Short Report

Synthesis, *in silico* Study and Antimicrobial Evaluation of New Selenoglycolicamides

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Nine new compounds derived from selenoglycolic acid were synthesized, and their structures were fully characterized by elemental analysis, infrared (IR), ^{1}H and ^{13}C nuclear magnetic resonance (NMR). The compounds were evaluated in an *in silico* study and showed strong to moderate antibacterial activity against several strains of *Staphylococcus aureus*. In particular, three compounds exhibited excellent antibacterial activity, with minimum inhibitory concentrations (MICs) between 16 and 64 μg mL^{-1}. Furthermore, two of the nine compounds showed antifungal activity, with MIC of 1024 and 512 μg mL^{-1}. *In silico* studies of the parameters of Lipinski's rule of five indicate that these compounds have potential to be new drug candidates.

Keywords: selenoglycolic acid, selenoglycolicamides, antibacterial activity, antifungal activity

Introduction

The treatment of bacteria and fungi has become a growing problem that concerns clinicians, the pharmaceutical industry and chemists, due to increased resistance to the available medicines in the market. These micro-organisms, when they become multidrug resistant, are one of the major causes of the increased mortality of the global population.[1]

Antibiotics shut down or subvert essential bacterial functions. The resistance mechanisms appear to exploit every possible strategy of preventing a drug from hitting its target. The major types of clinically relevant resistance mechanisms have been studied for a long time. The efflux phenomenon, found in many species of bacteria, fungi and mammalian cells, is shown to occur through default accumulation within cells and is responsible for exporting drugs from cells over the action of proteins, resulting in low intracellular drug concentrations.[2]

Many of these proteins are associated with treatment failure in nosocomial- and community-acquired infections by *Staphylococcus aureus*.[3] For example, TetK and MsrA are specific transporters that export tetracyclines and macrolides, respectively, and NorA is the most studied and was the first described multidrug and toxin extrusion protein in *S. aureus*,[4,5] which confers resistance to a wide range of compounds, including fluoroquinolones, ethidium bromide, acriflavine, ammonium compounds, chlorhexidine, dequalinium and others.[6]

The active efflux of drugs confers a moderate level of resistance, causing an increase of up to 64 times the minimum inhibitory concentration (MIC) at the expense of increased expression of efflux pumps[7] or through synergism with other forms of resistance.[8,9] Due to this, it has shown promise for improving the effectiveness of antimicrobial agents and its combination with efflux pump inhibitors (EPIs), which, besides expanding the usefulness of existing antibiotics, reduces the emergence of resistant mutant strains.[10,11]

One group of compounds that has been highlighted in the scientific community, both in the synthesis and the development of new therapeutic agents, is composed of organoselenium compounds. These compounds are obtained from reactions with elemental selenium[12] and electrophilic[13,14] or nucleophilic[15] reagents (inorganics

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or organics) containing selenium. The organoselenium compounds reported in the literature are presented as biologically active molecules with antibacterial,[15-17] antifungal,[18-21] anti-inflammatory,[22] antinociceptive,[22] antiparasitic[23-25] and antiviral activities.[26,27]

Considering the potential of organoselenium compounds in the synthesis of new pharmaceutical drug candidates, a series of nine selenoglycolicamides were planned for evaluating an *in silico* study as new structures of drug candidates, obeying the Lipinski's rules. The compounds were synthesized using our selenoglycolic acid synthesis protocol, and these are the early studies of microbiological activity against bacteria (*Staphylococcus aureus*) and fungi (*Candida albicans* and *Candida tropicalis*).

Results and Discussion

Chemistry

The synthesis of the compounds derived from selenoglycolic acid used our previously described procedures[28] with few modifications. The selenoglycolicamides (**7a-i**) were obtained in four synthetic steps, as described in Scheme 1.

The first stage produces α-chloro-*N*-arylacetamides (**2a-i**) by *N*-acetylation reaction with arylamines (**1a-i**) and 2-chloroacetyl chloride, in triethylamine and CH_2Cl_2 as solvent.[29] The second to fourth stages are a one-pot synthesis. The second stage produces sodium hydrogen selenide (**4**) by the powdered gray selenium (**3**) reaction with sodium borohydride in aqueous solution. In the third stage, NaHSe react with aroyl chlorides, forming sodium aroylselenides (**6**). The fourth stage produces

selenoglycolicamides (**7a-i**) with a yield of 70-80% by an aroylselenide (**6**) reaction with α-chloro-*N*-arylacetamides (**2a-i**). The purity of the compounds was checked by examining the melting ranges.

The selenoglycolicamide structures were confirmed using infrared (IR), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopic techniques. The principal characteristic of the IR spectra is the C=O stretch. All of the compounds (**7a-i**) presented two C=O functional group absorptions at 1651-1670 cm^{-1}, corresponding to (–NH)–C=O, and at 1670-1674 cm^{-1}, corresponding to (–Se)–C=O. For all compounds, the N–H stretch of the amide groups was approximately 3270 cm^{-1}. Specifically, for compounds **7b** and **7i**, the nitro group was characterized by two absorption bands in the ranges of 1529-1510 cm^{-1} and 1362-1342 cm^{-1}, respectively. In the ^{1}H NMR spectra for all compounds (**7a-i**), two α-hydrogen atoms (methylene group) had chemical shifts (δ) as singlets in the range of δ 3.77-4.01 ppm. The hydrogen atoms of the amide group had chemical shifts as singlets in the range of δ 8.24-10.91 ppm. The hydrogen atoms of the attached benzene ring had δ between 6.5 and 8.40 ppm. In the **7c** spectra, the ethyl group was characterized by a triplet at δ 1.19 ppm representing the three hydrogen atoms and by a quartet at δ 2.59 ppm for two secondary hydrogen. In the **7d** spectra, the methyl group was characterized by a singlet at δ 2.29 ppm for three primary hydrogens. Specifically, for the **7g** compound, the isopropyl group was characterized by a doublet at δ 1.21 ppm representing the six hydrogen atoms and by a septet at δ 2.68 ppm for the tertiary hydrogen. In the **7h** spectra, the methoxy group was characterized by a singlet at δ 3.77 ppm. For the ^{13}C NMR spectra, the carbon from the C=O corresponding to the
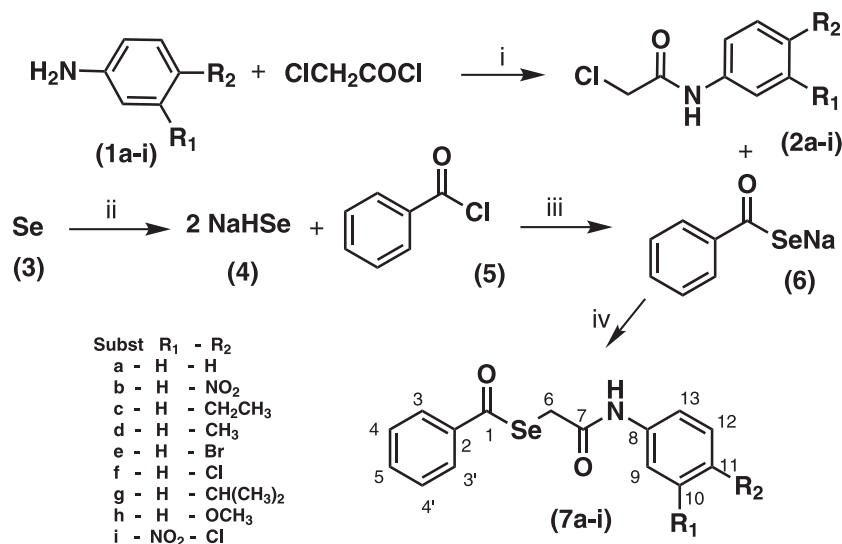
Scheme 1. Synthetic route for the synthesis of the target molecules. Reagents and conditions: (*i*) Et$_3$N, CH$_2$Cl$_2$, 0-rt (room temperature), 20 h, 95-85%; (*ii*) NaBH$_4$, H$_2$O, rt; (*iii*) H$_2$O, rt, 1 h; (*iv*) H$_2$O, rt, 70-80%.

selenol ester group was characterized by δ between 193.0 and 197.6 ppm, and the C=O corresponding to the amide group was characterized by δ between 167 and 168 ppm. The α-carbon was characterized by δ between 28.0 and 29.0 ppm, and all δ attributed to aromatic carbons were characterized by peaks between 117.0 and 156.5 ppm. In the spectra of the compound **7c**, two additional signals in the aliphatic region at δ 28.3 and 15.7 ppm, attributed to carbons of the ethyl group, were observed. In the spectra of the compound **7d**, an additional signal in the aliphatic region at δ 21.0 ppm for the carbon of the methyl group was observed. In the **7g** spectra, two additional signals at δ 33.7 and 24.1 ppm, attributed to the carbons of the isopropyl group, were observed. In the **7h** spectra, an additional signal at δ 55.6 ppm attributed to the carbon of the methoxy group was observed.

In silico study

The process of new drug development requires a great deal of time and resources. The theoretical studies have a fundamental role to minimize these factors because they show indications of potential drug applications. Several authors mention that it is not enough for a compound to present high biological activity and low toxicity to be tested as a drug; it is also necessary to meet the ADME pharmacokinetics parameters (absorption, distribution, metabolism and excretion), which determine the access and the concentration of the compound in the therapeutic target and its subsequent elimination by the organism.[30,31] Many drug candidates can be discarded for presenting unfavorable pharmacokinetics. The ADME parameters can be verified by *in silico* studies based on calculated physico-chemical standards. These standards emphasize lipophilicity, water solubility, molecule size and flexibility.

Prior analysis of these parameters drastically reduces the necessary time for the pharmacokinetic study in the clinical phase. Many studies relating physico-chemical standards with ADME parameters were performed in the 90s. The most widespread study was from the pioneer Lipinski *et al.*,[32] which presented a relationship between pharmacokinetics and physico-chemical parameters, indicating that a molecule will have high potential as drug if it highly resembles existing drugs, a phenomenon known as drug-likeness; the study established standards named "Rules of 5" by Lipinski, which consider only four factors (whose values are multiples of five): molar mass ≤ 500 g mol^{-1}, log P ≤ 5, number of hydrogen bond acceptors ≤ 10 (accounted in function of N or O atoms in the molecule), and number of hydrogen bond donors ≤ 5 (accounted in function of NH or OH groups in the molecule).

In this work, an *in silico* study of selenoglycolicamides **7a-i** was performed to verify the Lipinski parameters using OSIRIS Property Explorer,[33] Molinspiration[34] and Swiss ADME[35] software. The topological polar surface area (TPSA), absorption percentage (%ABS), drug-likeness and drug score were included in this study. The absorption percentage was calculated according to Zhao *et al.*,[36] and is expressed by the equation %ABS = 109 – (0.345 × TPSA). The calculated values for the studies are shown in Table 1.

According to the results obtained from the *in silico* approach, all of the compounds obeyed the Rules of 5 by Lipinski, thus indicating a good oral bioavailability during drug administration. The obtained TPSA values for all compounds were below 140 Å^2, indicating a great permeability of the compound in the cellular plasma membrane. The absorption percentage showed a great absorption of the compounds, as the lowest absorptions for the compounds **7b** and **7i** were 77.26%, and the best

Table 1. *In silico* study of the selenoglycolicamide compounds

Compound	Lipinski parameter					TPSA / Å^2	%ABS / %	log S	Class	Drug likeness	Drug score
	MW	HBD	HBA	milog P	nV						
7a	318.23	1	3	3.23	0	46.17	93.07	–3.53	soluble	–4.23	0.25
7b	363.23	1	6	3.19	0	91.99	77.26	–3.57	soluble	–17.9	0.23
7c	346.28	1	3	4.14	0	46.17	93.07	–4.16	moderately	–2.69	0.19
7d	332.26	1	3	3.68	0	46.17	93.07	–3.82	soluble	–3.88	0.24
7e	397.13	1	3	4.04	0	46.17	93.07	–4.43	moderately	–4.48	0.20
7f	352.67	1	3	3.91	0	46.17	93.07	–4.12	moderately	–1.39	0.26
7g	360.31	1	3	4.74	0	46.17	93.07	–4.37	moderately	–4.84	0.20
7h	348.26	1	4	3.29	0	55.40	89.88	–3.59	soluble	–3.6	0.25
7i	397.67	1	6	3.80	0	91.99	77.26	–4.16	moderately	–11.56	0.19

Physicochemical properties: MW: molecular weight; HBD: hydrogen bonding donor; HBA: hydrogen bonding acceptor; milog P: octanol/water partition coefficient based on Molinspiration milog P model; nV: number of violations; TPSA: total polar surface area; %ABS: adsorption percentage; log S: coefficient of solubility determined by the ESOL method calculated on SwissADME; Class: insoluble < –10 < poor < –6 < moderately < –4 < soluble < –2 < very < 0 < highly.

absorption for the compounds **7a**, **7c**, **7d**, **7e**, **7f** and **7g** was 93.08%, as shown in Table 1.

The log S (coefficient of solubility determined by the ESOL method calculated on SwissADME) of many commercial drugs shows a value greater than –4.00. As shown in Table 1, the log S presented values in the range of –4.43 to –3.53 for compounds **7a-i**. From these results, **7a**, **7b**, **7d** and **7h** compounds are considered soluble, while **7c**, **7e**, **7f**, **7g** and **7i** are considered moderately soluble.

The drug-likeness evaluates the comparison of the investigated compounds through fragments and/or physical properties similar to those of the most known drugs, and they must present positive values. The obtained values in this study are in the range of –17.9 and –1.39. The drug score value combines the records of drug-likeness, lipophilicity, solubility, molecular mass and toxicity risks into a single numerical value, which varies from 0.0 to 1.0 and can be used to predict the global potential of one compound as a new drug candidate. The obtained values from this approach were between 0.12 and 0.26, suggesting that these compounds have potential to become new drug candidates.

Biological study

Antibacterial activity

The selenoglycolicamides **7a**, **7f** and **7i** showed relevant antibacterial activity (MIC ≤ 64 µg mL^{-1}) against the strains *Staphylococcus aureus* IS-58, RN-4220 and SA-1199B, which over-express the effluent pumps TetK, MsrA and NorA, respectively. The other selenoglycolicamide compounds showed MIC values between 128-256 µg mL^{-1}, considered low inhibitory activity (Table 2).

According to Gibbons,[37] compounds (from natural products) with MIC above 100 µg mL^{-1} are considered poorly active antimicrobial agents, while those with MIC

below 10 µg mL^{-1} (ideal ≤ 2 µg mL^{-1}) are considered of great interest to pharmaceutical industries. However, according to Radhakrishna *et al.*,[38] ester derivatives from selenoglycolic acids and their analogues, are considered moderate to good active agents against *Staphylococcus aureus* bacteria with MIC values between 0.1-50 mg mL^{-1} and the organoselenium compounds from ebselen derivatives are considered highly active against *Staphylococcus aureus* with MIC values between 2.0-32 µg mL^{-1}.[39]

In general, for the efflux strains tested, of the nine compounds **7a-i**, only three compounds (**7a**, **7f** and **7i**) were more effective, presenting MIC values ≤ 64 µg mL^{-1}, while the other selenoglycolicamides presented MIC values between 256-128 µg mL^{-1}, considered low inhibitory activity. The **7a** compound presented activity against the strains IS-58 and RN-4220 with an MIC of 32 µg mL^{-1} and in the strain SA-1199B with an MIC of 128 µg mL^{-1}. The **7f** compound presented activity against the strain IS-58, with an MIC of 16 µg mL^{-1}, and the strains RN-4220 and SA-1199B, with an MIC of 32 µg mL^{-1}. The **7i** compound presented an MIC of 32 µg mL^{-1} for the strain IS-58, 64 µg mL^{-1} for the strain RN-4220 and 16 µg mL^{-1} for the strain SA-1199B. Thus, the compounds **7a**, **7f** and **7i** were shown to be better than the standard compound used in the experiment, chloramphenicol. On the other hand, the compounds **7b** and **7h** presented identical activities against the strains IS-58 and RN-4220, with an MIC of 128 µg mL^{-1}. The compounds **7c**, **7d** and **7e** presented an MIC of 256 µg mL^{-1} against three efflux species (IS-58, RN-4220 and SA-1199B). In the ATCC 25923 strain, the compounds showed low activity, with the exception of compound **7a**, which presented activity results identical to chloramphenicol.

According to Zloh and co-workers,[40,41] a characteristic of substances that possess a high degree of lipophilicity is

Table 2. Minimum inhibitory concentrations (MIC) of selenoglycolicamides against strains of *S. aureus*

Selenoglycolicamide	Minimum inhibitory concentration / (µg mL^{-1})			
	Strains (efflux pump)			
	ATCC 25923	IS-58 (TetK)	RN-4220 (MsrA)	SA-1199B (NorA)
7a	128	32	32	128
7b	256	128	128	256
7c	> 256	256	256	256
7d	> 256	256	256	256
7e	> 256	256	256	256
7f	> 256	16	32	32
7g	> 256	128	256	256
7h	256	128	128	256
7i	> 256	32	64	16
Cloramphenicol	128	64	64	64

TetK: tetracycline efflux protein; MsrA: macrolides efflux protein; NorA: fluoroquinolone efflux protein.

the ability to inhibit the multidrug-resistant bacteria (MRB) that have efflux proteins as a defense mechanism. This study showed that the obtained results were not consistent with the Zloh and co-workers[40,41] affirmation, i.e., this rule does not apply to glycolic amides. The compounds **7g**, **7c** and **7e** showed the highest lipophilicity values, and they should demonstrate antimicrobial action results consistent with the Zloh co-workers[40,41] affirmation; however, they were not the most active strains tested. The compounds with halogens in their structure can increase lipophilicity, as was shown by the compounds **7f** and **7i**, both with chlorine, which presented better results against the strains IS-58 (TetK), RN-4220 (MsrA) and SA-1199B (NorA). However, compound **7e**, with bromine, did not show significant antibacterial activity. Although compound **7b** possessed a nitro group, which is a pharmacophoric group capable of promoting an increase in the hydrophobic character of the molecule,[42,43] it did not show antibacterial activity. However, the **7i** compound, which contains a chlorine atom and a nitro group, demonstrated a relevant antibacterial effect. For the compounds **7c**, **7d**, **7g** and **7h** that possess activating groups on the benzene ring, effective antimicrobial action against the tested strains is not observed.

Antifungal activity

The *in vitro* antifungal activity of compounds **7a-i** was evaluated by the microdilution method with four strains of pathogenic fungi, *Candida albicans* (ATCC-76485 and LM-5) and *Candida tropicalis* (ATCC-13803 and LM-96), using nystatin as the standard drug. The compounds were tested at concentrations of 64 to 1024 μg mL^{-1} and solubilized in dimethyl sulfoxide (DMSO, Merck), in a proportion up to 10% to avoid interference with the

microorganisms. The antimicrobial activity of the products was interpreted and considered as active or inactive based on the following criteria: 50-500 μg mL^{-1} = strong/great activity; 600-1500 μg mL^{-1} = moderate activity; and above 1500 μg mL^{-1} = weak activity or inactive product.

Of the nine compounds tested, compounds **7b-7g** did not exhibit inhibitory activity against the strains mentioned in the evaluation. The **7a** compound demonstrated an inhibition of 100% and a moderate activity against the four strains of *Candida* (*C. albicans* ATCC-76485, *C. albicans* LM-5, *C. tropicalis* ATCC-13803 and *C. tropicalis* LM-96), at an MIC of 1024 μg mL^{-1}. However, the **7i** compound presented an inhibition of 50% with a great activity against two of the four strains of *Candida* (*C. tropicalis* ATCC-13803 and *C. tropicalis* LM-96), with an MIC of 512 μg mL^{-1}. The results (Table 3), therefore, were considered between good and moderate in terms of biological activity and taking into consideration the established parameters.[44]

Conclusions

In this short report, we performed an *in silico* study of selenoglycolicamides **7a-i**, all of which obeyed the Rules of 5 by Lipinski, thus indicating a good oral bioavailability during drug administration and a desirable profile as new drug candidates. Based on our *in silico* study, we synthesized nine novel selenoglycolicamides and characterized them using IR, ^{1}H and ^{13}C NMR spectroscopic techniques. All compounds were evaluated *in vitro* against different fungi and bacteria. In the antifungal activity study, only two of the nine compounds showed inhibition against the strains; the **7a** compound demonstrated inhibition against the fungi of the genus *Candida albicans* and *Candida tropicalis*, with

Table 3. Minimum inhibitory concentrations (MIC) of the selenoglycolicamides against strains of *Candida*

Compound	Minimum inhibitory concentration / (μg mL^{-1})			
	Candida strains			
	C. albicans ATCC-76485	*C. albicans* LM-5	*C. tropicalis* ATCC-13803	*C. tropicalis* LM-96
7a	1024	1024	1024	1024
7b	+	+	+	+
7c	+	+	+	+
7d	+	+	+	+
7e	+	+	+	+
7f	+	+	+	+
7g	+	+	+	+
7h	+	+	+	+
7i	+	+	512	512
Yeast control	+	+	+	+
Control of culture medium	−	−	−	−
Nystatin	−	−	−	−

−: there was no growth of the microorganism; +: growth of the microorganism.

an MIC of 1024 µg mL^{-1}, and the **7i** compound presented an inhibition against the fungi of the genus *Candida tropicalis*, with an MIC of 512 µg mL^{-1}. In the antibacterial activity study, of the nine compounds, only **7a**, **7f** and **7i** presented satisfactory antibacterial activity, with an MIC ≤ 64 µg mL^{-1}, being more potent than the standard drug chloramphenicol. Future studies will involve syntheses of new derivatives to carry out studies of the substances as antibiotic-resistant bacterial resistance modulators.

Experimental

Chemistry

All used reagents and solvents were purchased from commercial sources (Sigma-Aldrich) and used without further purification. The progress of the reactions was monitored by thin layer chromatography (TLC) on silica gel plates. The purification of the compounds was performed by recrystallization in ethanol and confirmed by determining the melting range on an MQAPF-302 hotplate (Microquímica). The structures of the new compounds **7a-j** were confirmed by elemental analysis on Carlo Erba EA1110 elemental analyzer. The IR spectra were obtained on a Shimadzu model IR Prestige-21 FTIR spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were obtained on two different machines: a Varian 200 NMR (200 MHz for ^{1}H and 50 MHz for ^{13}C) and Varian 500 NMR (500 MHz for ^{1}H and 126 MHz for ^{13}C). Deuterated chloroform (CDCl$_3$) and deuterated dimethyl sulfoxide (DMSO-d_6) were used as solvent, and tetramethylsilane (TMS) was used for the internal standard. Chemical shifts (δ) were measured in parts *per* million (ppm), and the coupling constants (J), in hertz (Hz).

General procedure for the preparation of α-chloro-*N*-arylacetamide (2a-i)[29]

To the mixture of substituted aromatic amine (0.020 mol) and Et$_3$N (0.024 mol), solubilized in 20 mL of CH$_2$Cl$_2$ at a temperature of 0 °C, maintained by an ice bath, 2-chloroacetyl chloride (0.024 mol) was slowly added. The ice bath was then removed, and the reaction stayed under agitation for 6 h at room temperature. The reaction mixture was monitored by TLC (hexane/methyl acetate 1:1). At the end of reaction, the reaction mixture was concentrated at reduced pressure and cold water was added to the reaction mixture, causing the formation of a solid. The solid was then filtered and washed with cold water (3 × 20 mL), and the final product was purified by recrystallization with an ethanol/water (1:1) mixture.

α-Chloro-*N*-phenylacetamide (2a)

Yield: 93%, mp 133-135 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 4.16 (s, 2H, CH$_2$), 7.15 (t, 1H, H-Ar), 7.33 (t, 2H, H-Ar), 7.52 (d, 2H, H-Ar), 8.28 (s, 1H, NH); ^{13}C NMR (50 MHz, CDCl$_3$) δ 164.0, 136.77, 129.2, 125.35, 120.27, 43.00; IR (KBr) ν / cm^{-1} 3267, 3205, 3143 (NH), 3098, 3049 (CH$_{Ar.}$), 2947, 2862 (CH$_{Alip.}$), 1672 (C=O), 1604, 1496 (C=C$_{Ar.}$), 1290, 1250 (C–Cl), 1078, 858 (CH$_{Ar.}$), 750 (NH), 557, 499 (C–C$_{Ar.}$).

α-Chloro-*N*-(p-nitrophenyl)acetamide (2b)

Yield: 85%, mp 188-190 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.36 (s, 2H, CH$_2$), 7.85 (d, 2H, H-Ar), 8.26 (d, 2H, Ar-H), 10.93 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 165.6, 144.6, 142.6, 125.07, 119.1, 43.6; IR (KBr) ν / cm^{-1} 3277, 3227, 3163 (NH), 3109, 3070 (CH$_{Ar.}$), 2939, 2825 (CH$_{Alip.}$), 1688 (C=O), 1624, 1506 (C=C$_{Ar.}$), 1597, 1570, 1338 (NO$_2$), 1294, 1255 (C–Cl), 1172, 869, 850 (C–N of ArNO$_2$), 1111, 829 (CH$_{Ar.}$), 748 (NH), 526 (C–C$_{Ar.}$).

α-Chloro-*N*-(p-ethylphenyl)acetamide (2c)

Yield: 92%, mp 140-142 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.22 (t, 3H, CH$_3$), 2.63 (q, 2H, CH$_2$), 4.17 (s, 2H, CH$_2$), 7.18 (d, 2H, H-Ar), 7.44 (d, 2H, H-Ar), 8.23 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 163.89, 141.53, 134.37, 128.56, 120.45, 43.0, 28.45, 15.7; IR (KBr) ν / cm^{-1} 3308, 3273, 3201 (NH), 3088, 2965 (CH$_{Ar.}$), 2964, 2868 (CH$_{Alip.}$), 1668 (C=O), 1614, 1512 (C=C$_{Ar.}$), 1292, 1254 (C–Cl), 1118, 864 (CH$_{Ar.}$), 740 (NH), 540, 487 (C–C$_{Ar.}$).

α-Chloro-*N*-(p-methylphenyl)acetamide (2d)

Yield: 95%, mp 182-184 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 2.33 (s, 3H, CH$_3$), 4.17 (s, 2H, CH$_2$), 7.15 (d, 2H, H-Ar), 7.42 (d, 2H, H-Ar), 8.21 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 163.86, 135.1, 134.2, 129.7, 120.3, 42.66, 21.0; IR (KBr) ν / cm^{-1} 3307, 3273, 3203 (NH), 3134, 3089 (CH$_{Ar.}$), 2953 (CH$_{Alip.}$), 1674 (C=O), 1616, 1552 (C=C$_{Ar.}$), 1292, 1252 (C–Cl), 114, 864 (CH$_{Ar.}$), 748 (NH), 505 (C–C$_{Ar.}$).

α-Chloro-*N*-(p-bromophenyl)acetamide (2e)

Yield: 85%, mp 184-186 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.27 (s, 2H, CH$_2$), 7.55 (m, 4H, H-Ar), 10.45 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 164.8, 137.8, 131.7, 121.28, 115.5, 43.56; IR (KBr) ν / cm^{-1} 3263, 3194 (NH), 3124, 3076 (CH$_{Ar.}$), 2999, 2953 (CH$_{Alip.}$), 1670 (C=O), 1610, 1550 (C=C$_{Ar.}$), 1281, 1246 (C–Cl), 1188 (C–Br), 1072, 860 (CH$_{Ar.}$), 736 (NH), 497 (C–C$_{Ar.}$).

α-Chloro-*N*-(p-chlorophenyl)acetamide (2f)

Yield: 79%, mp 170-172 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.27 (s, 2H, CH$_2$), 7.40 (d, 2H, H-Ar), 7.63

(d, 2H, H-Ar), 10.45 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 164.8, 137.4, 128.8, 127.4, 120.9, 43.5; IR (KBr) ν / cm^{-1} 3264, 3198 (NH), 3128, 3080 (CH$_{Ar.}$), 3003, 2951 (CH$_{Alip.}$), 1668 (C=O), 1612, 1551 (C=C$_{Ar.}$), 1281, 1246 (C–Cl), 1095 (C–Cl of ArCl), 1010, 862 (CH$_{Ar.}$), 737 (NH), 567, 501 (C–C$_{Ar.}$).

α-Chloro-*N*-(*p*-isopropylphenyl)acetamide (2g)

Yield: 77%, mp 141-143 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.24 (d, 6H, CH$_3$), 2.90 (s, 1H, CH), 4.17 (s, 2H, CH$_2$), 7.23 (d, 2H, H-Ar), 7.45 (d, 2H, H-Ar), 8.21 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 163.87, 146.19, 134.4, 127.16, 120.47, 43.0, 33.76, 24.1; IR (KBr) ν / cm^{-1} 3271, 3199 (NH), 3130 (CH$_{Ar.}$), 2960, 2870 (CH$_{Alip.}$), 1674 (C=O), 1612, 1548 (C=C$_{Ar.}$), 1282, 1250 (C–Cl), 1300, 1282 (*i*CH$_{Alip.}$), 1016, 837 (CH$_{Ar.}$), 779 (NH), 534 (C–C$_{Ar.}$).

α-Chloro-*N*-(*p*-methoxyphenyl)acetamide (2h)

Yield: 85%, mp 119-120 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.79 (s, 3H, OCH$_3$), 4.17 (s, 2H, CH$_2$), 6.88 (d, 2H, H-Ar), 7.43 (d, 2H, H-Ar), 8.20 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 163.89, 157.16, 129.79, 122.2, 114.3, 55.6, 42.97; IR (KBr) ν / cm^{-1} 3296, 3199 (NH), 3136, 3072 (CH$_{Ar.}$), 2956, 2835 (CH$_{Alip.}$), 1666 (C=O), 1605, 1548 (C=C$_{Ar.}$), 1346, 1301 (C–Cl), 1248, 1113 (C–O–C), 1029, 831 (CH$_{Ar.}$), 788 (NH), 582, 532 (C–C$_{Ar.}$).

α-Chloro-*N*-(*m*-nitro-*p*-chlorophenyl)acetamide (2i)

Yield: 80%, mp 120-122 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.32 (s, 2H, CH$_2$), 7.74 (d, 1H, H-Ar), 7.81 (dd, 1H, H-Ar), 8.40 (d, 1H, H-Ar), 10.87 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 165.5, 147.28, 138.3, 132.2, 124.27, 119.16, 115.76, 43.4; IR (KBr) ν / cm^{-1} 3313, 3269 (NH), 3122, 3094 (CH$_{Ar.}$), 2945, 2881 (CH$_{Alip.}$), 1691 (C=O), 1605, 1544 (C=C$_{Ar.}$), 1483, 1404, 1344 (NO$_2$), 1300, 1265 (C–Cl), 1045, 831 (CH$_{Ar.}$), 895 (C–N of ArNO$_2$), 785 (NH), 559 (C–C$_{Ar.}$).

General procedure for the preparation selenoglycolicamides[28]

Sodium borohydride (0.0139 mol) was dissolved in 6 mL of water and added, slowly, under a selenium suspension (0.0063 mol) in 6 mL of water, contained in a flask of 50 mL. The reaction was exothermic, and a liberation of hydrogen gas was observed. Then (*in situ*), the aroyl chloride (0.0063 mol) was added, slowly, at a temperature of 30 °C. After 60 min of agitation at room temperature, α-chloro-*N*-arylacetamides (0.0063 mol) in a small quantity of acetone were added, slowly, to the solution. The formation of a precipitate was observed at the end of reaction. The mixture was agitated for another 2 h at

room temperature. Then, the reaction mixture was subjected to extraction with dichloromethane (3 × 25 mL). The organic phase was filtered on a porcelain plate containing silica gel and then concentrated under reduced pressure, providing the final products that were recrystallized in ethanol.

N-Phenylbenzoselenoglycolicamide (7a)

Yield: 75%, white solid, mp 132-133 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.36 (s, 1H, NH), 7.92 (d, *J* 7.4 Hz, 2H, H-3 and H-3'), 7.65 (t, *J* 7.3 Hz, 1H, H-5), 7.54-7.45 (m, 4H, H-4, H-4', H-9 and H-13), 7.30 (t, *J* 7.3 Hz, 2H, H-10 and H-12), 7.08 (t, *J* 7.3 Hz, 1H, H-11), 3.78 (s, 2H, H-6); ^{13}C NMR (50 MHz, CDCl$_3$) δ 196.74 (C-1), 137.94 (C-2), 129.16 (C-3 and C-3'), 129.09 (C-4 and C-4'), 134.73 (C-5), 28.27 (C-6), 168.25 (C-7), 138.06 (C-8), 119.91 (C-9 and C-13), 127.38 (C-10 and C-12), 124.44 (C-11); IR (KBr) ν / cm^{-1} 3292 (N–H), 3053 (=C–H, Ar), 1654 (–N–CO), 1674 (–Se–CO), 1596 and 1444 (C=C, Ar). Anal. calcd. for C$_{15}$H$_{13}$NO$_2$Se: C, 56.61; H, 4.12; N, 4.40; found: C, 55.30; H, 3.90; N, 4.84.

N-(4-Nitrophenyl)benzoselenoglycolicamide (7b)

Yield: 70%, white solid, mp 192-193 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.82 (s, 1H, NH), 8.19 (d, *J* 9.2 Hz, 2H, H-10 and H-12), 7.94 (d, *J* 7.1 Hz, 2H, H-3 and H-3'), 7.73-7.66 (m, 3H, H-5 to H-9 and H-13), 7.52 (t, *J* 7.5 Hz, 2H, H-4 and H-4'), 3.80 (s, H, 2H, H-6); ^{13}C NMR (50 MHz, CDCl$_3$) δ 197.59 (C-1), 137.66 (C-2), 129.38 (C-3 and C-3'), 127.7 (C-4 and C-4'), 135.16 (C-5), 28.02 (C-6), 168.83 (C-7), 143.91 (C-8), 119.22 (C-9 and C-13), 125.2 (C-10 and C-12), 143.66 (C-11); IR (KBr) ν / cm^{-1} 3261 (N–H), 3207-3067 (=C–H, Ar), 1670 (–Se–CO and –N–CO overlapping), 1564 and 1404 (C=C, Ar), 1510 and 1342 (NO$_2$), 852 (=C–H, Ar). Anal. calcd. for C$_{15}$H$_{12}$N$_2$O$_4$Se: C, 49.60; H, 3.33; N, 7.71; found: C, 48.68; H, 3.19; N, 7.99.

N-(4-Ethylphenyl)benzoselenoglycolicamide (7c)

Yield: 72%, white solid, mp 124-125 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.30 (s, 1H, NH), 7.91 (d, *J* 7.6 Hz, 2H, H-3 and H-3'), 7.63 (t, *J* 7.4 Hz, 1H, H-5), 7.52-7.66 (m, 4H, H-4, H-4', H-9 and H-13), 7.12 (d, *J* 8.4 Hz, 2H, H-10 and H-12), 3.78 (s, 2H, H-6), 2.59 (q, 2H, C$\underline{H}_2$CH$_3$), 1.19 (t, 3H, CH$_2$C$\underline{H}_3$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 196.62 (C-1), 137.98 (C-2), 129.21 (C-3 and C-3'), 128.37 (C-4 and C-4'), 134.69 (C-5), 28.27 (C-6), 168.11 (C-7), 135.67 (C-8), 120.04 (C-9 and C-13), 127.55 (C-10 and C-12), 140.58 (C-11), 28.43 (CH$_2$), 15.83 (CH$_3$); IR (KBr) ν / cm^{-1} 3284 (N–H), 3068 (=C–H, Ar), 1674 (–Se–CO), 1651 (–N–CO), 1593 and 1413 (C=C, Ar), 883 (=C–H, Ar), 767

(N–H). Anal. calcd. for $C_{17}H_{17}NO_2Se$: C, 58.96; H, 4.95; N, 4.04; found: C, 59.78; H, 4.95; N, 4.90.

N-(4-Methylphenyl)benzoselenoglycolicamide (**7d**)

Yield: 77%, white solid, mp 132-134 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.28 (s, 1H, NH), 7.92 (d, *J* 7.6 Hz, 2H, H-3 and H-3'), 7.65 (t, *J* 7.4 Hz, 1H, H-5), 7.49 (t, *J* 7.4 Hz, 2H, H-4 and H-4'), 7.40 (d, *J* 8.4 Hz, 2H, H-9 and H-13), 7.10 (d, *J* 8.4 Hz, 2H, H-10 and H-12), 3.77 (s, 2H, H-6), 2.29 (s, 3H, C$\underline{H}_3$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 196.64 (C-1), 135.49 (C-2), 129.54 (C-3 and C-3'), 129.22 (C-4 and C-4'), 134.7 (C-5), 28.27 (C-6), 168.1 (C-7), 134.09 (C-8), 119.95 (C-9 and C-13), 127.56 (C-10 and C-12), 137.99 (C-11), 20.99 (CH$_3$); IR (KBr) ν / cm[-1] 3269.34 (N–H), 3037.89 (=C–H, Ar), 1674 (–Se–CO), 1651 (–N–CO), 1595 and 1402 (C=C, Ar), 889 (=C–H, Ar). Anal. calcd. for $C_{16}H_{15}NO_2Se$: C, 57.84; H, 4.55; N, 4.22; found: C, 58.37; H, 4.52; N, 5.01.

N-(4-Bromophenyl)benzoselenoglycolicamide (**7e**)

Yield: 73%, white solid, mp 165-167 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.40 (s, 1H, NH), 7.92 (d, *J* 7.6 Hz, 2H, H-3 and H-3'), 7.67 (t, *J* 6.9 Hz, 1H, H-5), 7.50 (t, *J* 7.5 Hz, 2H, H-4 and H-4'), 7.42 (s, 4H, H-9, H-10, H-12 and H-13), 3.77 (s, 2H, H-6); ^{13}C NMR (50 MHz, CDCl$_3$) δ 197.03 (C-1), 137.15 (C-2), 129.28 (C-3 and C-3'), 127.62 (C-4 and C-4'), 134.9 (C-5), 28.14 (C-6), 168.35 (C-7), 137.83 (C-8), 121.41 (C-9 and C-13), 132.02 (C-10 and C-12), 117.0 (C-11); IR (KBr) ν / cm[-1] 3263 (N–H), 3182-3059 (=C–H, Ar), 1672 (–Se–CO), 1651 (–N–CO), 1537 and 1487 (C=C, Ar), 815 (=C–H, Ar). Anal. calcd. for $C_{15}H_{12}BrNO_2Se$: C, 45.37; H, 3.05; N, 3.53; found: C, 46.08; H, 3.03; N, 4.05.

N-(4-Chlorophenyl)benzoselenoglycolicamide (**7f**)

Yield: 68%, white solid, mp 152-154 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.38 (s, 1H, NH), 7.92 (d, *J* 7.9 Hz, 2H, H-3 and H-3'), 7.66 (t, *J* 7.4 Hz, 1H, H-5), 7.51-7.49 (m, 4H, H-4, H-4', H-9 and H-13), 7.26 (d, 2H, *J* 8.4 Hz, H-10 and H-12), 3.77 (s, 2H, H-6); ^{13}C NMR (50 MHz, CDCl$_3$) δ 196.92 (C-1), 137.93 (C-2), 129.29 (C-3 and C-3'), 129.08 (C-4 and C-4'), 134.86 (C-5), 28.15 (C-6), 168.28 (C-7), 136.7 (C-8), 121.12 (C-9 and C-13), 127.62 (C-10 and C-12), 129.42 (C-11); IR (KBr) ν / cm[-1] 3278 (N–H), 3122-3068 (=C–H, Ar), 1674 (–Se–CO), 1654 (–N–CO), 1544 and 1400 (C=C, Ar), 833 (=C–H, Ar). Anal. calcd. for $C_{15}H_{12}ClNO_2Se$: C, 51.08; H, 3.48; N, 3.97; found: C, 51.98; H, 3.43; N, 4.10.

N-(4-Isopropylphenyl)benzoselenoglycolicamide (**7g**)

Yield: 70%, white solid, mp 108-110 °C; ^{1}H NMR

(200 MHz, CDCl$_3$) δ 8.25 (s, 1H, NH), 7.92 (d, *J* 7.5 Hz, 2H, H-3 and H-3'), 7.64 (t, *J* 7.4 Hz, 1H, H-5), 7.49 (t, *J* 7.8 Hz, 2H, H-4 and H-4'), 7.43 (d, *J* 8.4 Hz, 2H, H-9 and H-13), 7.16 (d, *J* 8.4 Hz, 2H, H-10 and H-12), 3.77 (s, 2H, H-6), 2.86 (sep, 1H, C$\underline{H}$(CH$_3$)$_2$), 1.21 (d, 6H, CH(C$\underline{H}_3$)$_2$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 196.63 (C-1), 138.07 (C-2), 129.23 (C-3 and C-3'), 127.58 (C-4 and C-4'), 134.7 (C-5), 28.29 (C-6), 168.1 (C-7), 135.78 (C-8), 120.1 (C-9 and C-13), 126.96 (C-10 and C-12), 145.26 (C-11), 33.74 ($\underline{C}$H(CH$_3$)$_2$), 24.1 (CH($\underline{C}$H$_3$)$_2$); IR (KBr) ν / cm[-1] 3282 (N–H), 3124-3061 (=C–H, Ar), 1674 (–Se–CO), 1656 (–N–CO), 1600-1446 (C=C, Ar), 889 (=C–H, Ar), 767 (N–H). Anal. calcd. for $C_{18}H_{19}NO_2Se$: C, 60.00; H, 5.32; N, 3.98; found: C, 60.76; H, 5.48; N, 4.55.

N-(4-Methoxyphenyl)benzoselenoglycolicamide (**7h**)

Yield: 75%, white solid, mp 139-140 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.24 (s, 1H, NH), 7.92 (d, *J* 7.1 Hz, 2H, H-3 and H-3'), 7.65 (t, *J* 7.1 Hz, 1H, H-5), 7.53-7.49 (m, 4H, H-4, H-4', H-9 and H-13), 6.48 (d, *J* 9.0 Hz, 2H, H-10 and H-12), 3.77 (s, 5H, H-6 and OC$\underline{H}_3$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 196.37 (C-1), 138.03 (C-2), 129.19 (C-3 and C-3'), 127.53 (C-4 and C-4'), 134.63 (C-5), 29.22 (C-6), 168.0 (C-7), 131.19 (C-8), 121.74 (C-9 and C-13), 114.22 (C-10 and C-12), 156.58 (C-11), 55.57 (O$\underline{C}$H$_3$); IR (KBr) ν / cm[-1] 3282 (N–H), 3124-3061 (=C–H, Ar), 1678 (–Se–CO), 1639 (–N–CO), 1541 (C=C, Ar), 1029 (C–O–C), 883 (=C–H, Ar), 767 (N–H). Anal. calcd. for $C_{16}H_{15}NO_3Se$: C, 55.18; H, 4.34; N, 4.02; found: C, 54.33; H, 4.25; N, 4.44.

N-(4-Chloro-3-nitrophenyl)benzoselenoglycolicamide (**7i**)

Yield: 72%, gray solid, mp 132-134 °C; ^{1}H NMR (200 MHz, CDCl$_3$) δ 10.91 (s, 1H, NH), 8.40 (d, *J* 2.2 Hz, 1H, H-9), 7.90 (d, *J* 7.1 Hz, 2H, H-3 and H-3'), 7.74 (m, 3H, H-5, H-12 and H-13), 7.58 (t, *J* 7.5 Hz, 2H, H-4 and H-4'), 4.01 (s, 2H, H-6); ^{13}C NMR (50 MHz, CDCl$_3$) δ 193.09 (C-1), 138.77 (C-2), 129.46 (C-3 and C-3'), 126.92 (C-4 and C-4'), 134.59 (C-5), 29.02 (C-6), 167.9 (C-7), 137.58 (C-8), 115.43 (C-9), 147.26 (C-10), 118.71 (C-11), 123.97 (C-12), 132.10 (C-13); IR (KBr) ν / cm[-1] 3238 (N–H), 3101-3059 (=C–H, Ar), 1674 (–Se–CO), 1654 (–N–CO), 1595 and 1408 (C=C, Ar), 1529 and 1361 (NO$_2$), 1047 (C–Cl), 835 (=C–H, Ar), 765 (N–H). Anal. calcd. for $C_{15}H_{11}ClN_2O_4Se$: C, 45.30; H, 2.79; N, 7.04; found: C, 45.80; H, 2.79; N, 7.47.

Biological activity

Antibacterial activity

Organoselenium compounds were screened *in vitro* for

antibacterial activity and antimicrobial modulatory effects in three effluxing strains: SA-1199B, which carries the gene encoding the NorA fluoroquinolone efflux protein;[5,45] RN-4220, which has the pUL5054 plasmid that carries the gene encoding the protein MsrA for macrolide efflux;[46] and IS-58, which has the efflux protein for tetracycline (TetK).[47] It was also used a standard strain ATCC 25923, an important strain for the quality test disk diffusion and MIC testing *Staphylococcus* spp., which does not produce beta-lactamase. All of these strains were cultured on nutrient agar slants (blood agar base (BAB), Difco Laboratories). Before being used, the cells were grown in nutrient broth infusion (brain heart infusion (BHI), Difco Laboratories) for 18-24 h at 37 °C. These strains were provided by Dr Simon Gibbons (University of London). The MICs of organoselenium compounds were determined by microdilution assay using a suspension of ca. 10^5 colony forming unit (CFU) mL^{-1} and drug concentration range of 512-4 µg mL^{-1}. MIC is defined as the lowest concentration at which no growth is observed.[48] The results of antibacterial activity are presented in Table 2.

Antifungal activity

In the antifungal assay of the synthesized compounds, the microorganisms used were *Candida albicans* (ATCC-76485 and LM-5) and *Candida tropicalis* (ATCC-13803 and LM-96). They were acquired at the Micoteca of the Mycology Laboratory, Department of Pharmaceutical Sciences (DCF), Health Sciences Center (CCS) at Federal University of Paraíba, Brazil. The fungus strains were maintained in appropriate medium, agar Sabouraud dextrose (ASD, Difco Laboratories), and stored at 4 and 35 °C. The microorganism suspension was prepared according with the 0.5 McFarland scale tube and was adjusted by the use of a spectrophotometer (Leitz-Phtometer 340-800) to 90% T (530 nm), corresponding to approximately 10^6 CFU mL^{-1}.[49-51] The antifungal tests were performed in Sabouraud dextrose broth (SDB, Difco Laboratories), which was prepared and used according the manufacturer's instructions. The MIC value was determined by the microdilution method using 96-well microtiter plates with "U" bottoms and in duplicate. To each plate well, 100 µL of SDB was added in double concentrated liquid medium. Then, 100 µL of the product solution (also double concentrated) was distributed in the wells of the first line of the plate. Using serial dilution (proportion of two), concentrations of 1024 to 64 µg mL^{-1} were obtained so that the higher concentrations were in the first line of the plate and the lower concentrations were in the last. Finally, 10 µL of inoculum was added to the wells, where each column of the plate referred to a specific strain. The same was done in the culture medium with the fungal

drug nystatin (100 µg mL^{-1}). The plates were incubated at 37 °C for 24-48 h. For each strain, the MIC was defined as the minimum concentration capable of inhibiting the fungal growth, by visual observation of the wells, when compared to the control. All of the tests were performed in duplicate, and the results were expressed as the geometric average of the MIC values obtained in the two assays. The antifungal activity results are presented in Table 3.

Supplementary Information

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

Acknowledgments

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