ar}$), 7.23 (dd, *J* 7.9 Hz, 2H, C$\underline{H}_{Ar}$), 4.34 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.61 (t, 2H, C$\underline{H}_2$Cl), 2.40 (s, 3H, C$\underline{H}_3C_{Ar}$), 1.94 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 143.7, 129.6, 129.2, 127.5, 64.0, 44.6, 29.4, 26.3, 21.7.

4-Chlorobutyl 4-nitrobenzoate (**6g**)

Yellow liquid; yield: 90%; MW 257.67 g mol^{-1}; IR (ATR) ν / cm^{-1} 1722 (C=O), 1271, 1103 (C–O), 1525, 1348 (N=O), 1116, 717 (C–Cl); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (dd, *J* 9.0 Hz, 2H, C$\underline{H}_{Ar}$), 8.18 (dd, *J* 9.0 Hz, 2H, C$\underline{H}_{Ar}$), 4.40 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.60 (t, 2H, C$\underline{H}_2$Cl), 2.06-1.82 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.6, 150.6, 135.6, 130.7, 123.6, 65.1, 44.4, 29.2, 26.1.

4-Chlorobutyl 3,5-dinitrobenzoate (**6h**)

Pale yellow solid; yield: 92%; MW 302.67 g mol^{-1}; mp 39-40 °C; IR (ATR) ν / cm^{-1} 1724 (C=O), 1271, 1076 (C–O), 1548, 1340 (N=O), 1164, 717 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, *J* 2.2 Hz, 1H, C$\underline{H}_{Ar}$), 9.13 (d, *J* 1.7 Hz, 2H, C$\underline{H}_{Ar}$), 4.50 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.63 (t,

2H, C$\underline{H}_2$Cl), 2.10-1.89 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.5, 148.7, 133.9, 129.4, 122.5, 66.2, 44.3, 29.0, 29.0, 26.0.

4-Chlorobutyl 4-chloro-3-nitrobenzoate (**6i**)

Yellow liquid; yield: 79%; MW 292.12 g mol^{-1}; IR (ATR) ν / cm^{-1} 1722 (C=O), 1280, 1111 (C–O), 1537, 1352 (N=O), 1153, 746 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* 2.0 Hz, 1H, C$\underline{H}_{Ar}$), 8.15 (dd, *J* 8.4, 2.0 Hz, 1H, C$\underline{H}_{Ar}$), 7.64 (d, *J* 8.4 Hz, 1H, C$\underline{H}_{Ar}$), 4.40 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.60 (t, 2H, C$\underline{H}_2$Cl), 2.03-1.83 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.7, 148.0, 133.6, 132.2, 131.7, 130.2, 126.6, 65.4, 44.4, 29.1, 26.1.

4-Chlorobutyl cinnamate (**6j**)

Yellow liquid; yield: 60%; MW 238.71 g mol^{-1}; IR (ATR) ν / cm^{-1} 1706 (C=O), 1309, 1165 (C–O), 1278, 767 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* 16.0 Hz, 1H, C$\underline{H}_{Olefin}$), 7.52 (dd, *J* 5.6, 4.1 Hz, 2H, C$\underline{H}_{Ar}$), 7.43-7.35 (m, 3H, C$\underline{H}_{Ar}$), 6.43 (d, *J* 16.0 Hz, 1H, C$\underline{H}_{Olefin}$), 4.24 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.59 (t, 2H, C$\underline{H}_2$Cl), 1.97-1.82 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 144.9, 134.4, 130.4, 128.9, 128.1, 118.0, 63.7, 44.5, 29.2, 26.2.

4-Chlorobutyl 3-nitro-cinnamate (**6k**)

Yellow solid; yield: 64%; MW 283.71 g mol^{-1}; mp 41-43 °C; IR (ATR) ν / cm^{-1} 1708 (C=O), 1323, 1176 (C–O), 1529, 1350 (N=O), 1205, 744 (C–Cl); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (t, *J* 1.9 Hz, 1H, C$\underline{H}_{Ar}$), 8.18 (dd, *J* 7.8, 1.8 Hz, 1H, C$\underline{H}_{Ar}$), 7.80 (d, *J* 7.7 Hz, 1H, C$\underline{H}_{Ar}$), 7.67 (d, *J* 16.0 Hz, 1H, C$\underline{H}_{Olefin}$), 7.56 (t, *J* 8.0 Hz, 1H, C$\underline{H}_{Ar}$), 6.52 (d, *J* 16.0 Hz, 1H, C$\underline{H}_{Olefin}$), 4.23 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.57 (t, 2H, C$\underline{H}_2$Cl), 1.94-1.81 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.0, 148.6, 141.9, 136.1, 133.6, 130.0, 124.5, 122.4, 121.1, 64.0, 44.5, 29.1, 26.1.

4-Chlorobutyl 4-trifluormethyl-cinnamate (**6l**)

Yellow liquid; yield: 55%; MW 306.71 g mol^{-1}; IR (ATR) ν / cm^{-1} 1714 (C=O), 1166, 1066 (C–O), 1321 (C–F), 1282, 831 (C–Cl); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (d, *J* 16.0 Hz, 1H, C$\underline{H}_{Olefin}$), 7.65-7.57 (m, 4H, C$\underline{H}_{Ar}$), 6.50 (d, *J* 15.5 Hz, 1H, C$\underline{H}_{Olefin}$), 4.25 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.59 (t, 2H, C$\underline{H}_2$Cl), 2.02-1.77 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 143.0, 137.8, 131.8, 128.2, 125.9, 125.0, 122.8, 120.6, 64.0, 44.5, 29.2, 26.2.

4-Chlorobutyl 3-(2-thienyl-acrylate) (**6m**)

Yellow liquid; yield: 60%; MW 244.74 g mol^{-1}; IR (ATR) ν / cm^{-1} 1705 (C=O), 1157, 1043 (C–O), 1201, 704 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* 15.7 Hz, 1H, C$\underline{H}_{Olefin}$), 7.37 (d, *J* 5.1 Hz, 1H, C$\underline{H}_{Ar}$), 7.25 (d, *J* 3.6 Hz, 1H, C$\underline{H}_{Ar}$), 7.05 (dd, *J* 5.1, 3.6 Hz, 1H, C$\underline{H}_{Ar}$), 6.23 (d, *J* 15.7 Hz, 1H, C$\underline{H}_{Olefin}$), 4.22 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.59 (t, 2H, C$\underline{H}_2$Cl), 2.00-1.79 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 139.5, 137.4, 131.0, 128.6, 128.1, 116.7, 63.7, 44.5, 29.2, 26.2.

4-Chlorobutyl 2-(4-methoxyphenyl)-acetate (**6n**)

Orange liquid; yield: 65%; MW 256.73 g mol^{-1}; IR (ATR) ν / cm^{-1} 1732 (C=O), 1246, 1031 (C–O), 1152, 819 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, *J* 8.7 Hz, 2H, C$\underline{H}_{Ar}$), 6.85 (d, *J* 8.7 Hz, 2H, C$\underline{H}_{Ar}$), 4.11 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.78 (s, 3H, C$\underline{H}_3$O), 3.55 (s, 2H, C$\underline{H}_2C_{Ar}$), 3.50 (t, 2H, C$\underline{H}_2$Cl), 1.81-1.74 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 158.7, 130.2, 126.1, 114.0, 63.9, 55.2, 44.4, 29.1, 26.0.

4-Chlorobutyl 2-(1,3-dioxoisoindolinyl)-acetate (**6o**)

Yellow liquid; yield: 67%; MW 295.72 g mol^{-1}; IR (ATR) ν / cm^{-1} 1749, 1718 (C=O), 1193, 1112 (C–O), 1392, 713 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92-7.81 (m, 2H, C$\underline{H}_{Ar}$), 7.78-7.65 (m, 2H, C$\underline{H}_{Ar}$), 4.42 (s, 2H, NC$\underline{H}_2$), 4.18 (t, 2H, OC$\underline{H}_{2Aliph}$), 3.52 (t, 2H, C$\underline{H}_2$Cl), 1.90-1.74 (m, 4H, C$\underline{H}_2$C$\underline{H}_{2Aliph}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 167.3, 134.4, 132.0, 123.7, 66.0, 44.3, 38.9, 28.9, 25.9.

General procedure for new piperine derivatives (**7a**-**7o**)

In a 25 mL flask containing 10 mL of DMF, 2.0 mmol of the δ-chloro-ester (**6a**-**6o**) and 2.0 mmol potassium iodide were added. Next, 2.2 mmol potassium piperate (**3**) were added, and after the salt addition, the reaction mixture was heated to 100 °C and remained under agitation for 24 h. Afterwards, the reaction was cooled, and cold distilled water was added. The precipitate formed was vacuum filtered, washed with water and recrystallized in ethanol.

Butyl 4-acetate-piperate (**7a**)

Pale yellow solid; yield: 70%; MW 332.35 g mol^{-1}; mp 76-78 °C; IR (ATR) ν / cm^{-1} 3057 (C–H_{Ar}), 2958, 2899 (C–H), 1722, 1707 (C=O), 1620, 1490 (C=C_{Ar}), 1251 (O–CH_2–O), 1142, 1035 (C–O), 813 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38 (ddd, *J* 15.2, 8.5, 1.8 Hz, 1H, H-3), 7.24 (d, *J* 1.5 Hz, 1H, H-7), 7.06-6.99 (m, 3H, H-4, H-5, H-11), 6.94 (d, *J* 8.0 Hz, 1H, H-10), 6.06 (s, 2H, H-12), 6.00 (d, *J* 15.2 Hz, 1H, H-2), 4.12 (t, 2H, H-16), 4.04 (t, 2H, H-13), 2.01 (s, 3H, H-18), 1.77-1.61 (m, 4H, H-15, H-14); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.4 (C-17), 166.2 (C-1), 148.2 (C-9), 148.0 (C-8), 145.1 (C-3), 140.5 (C-5), 130.4 (C-6), 124.6 (C-4), 123.2 (C-2), 119.7 (C-11), 108.5 (C-10), 105.7 (C-7), 101.4 (C-12), 63.4 (C-13), 63.3 (C-16), 24.9 (C-14), 24.8 (C-15), 20.7 (C-18).

Butyl 4-benzoate-piperate (**7b**)

Pale yellow solid; yield: 74%; MW 394.42 g mol^{-1}; mp 92-93 °C; IR (ATR) ν / cm^{-1} 3059 (C–H_{Ar}), 2958, 2885 (C–H), 1708, 1697 (C=O), 1604, 1444 (C=C_{Ar}), 1249 (O–CH_2–O), 1132, 1018 (C–O), 702 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (dd, *J* 8.4, 1.3 Hz, 2H, H-19, H-19'), 7.69-7.62 (m, 1H, H-21), 7.53 (t, *J* 7.6 Hz, 2H, H-20, H-20'), 7.36 (ddd, *J* 15.2, 7.3, 3.0 Hz, 1H, H-3), 7.22 (d, *J* 1.6 Hz, 1H, H-7), 7.03-6.96 (m, 3H, H-4, H-5, H-11), 6.93 (d, *J* 8.0 Hz, 1H, H-10), 6.05 (s, 2H, H-12), 6.00 (d, *J* 15.2 Hz, 1H, H-2), 4.32 (t, 2H, H-16), 4.17 (t, 2H, H-13), 1.95-1.68 (m, 4H, H-15, H-14); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.2 (C-1), 165.7 (C-1), 148.2 (C-9), 148.0 (C-8), 145.1 (C-3), 140.5 (C-5), 133.2 (C-21), 130.4 (C-6), 129.8 (C-18), 129.2 (C-19, C-19'), 128.6 (C-20, C-20'), 124.6 (C-4), 123.3 (C-11), 119.8 (C-2), 108.5 (C-10), 105.7 (C-7), 101.4 (C-12), 64.3 (C-16), 63.4 (C-13), 25.0 (C-15), 24.9 (C-14).

Butyl 4-(4-chlorobenzoate)-piperate (**7c**)

Pale yellow solid; yield: 71%; MW 428.87 g mol^{-1}; mp 124-125 °C; IR (ATR) ν / cm^{-1} 3068 (C–H_{Ar}), 2960, 2897 (C–H), 1708, 1697 (C=O), 1595, 1444 (C=C_{Ar}), 1249 (O–CH_2–O), 1134, 1020 (C–O), 1282 (C_{Ar}–Cl), 754 (C–H_{Ar}); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.96 (d, *J* 8.5 Hz, 2H, H-19, H-19'), 7.59 (d, *J* 8.4 Hz, 2H, H-20, H-20'), 7.35 (ddd, *J* 15.3, 8.2, 1.6 Hz, 1H, H-3), 7.21 (s, 1H, H-7), 7.02-6.94 (m, 3H, H-4, H-5, H11), 6.92 (d, *J* 8.0 Hz, 1H, H-10), 6.05 (s, 2H, H-12), 5.99 (d, *J* 15.2 Hz, 1H, H-2), 4.31 (t, 2H, H-16), 4.16 (t, 2H, H-13), 1.83-1.74 (m, 4H, H-15, H-14); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.2 (C-1), 164.9 (C-17), 148.2 (C-9), 147.9 (C-8), 145.1 (C-3), 140.4 (C-5), 138.2 (C-21), 130.9 (C-19, C-19'), 130.4 (C-18), 128.9 (C-20, C-20'), 128.6 (C-6), 124.6 (C-4), 123.2 (C-11), 119.7 (C-2), 108.5 (C-10), 105.7 (C-7), 101.3 (C-12), 65.5 (C-16), 63.4 (C-13), 24.9 (C-15), 24.8 (C-14).

Butyl 4-(4-bromobenzoate)-piperate (**7d**)

Brown solid; yield: 70%; MW 473.32 g mol^{-1}; mp 127-129 °C; IR (ATR) ν / cm^{-1} 3062 (C–H_{Ar}), 2949, 2897 (C–H), 1710, 1697 (C=O), 1604, 1442 (C=C_{Ar}), 1249 (O–CH_2–O), 1117, 1134, 1099 (C–O), 842 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, *J* 8.8 Hz, 2H, H-20, H-20'), 7.74 (d, *J* 8.8 Hz, 2H, H-19, H-19'), 7.36 (ddd, *J* 15.2, 7.5, 2.9 Hz, 1H, H-3), 7.22 (d, *J* 1.6 Hz, 1H, H-7), 7.03-6.97 (m, 3H, H-4, H-5, H-11), 6.93 (d, *J* 8.0 Hz, 1H, H-10), 6.05 (s, 2H, H-12), 5.99 (d, *J* 15.2 Hz, 1H, H-2), 4.31 (t, 2H, H-16), 4.16 (t, 2H, H-13), 1.87-1.65 (m, 4H, H-15, H-14); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.2 (C-1), 165.0 (C-17), 148.2 (C-9), 148.0 (C-8), 145.1 (C-3), 140.5 (C-5), 131.9 (C-19, C-19'), 131.1 (C-20, C-20'), 130.4 (C-6), 128.9 (C-18), 127.3 (C-21), 124.6 (C-4), 123.2 (C-11), 119.7 (C-2), 108.5 (C-10), 105.7 (C-7), 101.4 (C-12), 64.6 (C-16), 63.4 (C-13), 24.9 (C-15), 24.8 (C-14).

Butyl 4-(4-iodobenzoate)-piperate (**7e**)

Brown solid; yield: 64%; MW 520.32 g mol^{-1}; mp 162-163 °C; IR (ATR) ν / cm^{-1} 3066 (C–H_{Ar}), 2958, 2895 (C–H), 1706, 1697 (C=O), 1618, 1444 (C=C_{Ar}), 1249 (O–CH_2–O), 1174, 1134, 1037 (C–O), 748 (C–H_{Ar}); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.91 (d, *J* 8.5 Hz, 2H, H-20, H-20'), 7.71 (d, *J* 8.5 Hz, 2H, H-19, H-19'), 7.35 (ddd, *J* 15.2, 8.1, 2.2 Hz, 1H, H-3), 7.22 (d, *J* 1.6 Hz, 1H, H-7), 7.02-6.96 (m, 3H, H-4, H-5, H-11), 6.92 (d, *J* 8.0 Hz, 1H, H-10), 6.05 (s, 2H, H-12), 5.99 (d, *J* 15.2 Hz, 1H, H-2), 4.30 (t, 2H, H-16), 4.16 (t, 2H, H-13), 1.82-1.73 (m, 4H, H-15, H-14); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.2 (C-1), 165.4 (C-17), 148.2 (C-9), 148.0 (C-8), 145.1 (C-3), 140.5 (C-5), 137.7 (C-20, C-20'), 130.8 (C-19, C-19'), 130.4 (C-6), 129.2 (C-18), 124.6 (C-4), 123.2 (C-11), 119.7 (C-2), 108.5 (C-10), 105.7 (C-7), 101.7 (C-21), 101.4 (C-12), 64.5 (C-16), 63.4 (C-13), 24.9 (C-15), 24.9 (C-14).

Butyl 4-(4-methylbenzoate)-piperate (**7f**)

Pale yellow solid; yield: 76%; MW 408.45 g mol^{-1}; mp 124-125 °C; IR (ATR) ν / cm^{-1} 3068 (C–H_{Ar}), 2964, 2899 (C–H), 1703 (C=O), 1606, 1452 (C=C_{Ar}), 1267 (O–CH_2–O), 1236, 1134, 1014 (C–O), 850 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (d, *J* 8.2 Hz, 2H, H-19, H-19'), 7.40-7.34 (m, 1H, H-3), 7.32 (d, *J* 7.9 Hz, 2H, H-20, H-20'), 7.22 (d, *J* 1.5 Hz, 1H, H-7), 7.07-6.95 (m, 3H, H-4, H-5, H-11), 6.92 (d, *J* 8.0 Hz, 1H, H-10), 6.05 (s, 2H, H-12), 5.99 (d, *J* 15.2 Hz, 1H, H-2), 4.28 (t, 2H, H-16), 4.16 (t, 2H, H-13), 2.36 (s, 3H, H-22), 1.89-1.52 (m, 4H, H-15, H-14); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3 (C-1), 165.7 (C-17), 148.2 (C-9), 148.0 (C-8), 145.1 (C-3), 143.6 (C-21), 140.5 (C-5), 130.4 (C-6), 129.3 (C-19, C-19'), 129.1 (C-20, C-20'), 127.1 (C-18), 124.6 (C-4), 123.2 (C-11), 119.8 (C-2), 108.5 (C-10), 105.6 (C-7), 101.4 (C-12), 64.1 (C-16), 63.4 (C-13), 25.0 (C-15), 25.0 (C-14), 21.1 (C-22).

Butyl 4-(4-nitrobenzoate)-piperate (**7g**)

Yellow solid; yield: 78%; MW 439.42 g mol^{-1}; mp 128-129 °C; IR (ATR) ν / cm^{-1} 3016 (C–H_{Ar}), 2947, 2900 (C–H), 1712, 1695 (C=O), 1523, 1315 (N=O), 1604, 1444 (C=C_{Ar}), 1249 (O–CH_2–O), 1136, 1101, 1020 (C–O), 840 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (d, *J* 9.0 Hz, 2H, H-20, H-20'), 8.19 (d, *J* 9.0 Hz, 2H, H-19, H-19'), 7.35 (ddd, *J* 15.2, 7.7, 2.6 Hz, 1H, H-3), 7.21 (d, *J* 1.6 Hz, 1H, H-7), 7.10-6.95 (m, 3H, H-4, H-5, H-11), 6.92 (d, *J* 8.0 Hz, 1H, H-10), 6.05 (s, 2H, H-12), 5.98 (d,

J 15.2 Hz, 1H, H-2), 4.37 (t, 2H, H-16), 4.17 (t, 2H, H-13), 2.03-1.43 (m, 4H, H-15, H-14); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.2 (C-1), 164.3 (C-17), 150.2 (C-21), 148.2 (C-9), 148.0 (C-8), 145.1 (C-3), 140.5 (C-5), 135.2 (C-18), 130.6 (C-6), 130.4 (C-19, C-19'), 124.6 (C-4), 123.8 (C-20, C-20'), 123.2 (C-11), 119.7 (C-2), 108.5 (C-10), 105.7 (C-7), 101.4 (C-12), 65.2 (C-16), 63.4 (C-13), 24.9 (C-15), 24.8 (C-14).

Butyl 4-(3,5-dinitrobenzoate)-piperate (**7h**)

Yellow solid; yield: 84%; MW 484.42 g mol^{-1}; mp 175-176 °C; IR (ATR) ν / cm^{-1} 3103 (C–H_{Ar}), 2949 (C–H), 1730, 1701 (C=O), 1550, 1342 (N=O), 1618, 1448 (C=C_{Ar}), 1253 (O–CH_2–O), 1139, 1033, 1016 (C–O), 717 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (t, *J* 2.1 Hz, 1H, H-21), 8.91 (d, *J* 2.1 Hz, 2H, H-19, H-19'), 7.33 (dd, *J* 15.3, 9.7 Hz, 1H, H-3), 7.17 (s, 1H, H-7), 7.03-6.93 (m, 3H, H-4, H-5, H-11), 6.90 (d, *J* 8.0 Hz, 1H, H-10), 6.04 (s, 2H, H-12), 5.98 (d, *J* 15.2 Hz, 1H, H-2), 4.46 (t, 2H, H-16), 4.19 (t, 2H, H-13), 1.93-1.85 (m, 2H, H-15), 1.85-1.76 (m, 2H, H-14); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.2 (C-1), 162.5 (C-17), 148.3 (C-9), 148.1 (C-8), 147.9 (C-20, C-20'), 145.0 (C-3), 140.4 (C-5), 132.8 (C-18), 130.4 (C-6), 128.7 (C-19, C-19'), 124.6 (C-4), 123.0 (C-11), 122.4 (C-21), 119.8 (C-2), 108.4 (C-10), 105.7 (C-7), 101.3 (C-12), 65.9 (C-16), 63.3 (C-13), 24.9 (C-14), 24.7 (C-15).

Butyl 4-(4-chloro-3-nitrobenzoate)-piperate (**7i**)

Yellow solid; yield: 66%; MW 473.87 g mol^{-1}; mp 102-104 °C; IR (ATR) ν / cm^{-1} 3070 (C–H_{Ar}), 2958, 2899 (C–H), 1712, 1697 (C=O), 1604, 1313 (N=O), 1240 (C–Cl), 1541, 1447 (C=C_{Ar}), 1251 (O–CH_2–O), 1215, 1132, 1043 (C–O), 806 (C–H_{Ar}); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.52 (d, *J* 2.0 Hz, 1H, H-19), 8.20 (dd, *J* 8.4, 2.1 Hz, 1H, H-23), 7.93 (d, *J* 8.4 Hz, 1H, H-22), 7.34 (ddd, *J* 15.2, 8.7, 1.6 Hz, 1H, H-3), 7.21 (d, *J* 1.5 Hz, 1H, H-7), 7.02-6.95 (m, 3H, H-4, H-5, H-11), 6.92 (d, *J* 8.1 Hz, 1H, H-10), 6.05 (s, 2H, H-12), 5.98 (d, *J* 15.2 Hz, 1H, H-2), 4.36 (t, 2H, H-16), 4.16 (t, 2H, H-13), 1.87-1.69 (m, 4H, H-15, H-14); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.2 (C-1), 163.4 (C-17), 148.2 (C-9), 148.0 (C-8), 147.9 (C-20), 145.1 (C-3), 140.5 (C-5), 133.8 (C-23), 132.4 (C-22), 130.4 (C-21), 130.0 (C-6), 129.8 (C-18), 126.0 (C-19), 124.6 (C-4), 123.2 (C-11), 119.7 (C-2), 108.5 (C-10), 105.7 (C-7), 101.4 (C-12), 65.3 (C-16), 63.4 (C-13), 24.9 (C-14), 24.8 (C-15).

Butyl 4-(cinnamate)-piperate (**7j**)

Brown solid; yield: 60%; MW 420.46 g mol^{-1}; mp 86-88 °C; IR (ATR) ν / cm^{-1} 3034 (C–H_{Ar}), 2960, 2897 (C–H), 1705 (C=O), 1608, 1450 (C=C_{Ar}), 1267 (O–CH_2–O), 1172, 1136 (C–O), 765 (C–H_{Ar}); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.71 (dd, *J* 6.8, 2.9 Hz, 2H, H-21, H-21'), 7.66 (d, *J* 16.1 Hz, 1H, H-19), 7.42 (d, *J* 2.5 Hz, 3H, H-22, H-22', H-23), 7.37 (ddd, *J* 15.2, 8.4, 1.9 Hz, 1H, H-3), 7.22 (d, *J* 1.6 Hz, 1H, H-7), 7.04-6.95 (m, 3H, H-4, H-5, H-11), 6.92 (d, *J* 8.0 Hz, 1H, H-10), 6.63 (d, *J* 16.0 Hz, 1H, H-18), 6.05 (s, 2H, H-12), 6.00 (d, *J* 15.2 Hz, 1H, H-2), 4.19 (t, 2H, H-13), 4.15 (t, 2H, H-16), 1.79-1.69 (m, 4H, H-15, H-16); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.2 (C-1), 166.2 (C-17), 148.2 (C-9), 147.9 (C-8), 145.1 (C-3), 144.4 (C-19), 140.4 (C-5), 133.9 (C-20), 130.4 (C-6), 130.3 (C-23), 128.8 (C-22, C-22'), 128.3 (C-21, C-21'), 124.6 (C-4), 123.2 (C-11), 119.7 (C-2), 118.0 (C-18), 108.5 (C-10), 105.7 (C-7), 101.3 (C-12), 63.6 (C-13), 63.4 (C-16), 24.9 (C-14), 24.9 (C-15).

Butyl 4-(3-nitro-cinnamate)-piperate (**7k**)

Yellow solid; yield: 63%; MW 465.46 g mol^{-1}; mp 102-104 °C; IR (ATR) ν / cm^{-1} 3072 (C–H_{Ar}), 2945, 2902 (C–H), 1705, 1695 (C=O), 1529, 1352 (N=O), 1604, 1446 (C=C_{Ar}), 1253 (O–CH_2–O), 1176, 1026 (C–O), 862 (C–H_{Ar}); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.54 (s, 1H, H-21), 8.23 (d, *J* 8.1 Hz, 1H, H-23), 8.19 (d, *J* 7.4 Hz, 1H, H-25), 7.78 (d, *J* 16.1 Hz, 1H, H-19), 7.69 (t, *J* 8.0 Hz, 1H, H-24), 7.36 (dd, *J* 15.9, 9.2 Hz, 1H, H-3), 7.19 (s, 1H, H-7), 6.98 (m, 3H, H-4, H-5, H-11), 6.91 (d, *J* 8.0 Hz, 1H, H-10), 6.84 (d, *J* 16.1 Hz, 1H, H-18), 6.04 (s, 2H, H-12), 5.99 (d, *J* 15.2 Hz, 1H, H-2), 4.21 (t, 2H, H-16), 4.16 (s, 2H, H-13), 1.84-1.68 (m, 4H, H-15, H-14); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.2 (C-1), 165.8 (C-17), 148.3 (C-22), 148.2 (C-9), 147.9 (C-8), 145.1 (C-3), 142.0 (C-19), 140.5 (C-5), 135.9 (C-20), 134.1 (C-25), 130.3 (C-6), 130.3 (C-24), 124.6 (C-4), 124.5 (C-23), 123.2 (C-11), 123.0 (C-21), 121.0 (C-18), 119.7 (C-2), 108.5 (C-10), 105.7 (C-7), 101.4 (C-12), 63.9 (C-13), 63.4 (C-16), 24.9 (C-14), 24.9 (C-15).

Butyl 4-(4-trifluormethyl-cinnamate)-piperate (**7l**)

Yellow solid; yield: 55%; MW 488.46 g mol^{-1}; mp 123-124 °C; IR (ATR) ν / cm^{-1} 3007 (C–H_{Ar}), 2964, 2893 (C–H), 1714, 1701 (C=O), 1610, 1442 (C=C_{Ar}), 1249 (O–CH_2–O), 1165, 1066 (C–O), 1111 (C–F), 844 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (d, *J* 8.1 Hz, 1H, H-21, H-21'), 7.73 (dd, *J* 15.7, 9.6 Hz, 3H, H-22, H-22', H-19), 7.36 (ddd, *J* 15.3, 7.4, 3.0 Hz, 1H, H-3), 7.21 (d, *J* 1.5 Hz, 1H, H-7), 7.02-6.95 (m, 3H, H-4, H-5, H-11), 6.91 (d, *J* 8.0 Hz, 1H, H-10), 6.78 (d, *J* 16.1 Hz, 1H, H-18), 6.04 (s, 1H, H-12), 6.00 (d, *J* 15.2 Hz, 1H, H-2), 4.21 (t, 2H, H-16), 4.15 (t, 2H, H-13), 1.81-1.62 (m, 4H, H-15, H-14); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3 (C-1), 165.9 (C-17), 148.2 (C-9),

148.0 (C-8), 145.2 (C-3), 142.6 (C-19), 140.5 (C-5), 138.0 (C-20), 130.4 (C-6), 129.9 (C-23), 129.0 (C-21, C-21'), 125.8 (C-22, C-22'), 125.7 (C-22, C-22'), 125.3 (C-24), 124.6 (C-4), 123.3 (C-11), 121.0 (C-18), 119.8 (C-2), 108.5 (C-10), 105.7 (C-7), 101.4 (C-12), 63.9 (C-13), 63.4 (C-16), 25.0 (C-14), 24.9 (C-15).

Butyl 4-(3-(2-thienyl acrylate))-piperate (**7m**)

Yellow solid; yield: 52%; MW 426.46 g mol^{-1}; mp 87-88 °C; IR (ATR) ν / cm^{-1} 3020 (C–H_{Ar}), 2897 (C–H), 1703 (C=O), 1618, 1452 (C=C_{Ar}), 1267 (O–CH_2–O), 1163, 1136 (C–O), 835 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (d, *J* 15.8 Hz, 1H, H-21), 7.71 (d, *J* 5.1 Hz, 1H, H-23), 7.54 (d, *J* 3.5 Hz, 1H, H-19), 7.36 (ddd, *J* 15.2, 7.8, 2.5 Hz, 1H, H-3), 7.21 (d, *J* 1.5 Hz, 1H, H-7), 7.14 (dd, *J* 5.0, 3.6 Hz, 1H, H-22), 7.04-6.95 (m, 3H, H-4, H-5, H-11), 6.91 (d, *J* 8.0 Hz, 1H, H-10), 6.26 (d, *J* 15.8 Hz, 1H, H-18), 6.04 (s, 2H, H-12), 5.99 (d, *J* 15.2 Hz, 1H, H-2), 4.23-4.05 (m, 4H, H-16, H-13), 1.82-1.63 (m, 4H, H-15, H-14); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.7 (C-1), 166.4 (C-17), 148.7 (C-9), 148.4 (C-8), 145.6 (C-3), 140.9 (C-5), 139.1 (C-20), 137.6 (C-19), 132.5 (C-21), 130.8 (C-6), 130.3 (C-23), 129.0 (C-22), 125.1 (C-4), 123.7 (C-11), 120.2 (C-2), 116.6 (C-18), 108.9 (C-10), 106.2 (C-7), 101.8 (C-12), 64.1 (C-13), 63.8 (C-16), 25.4 (C-14), 25.4 (C-15).

Butyl 4-(2-(4-methoxyphenyl)-acetate)-piperate (**7n**)

Yellow-colored viscous liquid; yield: 50%; MW 438.48 g mol^{-1}; IR (ATR) ν / cm^{-1} 3030 (C–H_{Ar}), 2956, 2900 (C–H), 1730, 1705 (C=O), 1608, 1446 (C=C_{Ar}), 1246 (O–CH_2–O), 1168, 1132, 1033 (C–O), 808 (C–H_{Ar}); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.36 (m, 1H, H-3), 7.19 (d, *J* 8.7 Hz, 2H, H-20, H-20'), 6.98 (d, *J* 1.6 Hz, 1H, H-7), 6.92-6.76 (m, 5H, H-5, H-11, H-21, H-21', H-10), 6.69 (dd, *J* 15.5, 10.8 Hz, 1H, H-4), 5.97 (s, 2H, H-12), 5.92 (d, *J* 15.2 Hz, 1H, H-2), 4.28-4.05 (m, 4H, H-13, H-16), 3.77 (s, 3H, H-23), 3.55 (s, 2H, H-18), 1.86-1.61 (m, 4H, H-15, H-14); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9 (C-17), 167.0 (C-1), 158.6 (C-22), 148.5 (C-9), 148.2 (C-8), 144.9 (C-3), 140.3 (C-19), 130.5 (C-6), 130.2 (C-20, C-20'), 126.1 (C-19), 124.4 (C-4), 122.9 (C-11), 120.1 (C-2), 113.9 (C-21, C-21'), 108.5 (C-10), 105.8 (C-7), 101.4 (C-12), 64.3 (C-16), 63.7 (C-13), 55.2 (C-23), 40.4 (C-18), 25.3 (C-15), 25.3 (C-14).

Butyl 4-(2-(1,3-dioxoisoindolinyl)-acetate)-piperate (**7o**)

Brown solid; yield: 58%; MW 477.47 g mol^{-1}; mp 97-99 °C; IR (ATR) ν / cm^{-1} 3014 (C–H_{Ar}), 2947, 2889 (C–H), 1745, 1707 (C=O), 1618, 1492 (C=C_{Ar}), 1255 (O–CH_2–O), 1138, 1031 (C–O), 711 (C–H_{Ar}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94-7.89 (m, 2H, H-22, H-22'), 7.89-7.85 (m, 2H, H-21, H-21'), 7.34 (ddd, *J* 15.3, 8.5, 1.7 Hz, 1H, H-3), 7.20 (d, *J* 1.1 Hz, 1H, H-7), 7.02-6.95 (m, 3H, H-4, H-5, H-11), 6.90 (d, *J* 8.0 Hz, 1H, H-10), 6.02 (s, 2H, H-12), 5.98 (dd, *J* 15.2, 5.9 Hz, 1H, H-2), 4.43 (s, 2H, H-18), 4.14 (t, 2H, H-16), 4.08 (t, 2H, H-13), 1.74-1.49 (m, 4H, H-15, H-14); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7 (C-17), 167.2 (C-1), 166.3 (C-19, C-19'), 148.3 (C-9), 148.1 (C-8), 145.2 (C-3), 140.6 (C-5), 135.0 (C-22, C-22'), 131.4 (C-20, C-20'), 130.5 (C-6), 124.7 (C-4), 123.5 (C-21, C-21'), 123.3 (C-11), 119.8 (C-2), 108.6 (C-10), 105.8 (C-7), 101.5 (C-12), 65.0 (C-16), 63.4 (C-13), 38.9 (C-18), 24.8 (C-15), 24.8 (C-14).

In silico study

The ADME (absorption, distribution, metabolism, and excretion) properties of the final compounds were calculated using the Molinspiration online program,[26] in order to calculate the parameters of Lipinski's rule of five: clogP, MW, HBA, HBD and TPSA. The parameters log S, drug-likeness and drug-score were calculated with the OSIRIS Property Explorer software.[24] The compounds absorption percentage was calculated using the equation: ABS(%) = 109 – 0.345 TPSA.[27]

Antimicrobial activity

Culture media

For maintenance of bacterial and fungal strains, the culture media used were brain heart infusion (BHI) and Sabouraud dextrose agar (SDA) (acquired from Difco Laboratories Ltd., Detroit, USA), respectively. For biological activity assays, BHI liquid nutrient medium was used 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). All media have been prepared according to the descriptions of the manufacturers.

Microorganisms

In the antimicrobial activity assays of the compounds, the following strains were used: bacteria: *Staphylococcus aureus* (American Type Culture Collection (ATCC)-25923), *Pseudomonas aeruginosa* (ATCC-25853); yeasts: *Candida albicans* (ATCC-60193; LM-92), *Candida tropicalis* (ATCC-13803; LM-18); filamentous fungi: *Aspergillus fumigatus* (ATCC-40640; IPP-210), *Aspergillus flavus* (LM-714), *Aspergillus niger* (LM-108). The microorganisms were obtained from the collection of the Mycology Laboratory, Department of

Pharmaceutical Sciences (DCF), Health Sciences Center (CCS) of the Federal University of Paraíba (UFPB), Brazil. The fungal and bacterial strains were maintained at 4 °C in SDA and BHI, respectively. For use in the assays, the fungi and bacteria were harvested in SDA and BHI, respectively, and incubated at 35 ± 2 °C for 24-48 h. The filamentous fungi were harvested in SDA and incubated at room temperature (28 ± 2 °C) for 7-14 days. The microorganism suspension was prepared in sterile saline solution (0.9% NaCl) and compared to the 0.5 McFarland scale tube in order to obtain an inoculum of approximately 10^6 and 10^8 colony forming units (CFU) mL^{-1} for fungi and bacteria, respectively.

Determination of minimum inhibitory concentration (MIC)

The determination of the samples MIC on the selected strains was performed by the broth microdilution method in a 96-wells microplate for cellular culture with U-shaped bottom (TPP, Trasadingen, Switzerland). The tested compounds were weighed and solubilized in DMSO at 5% and Tween-80 at 2%, completing the volume with sterile distilled water for obtaining an emulsion of the products with an initial concentration of 1024 μg mL^{-1}.[28-30] Initially, 100 μL of double-concentrated RPMI/BHI broth was distributed in the wells of the microdilution plates. Then, 100 μL of the substances were dispensed into the wells of the first line of the plate. By means of a serial dilution at a ratio of two, concentrations of 1024 to 64 μg mL^{-1} were obtained. Finally, 10 μL of the bacterial and fungal inoculum was added to the wells, in which each column of the plate referred specifically to a species.

In parallel, the following controls were performed: microorganisms (BHI + bacteria and RPMI + yeasts or filamentous fungi) and culture medium (RPMI/BHI), to assure the viability of the strains and sterility of the medium; and negative control with antibiotics: gentamicin (64 μg mL^{-1}) for bacterial inhibition and amphotericin B (32 μg mL^{-1}) for fungal inhibition. The plates were incubated at 35 ± 2 °C during 24-48 h for the assays with bacteria and yeasts, while the filamentous fungi were incubated at 28 ± 2 °C for 7 days. In the biological assay with bacteria, 20 μL of 0.01% resazurin dye solution (INLAB, São Paulo, Brazil) was added after 24 h of incubation, recognized as a colorimetric indicator of oxide-reduction.[31] The color change of the dye (blue to red) was considered as an indicator of microbial growth, in which the permanence of the blue color means the absence of microbial growth. The MIC for each product was defined as the lowest concentration capable of visually inhibiting microbial growth and/or verified by the permanence of the color of the indicator dye.

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 designated the experiment; Emmely O. Trindade, Thalisson F. Dutra, Maria C. R. Brandão and Bruno F. Lira performed the experiments; Emmely O. Trindade and Maria C. R. Brandão performed the *in silico* study and analyzed the data; Hermes Diniz Neto and Edeltrudes O. Lima performed the antimicrobial study; Emmely O. Trindade, Petrônio F. de Athayde-Filho and José M. Barbosa-Filho wrote the paper.

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