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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of piplartine analogs and preliminary findings on structure–antimicrobial activity relationship

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Abstract In this work it is described the synthesis, characterization and antimicrobial and toxicity evaluation of a series of analogs of piplartine, a piperamide found in *Piper* sp. The compounds structures were confirmed by infrared spectroscopy, ^{1}H, ^{13}C nuclear magnetic resonance, high resolution mass spectroscopy and were evaluated against strains of *Candida* spp., *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Derivative **24** was almost four-fold more potent (IC_{50}: 48.83 µM) and five-fold less toxic (SI > 3) than piplartine (IC_{50}: 189.2 µM; SI: 0.21) against *Candida krusei*, as well as two-fold more potent than fluconazole (IC_{50}: 104.48 µM). This compound was also active against *Candida tropicalis* at 97.67 µM. Benzoyl derivative **17** was three-fold more potent (IC_{50}: 85.2 µM) and more than five-fold less toxic (CC_{50}: 231.71 µM) than piplartine (IC_{50}: 315.33 µM and CC_{50}: 41.14 µM) against *Staphylococcus aureus*. Given these findings, we have found analogs of piplartine which can be assumed as prototypes for the optimization and the development of new antimicrobial (compounds **24** and **17**) agents.

Keywords Piplartine · Analogs · Antifungal activity · Antibacterial activity

Introduction

Fungal and bacterial infections represent a serious problem of public health and, especially among immunocompromised patients related to AIDS, cancer, organ transplants, old age, and others factors. The increase in the cases of microbial resistance to available drugs has contributed to the rise in mortality rates associated with those infections. Thus, these factors justify the need for research and development of new antibacterial and antifungal agents as an alternative to improve the therapeutic arsenal. (Ling et al. 2015; Low and Rotstein 2011).

Natural products are one of the most rational sources of new drugs. They evolved naturally to perform specialized functions in plants and other organisms, so their refined structural backbones often allow the possibility for biological activity or structural modification to improve potency and pharmacokinetics (Chen et al. 2015). Many plant species produce secondary metabolites that are naturally toxic to bacteria, fungi and other parasitic organisms, as a defense response and fight for survival in competitive environments (Chin et al. 2006; Balunas and Kinglorn 2005; Paterson and Anderson 2005). Moreover, edible plant species often contain bioactive compounds, which justify the medicinal effects resulting from observations of their intake (Lampe 2003; Cragg and Newman 2005).

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As members of Piperaceae family, species of the genus *Piper* sp. are widely consumed around the world, especially among those populations that often use peppers in the preparation of traditional menus. Different biological activities have been reported for extracts of these species, their isolated constituents and derivatives thereof, such as anti-inflammatory (Sun et al. 2015), antiplatelet (Park et al. 2008), anti-diabetic (Rao et al. 2012), anti-microbial (Bezerra et al. 2013), anti-parasitary (Moraes et al. 2012; Cotinguiba et al. 2009), antitumor (Bezerra et al. 2006), and insecticide actions (Alécio et al. 1998). For a long time, it has been known that the main bioactive components found in these species, mainly in the roots, are alkaloid amides called piperamides (Okwute and Egharevba 2013). Piplartine, also known as piperlongumine, is an important piperamide found in *Piper* sp., accounting for many of the biological actions associated with the consumption of these species or their extracts.

Piplartine discretely inhibits the phytopathogenic fungus *Pyricularia grisea* (Lee et al. 2001), has antifungal activities against *Cladosporium sphaerospermum* and *C. cladosporioides*, comparable to nystatin and miconazole (Navickiene et al. 2006; Silva et al. 2012) and is also able to inhibit the production of mycotoxins by some species of *Aspergillus* and *Penicillium* in agricultural products (Lee et al. 2001, 2007). An antibacterial study of isolates from *P. longum* showed that piplartine was four times more potent than benzylpenicillin against Gram-positive bacteria *Bacillus subtilis* (Reddy et al. 2011). Another study found that piplartine could also inhibit the growth of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in a concentration-dependent manner (Naika et al. 2010). Some derivatives showed promising activities of a set of piplartine-like piperamides against *S. aureus*, *B. subtilis*, *Aspergillus flavus* and *Candida albicans* (Prashanth et al. 2012). Analogs of piplartine with a 4-alkoxy cinnamic moiety have exhibited submicromolar minimum inhibitory concentrations against *Mycobacterium tuberculosis* (Prithwiraj et al. 2011). More recently, derivatives of piplartine were synthesized and evaluated as antibacterial and cytotoxic agents (Kumar et al. 2013; Adams et al. 2012); those derivatives that possessed substituents such as phenyl, 2-nitrophenyl and 2-thiophenyl at the dihydropyridone ring showed cytostatic effects in bacteria and cancer cell lines. In another recent work, the synthesis of many structural analogs of piplartine was also described and the need for electrophilic sites in these compounds for the maintenance of this biological action was proven by cytotoxicity evaluations (Adams et al. 2012).

In view of these findings and as part of our interest in synthesizing natural analogs for bioactivity and selectivity optimization (Abrão et al. 2015; Souza et al. 2014, 2015), we report herein the preparation of new piplartine-like compounds and their antifungal, antibacterial and cytotoxic activities. (Fig. 1)

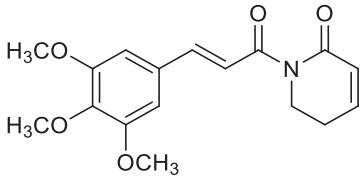


Fig. 1 Chemical structure of piplartine

Materials and methods

Chemistry

Melting point of the compounds was determined on Microquímica MOAs 301 apparatus and was uncorrected. IR spectroscopy was performed on a Shimadzu a FTIR-Affinity-1 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker AC-300 spectrometer (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C spectra) in deuterated chloroform, dimethylsulfoxide or methanol. Chemical shifts (δ) were reported in parts per million (ppm) with reference to tetramethylsilane as internal standard and coupling constants (J) were reported in Hertz (Hz). The following abbreviations were used for the ^{1}H multiplicities: singlet (*s*), doublet (*d*) and multiplet (*m*). High resolution mass spectra (HRMS) were acquired using a LCMS-IT-TOF mass spectrometer and the samples were solubilized in MeOH + 0.1% formic acid, following manual injection. Reaction courses and product mixtures were monitored by thin-layer chromatography (TLC) on commercial silica gel 60 plates. For chromatography, column grade silica gel (0.040–0.063 mm mesh size) was employed. The log*P* values of the compounds were determined by ChemBioDraw Ultra 12.0 program.

General procedure for the preparation of acyl chlorides ***5–8***

To a solution of 2.5 mmol of cinnamic acid in 10 mL of dichloromethane were added 5 mmol of thionyl chloride and 10% mmol of *N,N*-dimethylformamide. After this addition, the mixture was stirred at room temperature until completion of the reaction, as noted by TLC. The crude solution of the acyl chlorides in dichloromethane were used in further steps without prior treatment or purification.

*General procedure for the preparation of hydrazides **9–12***

To a solution of 12.5 mmol of hydrazine hydrate in 10 mL of acetonitrile at 5 °C was added dropwise 2.5 mmol of acid chlorides **5–8**. After this addition, the mixture was allowed to warm up to room temperature until completion of the reaction, as noted by TLC. Then, 50 mL of water were added to the mixture and the product was extracted with ethyl acetate (3 × 25 mL). The organic layer was dried by anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure. The hydrazide derivatives were used without previous purification.

*General procedure for the preparation of phthalimides **13–16***

To a solution of **9–12** (1.5 mmol) in glacial acetic acid (30 mL) was added 1.5 mol of phthalic anhydride and this mixture was heated under reflux for 3 h. The mixture was cooled and poured into iced water and the formed solid was filtered.

(E)-N-(1,3-dioxoisoindolin-2-yl)cinnamamide **(13)** This compound was prepared by 1.5 mmol of **9** and 1.5 mmol of phthalic anhydride, according to the general procedure. The product was obtained in 90% yield as white crystals; mp 181–182 °C; IR ($\bar{\upsilon}$/cm^{-1}): 3376, 2887, 1738, 1677, 1633, 1566, 1496, 1467. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.86–7.79 (4H, m, Ar–H), 7.63(1H, d, olefin, $J^3 = 12.0$ Hz), 7.55–7.53 (2H, m, Ar–H), 7.34–7.32 (3H, Ar–H), 6.68 (1H, d, olefin, $J^3 = 12.0$ Hz), 5.38 (1H, s, N–H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 166.1 (1C, C-9), 165.2 (2C, C-10), 143.5 (1C, C-7); 134.7 (2C, C-13), 134.3 (1C, C-1), 130.1 (2C, C-11), 130.0 (1C, C-4), 128.6 (2C, C-3/C-5), 127.8 (2C, C-2/C-6), 123.3 (2C, C-12), 116.3 (1C, C-8). HRMS-ESI: *m/z* calcd. for $C_{17}H_{12}N_2O_3$ $(M+Na)^+$: 315.0740; found: 315.0564.

(E)-N-(1,3-dioxoisoindolin-2-yl)-3-(4-methoxyphenyl) acrylamide **(14)** This compound was prepared by 1.5 mmol of **10** and 1.5 mmol of phthalic anhydride, according to the general procedure. The product was obtained in 85% yield as white crystals; mp 233–234 °C; IR ($\bar{\upsilon}$/cm^{-1}): 3223, 3008, 1737, 1655, 1623, 1599, 1570, 1520.^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.77 (1H, s, N–H), 8.00–7.92(4H, m, Ar–H), 7.64–7.56 (3H, m, olefin, Ar–H), 7.02 (2H, d, Ar–H, $J^3 =$ 8,8 Hz), 6.66 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.80 (3H, s, methylic). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 165.7 (1C, C-9), 165.0 (2C, C-10), 161.4 (1C, C-4), 142.4 (1C, C-7), 135.6 (2C, C-13); 130.1 (2C, C-11), 129.9 (2C, C-2/C-6), 127.1 (1C, C-1), 124.1 (2C, C-12), 115.4 (1C, C-8), 114.9 (2C, C-3/C-5), 55.7 (1C, O$\underline{C}H_3$). HRMS-ESI: *m/z* calcd. for $C_{18}H_{14}N_2O_4$ $(M+Na)^+$: 345.0846; found: 345.0678.

(E)-3-(3,4-dimethoxyphenyl)-N-(1,3-dioxoisoindolin-2-yl)acrylamide **(15)** This compound was prepared by 1.5 mmol of **11** and 1.5 mmol of phthalic anhydride, according to the general procedure. The product was obtained in 70% yield as white crystals; mp 189–190 °C; IR ($\bar{\upsilon}$/cm^{-1}): 3213, 2980, 1739, 1654, 1619, 1596, 1581, 1512, 1467. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.73 (1H, s, N-H), 7.99–7.93 (4H, m, Ar–H), 7.57 (1H, d, olefin, $J^3 = 12.0$ Hz), 7.27–7.22 (2H, m, Ar–H), 7.02 (1H, d, Ar–H, $J^5 = 6{,}2$ Hz), 6.69 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.81 (3H, s, methylic), 3.80 (3H, s, methylic). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 165.3 (1C, C-9), 164.6 (2C, C-10), 150.8 (1C, C-3), 148.9 (1C, C-4), 142.3 (1C, C-7), 135.2 (2C, C-13), 129.5 (2C, C-11), 126.9 (1C, C-1), 123.7 (2C, C-12), 122.1 (1C, C-6), 115.2 (1C, C-8), 111.7 (1C, C-5), 110.4 (1C, C-2), 55.5 (1C, O$\underline{C}H_3$), 55.4 (1C, O$\underline{C}H_3$). HRMS-ESI: *m/z* calcd. for $C_{19}H_{16}N_2O_5$ $(M+H)^+$: 353.1132; found: 353.0984.

(E)-N-(1,3-dioxoisoindolin-2-yl)-3-(3,4,5-trimethoxyphenyl)acrylamide **(16)** This compound was prepared by 1.5 mmol of **12** and 1.5 mmol of phthalic anhydride, according to the general procedure. The product was obtained in 85% yield as white crystals; mp >230 °C; IR ($\bar{\upsilon}$/cm^{-1}): 3187, 2979, 1791, 1682, 1651, 1624, 1588. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.78 (1H, s, N–H), 8.00–7.93(4H, m, Ar–H), 7.58 (1H, d, olefin, $J^3 = 12.0$ Hz), 7.02 (2H, s, Ar–H), 6.78 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.83 (6H, s, methylic), 3.71 (3H, s, methylic). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 165.6 (1C, C-9), 164.8 (2C, C-10), 153.5 (2C, C-3/C-5), 142.3 (1C, C-7), 139.7 (1C, C-4), 135.6 (2C, C-13), 130.2 (1C, C-1), 129.9 (2C, C-11), 124.1 (2C, C-12), 117.5 (1C, C-8), 105.9 (2C, C-2/C-6), 60.5 (1C, O$\underline{C}H_3$), 56.3 (2C, O$\underline{C}H_3$). HRMS-ESI: *m/z* calcd. for $C_{20}H_{18}N_2O_6$ $(M+Na)^+$: 405.1057; found: 405.0894.

*General procedure for the preparation of benzoylhydrazides **17–20***

To a solution of **9–12** (1.5 mmol) in 5 mL of pyridine at 5 °C was added dropwise 1.5 mmol of benzoyl chloride and after addition, the mixture was allowed to warm up to room temperature until completion of the reaction, as reported by TLC. Then 1 M HCl was added to the mixture until pH 1 and the precipitate formed was filtered affording the pure products.

(E)-N′-cinnamoylbenzohydrazide **(17)** This compound was prepared by 1.5 mmol of **9** and 1.5 mmol of benzoyl chloride, according to the general procedure. The product was obtained in 94% yield as white crystals; mp 210–211 °C; IR ($\bar{\upsilon}$/cm^{-1}): 3290, 3213, 3023, 1692, 1641, 1630, 1601, 1587, 1511, 1487. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.51 (1H, d, N–H, $J^3 = 1.5$ Hz), 10.21 (1H, d, N–H, $J^3 = 1.5$ Hz), 7.92–7.89(2H, m, Ar–H), 7.64–7.42 (9H, m, Ar–H, olefin), 6.75 (1H, d, olefin, $J^3 = 12.0$ Hz). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 165.4 (1C, C-9), 164.4 (1C, C-10), 140.3 (1C, C-7), 134.5 (1C, C-1), 132.4 (1C, C-14), 131.8

(1C, C-11), 129.8 (1C, C-4), 129.0 (2C, C-3/C-5), 128.5 (2C, C-2/C-6), 127.7 (2C, C-13), 127.4 (2C, C-12), 119.4 (1C, C-8). HRMS-ESI: *m/z* calcd. for $C_{16}H_{14}N_2O_2$ $(M+Na)^+$: 289.0947; found: 289.0750.

(E)-N′-(3-(4-methoxyphenyl)acryloyl)benzohydrazide **(18)** This compound was prepared by 1.5 mmol of **10** and 1.5 mmol of benzoyl chloride, according to the general procedure. The product was obtained in 86% yield as white crystals; mp 182–183 °C; IR ($\bar{\upsilon}/cm^{-1}$): 3203, 2999, 1693, 1642, 1627, 1601, 1577, 1508, 1479. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.47 (1H, d, N–H, $J^3 = 1.5$ Hz), 10.10 (1H, d, N–H, $J^3 = 1.5$ Hz), 7.90 (2H, d, Ar–H, $J^3 = 8.5$ Hz), 7.59–7.50 (6H, m, Ar–H, olefin), 7.00 (2H, d, Ar–H, $J^3 = 8.5$ Hz), 6.60 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.80 (3H, s, methylic). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 165.4 (1C, C-9), 164.7 (1C, C-10), 160.6 (1C, C-4), 140.0 (1C, C-7), 132.4 (1C, C-14); 131.8 (1C, C-11), 129.3 (2C, C-2/C-6), 128.4 (2C, C-13), 127.4 (2C, C-12), 127.1 (1C, C-1), 116.9 (1C, C-8), 114.4 (2C, C-3/C-5), 55.3 (1C, O$\underline{C}H_3$). HRMS-ESI: *m/z* calcd. for $C_{17}H_{16}N_2O_3$ $(M+Na)^+$: 319.1053; found: 319.0862.

(E)-N′-(3-(3,4-dimethoxyphenyl)acryloyl)benzohydrazide **(19)** This compound was prepared by 1.5 mmol of **11** and 1.5 mmol of benzoyl chloride, according to the general procedure. The product was obtained in 82% yield as white crystals; mp 188–189 °C; IR ($\bar{\upsilon}/cm^{-1}$): 3292, 3188, 1667, 1642, 1622, 1564, 1513, 1467. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.48 (1H, d, N–H, $J^3 = 1.5$ Hz), 10.06 (1H, d, N–H, $J^3 = 1.5$ Hz), 7.92–7.88 (1H, m, Ar–H), 7.58–7.45 (3H, m, Ar–H, olefin), 7.22–7.17 (2H, m, Ar–H), 7.02–6.98 (1H, m, Ar–H), 6.64 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.81 (3H, s, methylic), 3.79 (3H, s, methylic). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 165.7 (1C, C-9), 164.8 (1C, C-10), 150.4 (1C, C-5), 148.9 (1C, C-4), 140.4 (1C, C-7), 140.1 (1C, C-1), 132.5 (1C, C-14), 131.8 (1C, C-11), 128.5 (2C, C-13), 127.4 (2C, C-12), 121.6 (1C, C-2), 117.1 (1C, C-8), 111.8 (2C, C-3), 110.3 (1C, C-6), 55.6 (1C, O$\underline{C}H_3$), 55.5 (1C, O$\underline{C}H_3$). HRMS-ESI: *m/z* calcd. for $C_{18}H_{18}N_2O_4$ $(M+Na)^+$: 349.1159; found: 349.1004.

(E)-N′-(3-(3,4,5-trimethoxyphenyl)acryloyl)benzohydrazide **(20)** This compound was prepared by 1.5 mmol of **12** and 1.5 mmol of benzoyl chloride, according to the general procedure. The product was obtained in 87% yield as white crystals; mp 183–184 °C; IR ($\bar{\upsilon}/cm^{-1}$): 3445, 3326, 3069, 2998, 1665, 1644, 1641, 1602, 1581, 1478. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.50 (1H, d, N–H, $J^3 = 1.5$ Hz), 10.10 (1H, d, N–H, $J^3 = 1.5$ Hz), 7.92–7.89 (2H, m, Ar–H), 7.54–7.49 (4H, m, Ar–H, olefin), 6.69 (2H, s, Ar–H), 6.60 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.83 (6H, s, methylic), 3.69 (3H, s, methylic). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 165.3 (1C, C-9), 164.5 (1C, C-10), 153.1 (2C, C-3/C-5), 140.4 (1C, C-7), 138.9 (1C, C-4); 132.4 (1C, C-14), 131.8 (1C, C-11), 130.2 (1C, C-1), 128.4 (2C, C-13), 127.4 (2C, C-12), 118.8 (1C, C-8), 105.2 (2C, C-2/C-6), 60.1 (1C, O$\underline{C}H_3$), 55.9 (2C, O$\underline{C}H_3$). HRMS-ESI: *m/z* calcd. for $C_{19}H_{20}N_2O_5$ $(M+Na)^+$: 379.1264; found: 379.1096.

*General procedure for the preparation of amides **21–24***

To a solution of 5.6 mmol of morpholine in 10 mL of dichloromethane at 5 °C was added dropwise 1.4 mmol of acid chlorides **5–8** and after the addition, the mixture was allowed to warm up to room temperature until completion of the reaction, as noticed by TLC. Then, 50 mL de water were added to the mixture and the product was extracted with dichloromethane (3 × 25 mL). The organic layer was dried by anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure, affording the pure amides.

(E)-1-morpholino-3-phenylprop-2-en-1-one **(21)** This compound was prepared by 1.4 mmol of **5** and 5.6 mmol of morpholine, according to the general procedure. The product was obtained in 72% yield as white crystals; mp 90–91 °C; IR ($\bar{\upsilon}/cm^{-1}$): 3080, 3026, 2964, 2922, 2856, 1644, 1595, 1577, 1494. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.69 (1H, d, olefin, $J^3 = 12.0$ Hz), 7.53–7.33(5H, m, Ar–H), 6.84 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.71 (8H, s, methylenic). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 165.5 (1C, C-9), 143.1 (1C, C-7), 135.0 (1C, C-1), 129.7 (1C, C-4), 128.7 (2C, C-3/C-5), 127.7 (2C, C-2/C-6), 116.5 (1C, C-8), 66.8 (4C, C-10, C-11). HRMS-ESI: *m/z* calcd. for $C_{13}H_{15}NO_2$ $(M+Na)^+$: 240.0995 found: 240.0846.

(E)-3-(4-methoxyphenyl)-1-morpholinoprop-2-en-1-one **(22)** This compound was prepared by 1.4 mmol of **6** and 5.6 mmol of morpholine, according to the general procedure. The product was obtained in 75% yield as white crystals; mp 105–106 °C; IR ($\bar{\upsilon}/cm^{-1}$): 2963, 2909, 2860, 1644, 1593, 1573, 1510. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.65 (1H, d, olefin, $J^3 = 12.0$ Hz), 7.46 (2H, dd, Ar–H, $J^3 = 8{,}50$ $J^4 = 4{,}7$ Hz), 6.88 (2H, dd, Ar–H, $J^3 = 8{,}50$ $J^4 = 4{,}7$ Hz), 6.70 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.82 (3H, s, methylic), 3.70 (8H, s, methylenic). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 165.8 (1C, C-9), 160.9 (1C, C-4), 142.9 (1C, C-7), 129.3 (2C, C-2/C-6), 127.8 (1C, C-1), 114.2 (2C, C-3/C-5), 113.9 (1C, C-8), 66.8 (4C, C-10, C-11), 55.3 (1C, O$\underline{C}H_3$),. HRMS-ESI: *m/z* calcd. for $C_{14}H_{17}NO_3$ $(M+Na)^+$: 270.1101 found: 270.0921.

(E)-3-(3,4-dimethoxyphenyl)-1-morpholinoprop-2-en-1-one **(23)** This compound was prepared by 1.4 mmol of **7** and 5.6 mmol of morpholine, according to the general procedure. The product was obtained in 72% yield as white crystals; mp 120–121 °C; IR ($\bar{\upsilon}/cm^{-1}$): 2980, 2937, 2857, 1643, 1591, 1514, 1455. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.63 (1H, d, olefin, $J^3 = 12.0$Hz), 7.10 (1H, dd, Ar–H, $J^3 = 8{,}50$ Hz, $J^4 = 2{,}0$ Hz), 7.02 (1H, d, Ar–H, $J^4 = 2{,}0$ Hz), 6.84 (1H, d, Ar–H, $J^3 = 8.5$ Hz), 6.68 (1H, d, olefin, $J^3 = 12.0$

Hz), 3.90 (6H, s, methylic), 3.70 (8H, s, methylenic). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 165.7 (1C, C-9), 150.6 (1C, C-3), 149.1 (1C, C-4), 143.2 (1C, C-7), 128.0 (1C, C-1), 121.8 (1C, C-6), 114.1 (1C, C-8), 111.0 (1C, C-5), 109.8 (1C, C-2), 66.8 (4C, C-10, C-11), 55.9 (2C, O$\underline{C}H_3$). HRMS-ESI: *m/z* calcd. for $C_{15}H_{19}NO_4$ $(M+H)^+$: 278.1387 found: 278.1215.

(E)-1-morpholino-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **(24)** This compound was prepared by 1.4 mmol of **8** and 5.6 mmol of morpholine, according to the general procedure. The product was obtained in 86% yield as white crystals; mp 127–128 °C; IR ($\bar{\upsilon}$/cm^{-1}): 2951, 2896, 2846, 1640, 1580, 1504, 1498. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.59 (1H, d, olefin, $J^3 = 12.0$ Hz), 6.72–6.67 (3H, m, Ar–H, olefin), 3.87 (9H, s, methylic), 3.70 (8H, s, methylenic). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 165.5 (1C, C-9), 153.4 (2C, C-3/C-5), 143.3 (1C, C-7), 139.7 (1C, C-4), 130.6 (1C, C-1), 115.6 (1C, C-8), 105.0 (2C, C-2, C-6), 66.8 (4C, C-10, C-11), 60.9 (1C, O$\underline{C}H_3$), 56.1 (2C, O$\underline{C}H_3$). HRMS-ESI: *m/z* calcd. for $C_{16}H_{21}NO_5$ $(M+H)^+$: 308.1492 found: 308.1294.

General procedure for the preparation of derivatives N-*hydroxysuccinimides* ***25–28***

To a solution of 1.4 mmol of **5–8** in 10 mL of dichloromethane were added 2.1 mmol of *N*-hydroxysuccinimide and 2.1 mmol of pyridine. The mixture was stirred at room temperature until completion of the reaction, noticed by TLC. Then 2 M HCl was added to the mixture until pH 1 and the product was extracted with ethyl acetate (3 × 25 mL). The organic layer was washed with NaOH 10% (3 × 25 mL), dried with anhydrous sodium sulfate, filtered and removed under reduced pressure affording the pure acyl derivatives after purified by recrystallization from ethanol/water solution 80:20.

(E)-2,5-dioxopyrrolidin-1-yl cinnamate **(25)** This compound was prepared by 1.4 mmol of **5** and 2.8 mmol of *N*-hydroxysuccinimide, according to the general procedure. The product was obtained in 83% yield as white crystals after purified by recrystallization from ethanol/water solution 80:20; mp 177–178 °C; IR ($\bar{\upsilon}$/cm^{-1}): 3065, 2943, 1755, 1723, 1625, 1574. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.92 (1H, d, olefin, $J^3 = 12.0$ Hz), 7.58–7.25(5H, m, Ar–H), 6.68 (1H, d, olefin, $J^3 = 12.0$ Hz), 2.88 (4H, s, methylenic). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 169.2 (1C, C-9), 162.0 (2C, C-10), 150.0 (1C, C-7), 133.4 (1C, C-1), 131.5 (1C, C-4), 129.0 (2C, C-3/C-5), 128.6 (2C, C-2/C-6), 111.5 (1C, C-8), 25.6 (2C, C-11). HRMS-ESI: *m/z* calcd. for $C_{13}H_{11}NO_4$ $(M+Na)^+$: 268.0580 found: 268.0433.

(E)-2,5-dioxopyrrolidin-1-yl 3-(4-methoxyphenyl)acrylate **(26)** This compound was prepared by 1.4 mmol of **6** and 2.8 mmol of *N*-hydroxysuccinimide, according to the general procedure. The product was obtained in 75% yield as white crystals after purified by recrystallization from ethanol/water solution 80:20; mp 148–149 °C; IR ($\bar{\upsilon}$/cm^{-1}): 3061, 2937, 1757, 1725, 1623, 1595. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.65 (1H, d, olefin, $J^3 = 12.0$ Hz), 7.51 (2H, d, Ar–H, $J^3 = 8{,}50$ Hz), 6.91 (2H, d, Ar–H, $J^3 = 8{,}50$ Hz), 6.43 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.84 (3H, s, methylic), 2.86 (4H, s, methylenic). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 169.4 (1C, C-9), 162.4 (2C, C-10), 162.3 (1C, C-4), 149.7 (1C, C-7), 130.5 (2C, C-2/C-6), 126.2 (1C, C-1), 114.5 (2C, C-3/C-5), 108.5 (1C, C-8), 55.4 (1C, O$\underline{C}H_3$), 25.5 (2C, C-11). HRMS-ESI: *m/z* calcd. for $C_{14}H_{13}NO_5$ $(M+Na)^+$: 298.0686 found: 298.0526.

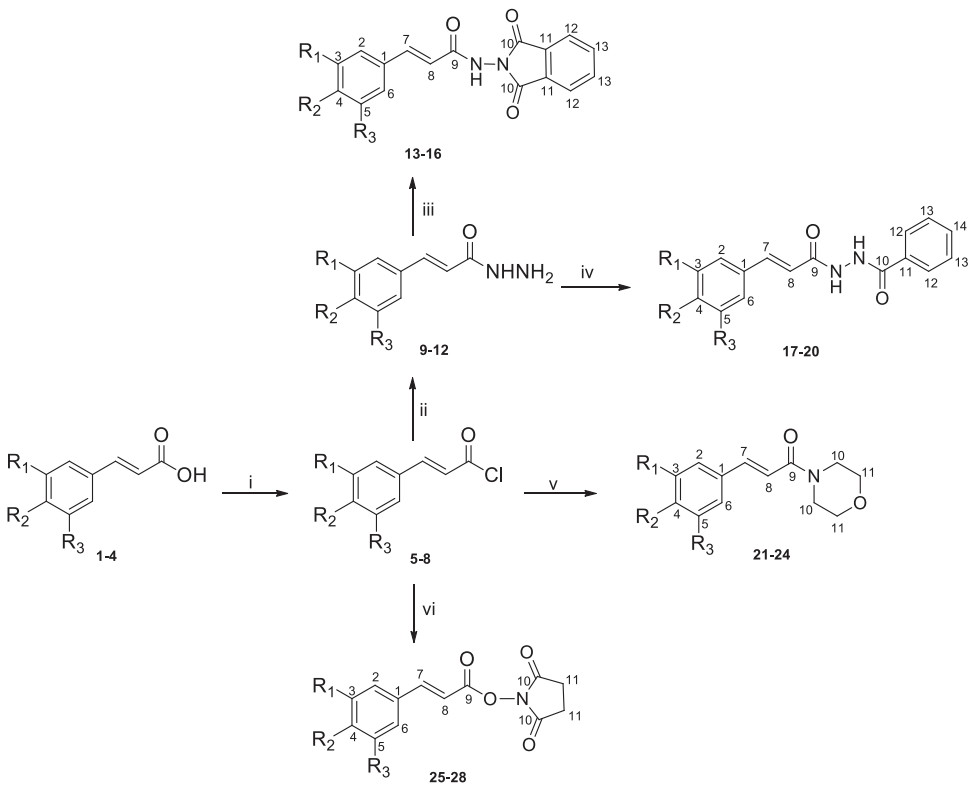
(E)-2,5-dioxopyrrolidin-1-yl 3-(3,4-dimethoxyphenyl) acrylate **(27)** This compound was prepared by 1.4 mmol of **7** and 2.8 mmol of *N*-hydroxysuccinimide, according to the general procedure. The product was obtained in 88% yield as white crystals after purified by recrystallization from ethanol/water solution 80:20; mp 149–150 °C; IR ($\bar{\upsilon}$/cm^{-1}): 3074, 2967, 1759, 1728, 1621, 1598. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.84 (1H, d, olefin, $J^3 = 12.0$ Hz), 7.15 (1H, d, Ar–H, $J^3 = 8{,}50$ Hz), 7.06 (1H, s, Ar–H), 6.88 (1H, d, Ar–H, $J^3 = 8.5$ Hz), 6.43 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.91 (6H, s, methylic), 2.86 (4H, s, methylenic). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 169.4 (1C, C-9), 162.2 (2C, C-10), 152.2 (1C, C-5), 149.9 (1C, C-7), 149.3 (1C, C-4), 126.4 (1C, C-1), 123.8 (1C, C-2), 111.3 (1C, C-8), 109.8 (1C, C-3), 108.8 (1C, C-6), 55.9 (2C, O$\underline{C}H_3$), 25.6 (2C, C-11). HRMS-ESI: *m/z* calcd. for $C_{15}H_{15}NO_6$ $(M+Na)^+$ 328.0792 found: 328.0600.

(E)-2,5-dioxopyrrolidin-1-yl 3-(3,4,5-trimethoxyphenyl) acrylate **(28)** This compound was prepared by 1.4 mmol of **8** and 2.8 mmol of *N*-hydroxysuccinimide, according to the general procedure. The product was obtained in 70% yield as white crystals after purified by recrystallization from aqueous ethanol (80% v/v); mp 176–177 °C; IR ($\bar{\upsilon}$/cm^{-1}): 3009, 2940, 1758, 1728, 1625, 1578. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.82 (1H, d, olefin, $J^3 = 12.0$ Hz), 6.78 (2H, s, Ar–H), 6.48 (1H, d, olefin, $J^3 = 12.0$ Hz), 3.89 (9H, s, methylic), 2.86 (4H, s, methylenic). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 169.3 (1C, C-9), 162.0 (2C, C-10), 153.4 (2C, C-3/C-5), 149.9 (1C, C-7), 141.2 (1C, C-4), 128.8 (1C, C-1), 110.5 (1C, C-8), 105.8 (2C, C-2/C-6), 60.9 (1C, O$\underline{C}H_3$), 56.1 (2C, O$\underline{C}H_3$), 25.6 (2C, C-11). HRMS-ESI: *m/z* calcd. for $C_{16}H_{17}NO_7$ $(M+Na)^+$: 358.0897 found: 358.0721.

In vitro bioassays

Antibacterial and antifungal activities evaluation

The piplartine analogs were evaluated in vitro for their antimicrobial activities against the fungi through a Mueller Hinton broth microdilution method and with the methodology and interpretative criteria proposed by document M27A3 (CLSI 2008) and through a standard Mueller



Scheme 1 Synthesis of piplartine analogs. **i** $SOCl_2$, DMF, CH_2Cl_2, r.t.; **ii** NH_2NH_2. H_2O, CH_3CN, 0 °C; **iii** phthalic anhydride, HAc, reflux; **iv** benzoyl chloride, piridine, 0 °C; **v** morpholine, CH_2Cl_2, r.t.; **vi** NHS, CH_2Cl_2, r.t

Hinton broth microdilution method for bacteria proposed by document M7A6 (CLSI 2012). The stock solutions of all the compounds were prepared in DMSO 1% at final concentration and tested at concentrations (μg/mL) 100, 60, 30, 15, 7.5, 3.75, 1.875, 0.468, 0.23, 0.06. The standard drug fluconazole was applied as control of fungistatic action at concentrations from 64 to 0.031 g/mL and the standard drug chloramphenicol as a control of bacteriostatic action at concentrations from 125 to 0.06 g/mL. The microplates were incubated at 35 °C for 24 h for bacteria and 37 °C and for 24 h for fungi. Results were visualized and analyzed at 530 nm in an Anthos Zenyth 200rt Microplate Reader. The inhibitory concentrations of microbial growth were determined at 50% (IC_{50}) and 90% (IC_{90}) in μmol/mL and compared among the microorganisms. The tests were all done in duplicates.

Toxicity evaluation

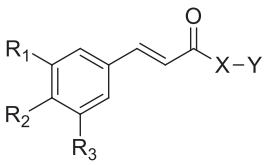
The toxicity of the compounds (200–1.5 μg/mL) to non-tumor cell line, BHK-21 cells (kidney cells of hamster), was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method (Souza et al. 2015). The BHK-21 cells at a concentration of 1.0×10^5 cells/mL were distributed in a 96-well plate, 98 μL in each well with 2 μL of test compounds at different concentrations. The plate was incubated at 37 °C in an incubator at 5% CO_2 for additional 48 h. After, 10 μL of MTT dye (5 mg/mL) was added and the cells were incubated again for an additional 4 h period. Then, the medium was carefully removed and added to each well 100 μL of DMSO. The plates were shaken for 5 min and absorbance for each sample was measured in a spectrophotometric microplate reader at 560 nm. The percentage of cytotoxicity was calculated as $[(A-B)/A \times 100)]$, where A and B are the absorbance of control and treated cells, respectively. Data were analyzed using linear regression to obtain values for CC_{50} and CC_{90} (cytotoxic concentration for 50 and 90% of cells, respectively). Selectivity indexes were expressed as the ratio CC_{50}/IC_{50}.

Results and discussion

Chemistry

The proposed piplartine analogs were obtained by a simple and objective synthetic route (Scheme 1); the compounds were achieved in good yields and high purity and characterized by infrared, ^1H and ^{13}C nuclear magnetic resonance and high resolution mass spectroscopy. Here in, several classes of compounds containing different groups have been synthesized in substitution for the heterocyclic ring of piplartin, such as α,β-unsaturated carbonyl heterocyclic derivatives (phthalimides), α,β-saturated carbonyl heterocyclic derivatives (succinimides), non-carbonylated

Table 1 Chemical structures, yields and logP values of the synthesized compounds



13-28

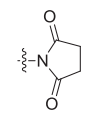
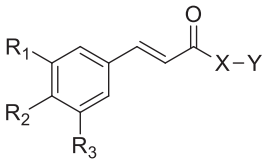
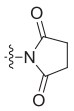
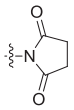
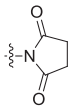
Compound	R_1	R_2	R_3	X	Y	cLog$P$[a]	Yield (%)
13	H	H	H	NH		2.45	90
14	H	OCH_3	H	NH		2.33	85
15	OCH_3	OCH_3	H	NH		2.2	70
16	OCH_3	OCH_3	OCH_3	NH		2.08	85
17	H	H	H	NH		2.64	94
18	H	OCH_3	H	NH		2.52	86
19	OCH_3	OCH_3	H	NH		2.39	82
20	OCH_3	OCH_3	OCH_3	NH		2.26	87
21	H	H	H	–		1.35	72
22	H	OCH_3	H	–		1.23	75
23	OCH_3	OCH_3	H	–		1.1	72
24	OCH_3	OCH_3	OCH_3	–		0.97	86
25	H	H	H	O		2.1	83

Table 1 continued



13-28

Compound	R_1	R_2	R_3	X	Y	cLog$P$[a]	Yield (%)
26	H	OCH_3	H	O		2.0	75
27	OCH_3	OCH_3	H	O		1.7	88
28	OCH_3	OCH_3	OCH_3	O		1.4	70
Piplartine	OCH_3	OCH_3	OCH_3	–		1.6	–

[a] The log$P$ values were determined by ChemBioDraw Ultra 12.0 program

heterocyclic derivatives (amides) and finally non-heterocyclic compounds (acylhydrazides). With these chemical variations combined with the variations of the trimethoxylated ring of piplartine, preliminary information about structure–antimicrobial activity relationship could be determined.

The initial step of the synthetic route involved the synthesis of acyl chlorides (**5–8**) from four commercial cinnamic acids (cinnamic acid, 4-methoxycinnamic acid, 3,4-dimethoxycinnamic acid and 3,4,5-trimethoxycinnamic acid) (Carvalho et al. 2012). The crude dichloromethane solution of these acyl chlorides was employed in the synthesis of hydrazides **9–12** which were also used without prior purification in the following steps. Phthalimides **13–16** were obtained in yields higher than 70% from the reaction of hydrazides **9–12** with phthalic anhydride in glacial acetic acid under heating (Joshi et al. 2014). The analysis of ^{13}C-NMR spectra of phthalimides showed two signals corresponding to the carbonyl carbons of the compounds between 166.1–164.6 ppm, confirming the cyclization reaction and formation of phthalimide derivatives. The IR spectra of these compounds also showed bands corresponding to carbonyl groups between 1737 and 1791 cm^{-1}. The singlet corresponding to the NH proton was observed between 11 and 10 ppm in ^{1}H-NMR spectra. The reaction of hydrazides with benzoyl chloride provided the benzoyl derivatives **17–20** in yields higher than 82%. In the ^{1}H-NMR spectra, it was possible to observe a coupling constant (J^3) of 1.5 Hz for the two NH protons and these doublets were registered near 10.5 and 10.2 ppm. Two signals corresponding to the two carbonyl groups were observed between 165.7–164.4 ppm, confirming the successful benzoylation of hydrazides. The amides **21–24** were smoothly synthesized by the reaction of acyl chlorides **5–8** with morpholine and were obtained in high yields (>72%). It was possible to notice a broad signal at 3.7 ppm relative to the eight methylene protons of the morpholine moiety in amides **21–24** in their ^{1}H-NMR spectra and one signal near 165 ppm corresponding to the amidic carbonyl in their ^{13}C-NMR spectra. The bands referring to the amidic carbonyl were recorded near 1640 cm^{-1} in their infrared spectra. The reaction of acyl chlorides **5–8** with *N*-hydroxysuccinimide afforded the pure acylated derivatives **25–28** in yields higher than 70% after recrystallization from ethanol/water. In their ^{1}H-NMR spectra, it was possible to observe a singlet at 2.8 ppm relative to the four methylene protons and two signals between 169.4–162.0 ppm in their ^{13}C-NMR spectra, corresponding to the carbonyl groups of the compounds. Finally, it is important to note that the protons related to the cinnamic subunit olefin have been preserved ($J^3 = 12$ Hz), which proves the absence of

conjugate addition products and the maintenance of *E* configuration. (Table 1).

Antimicrobial and toxic assays

The toxicity action of piplartine and its analogs and reference drugs on healthy hamster kidney cells (BHK-21) was evaluated and the results are shown in Table 2. This evaluation was necessary to establish the indices of selectivity with respect to antifungal and antibacterial actions.

Piplartine and its analogs were evaluated against *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. parapsilosis*; the results of the investigation on these yeasts are reported here for the first time, along with their selectivity indexes (Table 3).

Fluconazole was used as a reference fungistatic drug and the results were estimated as the minimum concentration required to inhibit 50% (IC_{50}, related to fungistatic activity) and 90% (IC_{90}, related to fungicidal activity) of fungal cell growth. Piplartine presented the broadest spectrum of antifungal activity, and was shown to be fungistatic against all fungi stains evaluated, except *C. tropicalis*, in the range of 94.60–189.20 μM. However, piplartine was the most toxic compound to BHK-21 cell culture with the lowest selective index range, between 0.2 and 0.4. This high toxicity prevents the substances for future in vivo testing.

Table 2 In vitro cytotoxicity (μM) against hamster kidney cell culture (BHK-21)

Compound	CC_{50}	CC_{90}
13	285.12	452.13
14	252.91	396.65
15	239.98	379.21
16	156.73	427.23
17	231.71	439.04
18	212.75	402.75
19	188.18	361.64
20	125.20	333.19
21	354.75	586.79
22	295.28	506.59
23	281.96	458.91
24	185,55	384.38
25	259.93	462.70
26	249.93	429.70
27	196.34	397.76
28	174.99	415.13
Piplartine	40.14	80.31
Fluconazole	382.60	521.13
Chloramphenicol	269.18	486.52

Among the synthesized piplartine analogs, morpholine 3,4,5-trimethoxylated derivative **24** was the most promising analog showing activity against *C. krusei* at 48.83 μM, and a selectivity index greater than three. This compound was almost four-fold more potent and about five-fold less toxic than piplartine (IC_{50}: 189.2 μM; selectivity index of 0.21). Furthermore, derivative **24** was more than two-fold more effective than fluconazole (IC_{50}: 104.48 μM) against this strain. Although less active than fluconazole, derivative **24** also showed activity against *C. tropicalis* at 97.67 μM, while piplartine was inactive up to the highest evaluated concentration. It is also worth noting that piperazine or morpholine bioisosteric rings play a crucial role in antimicrobial activity, as in drugs such as linezolid, eperezolid and itraconazole or in morpholine–eugenol derivatives reported in our recent paper (26). Moreover, it is important to note that derivative **24** showed the lowest logP value (0.97) among all the synthesized compounds, suggesting an interesting value for anti-*Candida* activity (Table 1).

Eleven other analogs of piplartine were moderately active against different *Candida* species. Phthalimide derivatives **13**, **14**, and **15** showed some activity against *C. tropicalis*; for these three compounds, there is a clear and direct correlation between the number of methoxyl substituents on the aromatic ring and the activity against this strain (IC_{50} of **13**: 342.32 μM; IC_{50} of **14**: 310.47 μM; IC_{50} of **15**: 170.40 μM), which may suggest that the investigation of other ring substitution patterns may lead to the discovery of more active antifungal agents. Again, it can be seen that a lower logP value is related to a better antifungal activities, since derivative **15** has a logP 2.2. On the other hand, only the phthalimide derivative **16** (3,4,5-trimethoxy-substituted) showed some activity against *C. krusei* and it was not active against *C. tropicalis*, suggesting that more than two methoxyl groups at this aromatic ring is unfavorable for activity against this species.

Among the benzoylhydrazides (**17–20**), derivative **17** showed a broader spectrum of action, presenting activity against all species evaluated, except *C. krusei*, although at a low potency (IC_{50}: 375.7 μM). Derivative **19** was the most potent of the four acylhydrazides, showing IC_{50} of 183.9 μM against *C. glabrata*, and once again it was noticed that a 3,4-disubstituted cinnamic ring may be related to a better antifungal activity. Tri-substituted acylhydrazide **20** was also moderately active at 280.7 μM against *C. albicans*, *C. tropicalis*, and *C. krusei*.

It was also observed that the increasing in the number of methoxyl groups in the cinnamic ring of the *O*-acyl-succinic derivatives and the decreasing of their logP values led to an increase in activity against *C. tropicalis*, as noted for the phthalic derivatives. Succinic unsubstituted derivative **25** (logP 2.1) was inactive against *C. tropicalis*, while the three-substituted (**26**; logP 2.0), 3,4-disubstituted (**27**; logP

Table 3 In vitro antifungal activity (μM) and selectivity index (SI) of piplartine and synthesized analogs

Compound		*C. albicans* ATCC 10231 (SI)	*C. tropicalis* ATCC 750 (SI)	*C. krusei* ATCC 6258 (SI)	*C. glabrata* ATCC 90030 (SI)	*C. parapsilosis* ATCC 22019 (SI)
13	IC_{50}	–[a]	342.32 (0.83)	–	–	–
	IC_{90}	–	–	–	–	–
14	IC_{50}	–	310.47 (0.81)	–	–	–
	IC_{90}	–	–	–	–	–
15	IC_{50}	–	170.40 (1.4)	–	–	–
	IC_{90}	–	–	–	–	–
16	IC_{50}	–	–	261.70 (0.59)	–	–
	IC_{90}	–	–	–	–	–
17	IC_{50}	375.79 (0.61)	375.79 (0.61)	–	375.79 (0.61)	375.79 (0.61)
	IC_{90}	–	–	–	–	–
18	IC_{50}	–	–	–	–	–
	IC_{90}	–	–	–	–	–
19	IC_{50}	–	–	–	183.98 (1.02)	–
	IC_{90}	–	–	–	–	–
20	IC_{50}	280.79 (0.44)	280.79 (0.44)	280.79 (0.44)	–	–
	IC_{90}	–	–	–	280.79 (1.18)	280.79 (1.18)
21	IC_{50}	–	460.59 (0.77)	460.59 (0,77)	–	460.59 (0.77)
	IC_{90}	–	–	–	–	–
22	IC_{50}	–	–	–	–	–
	IC_{90}	–	–	–	–	–
23	IC_{50}	–	–	–	–	–
	IC_{90}	–	–	–	–	–
24	IC_{50}	–	97.67 (1.89)	48.83 (3.79)	–	–
	IC_{90}	–	–	–	–	–
25	IC_{50}	–	–	–	–	–
	IC_{90}	–	–	–	–	–
26	IC_{50}	–	218.12 (1.14)	363.54 (0.68)	–	–
	IC_{90}	–	–	–	–	–
27	IC_{50}	–	196.66 (0.99)	–	–	–
	IC_{90}	–	–	–	–	–
28	IC_{50}	–	179.05 (0.97)	–	–	–
	IC_{90}	–	–	–	–	–
Pip.	IC_{50}	94.60 (0.42)	–	189.20 (0.21)	189.20 (0.21)	94.60 (0.42)
	IC_{90}	–	–	–	–	–
Fluc.	IC_{50}	1.63 (234)	3.26 (117)	104.48 (3.6)	52.24 (7.3)	3.26 (117)

Pip. Piplartine, *Fluc.* Fluconazole

[a] Inactive at highest evaluated concentration

1.7) and 3,4,5-trisubstituted (**28**, logP 1.4) derivatives were active at 218.12, 196.66, and 179.05 μM, respectively, against this strain.

Piplartine, as well as its synthetic analogs, were also assessed for their antibacterial potential against *Staphylococcus aureus*, *Eschericha coli* and *Pseudomonas aeruginosa* strains using the same concentrations and dilutions employed in the antifungal assays.

The results and selectivity indexes on BHK-21 cells are shown in Table 4. Three piplartine analogs were active against the same strain and it was noticed that all of these active derivatives bear an unsubstituted cinnamic ring (**13**, **17** and **25** compounds). Among them, benzyl hydrazide **17** was more than three-fold more potent than the prototype piplartine, showing an IC_{50} value of 85.20 μM and a greater selectivity index (2.71). This suggests that new

Table 4 In vitro antibacterial activity (μM) and selectivity index (SI) of piplartine and synthesized analogs

Compound		*S. aureus* ATCC 6538 (SI)	*E. coli* ATCC 25922 (SI)	*P. aeruginosa* ATCC 9027 (SI)
13	IC_{50}	205.42 (1.38)	–[a]	–
	IC_{90}	–	–	–
14	IC_{50}	–	–	–
	IC_{90}	–	–	–
15	IC_{50}	–	–	–
	IC_{90}	–	–	–
16	IC_{50}	–	–	–
	IC_{90}	–	–	–
17	IC_{50}	85.20 (2.71)	–	–
	IC_{90}	–	–	–
18	IC_{50}	–	–	–
	IC_{90}	–	–	–
19	IC_{50}	–	–	–
	IC_{90}	–	–	–
20	IC_{50}	–	–	–
	IC_{90}	–	–	–
21	IC_{50}	–	–	–
	IC_{90}	–	–	–
22	IC_{50}	–	–	–
	IC_{90}	–	–	–
23	IC_{50}	–	–	–
	IC_{90}	–	–	–
24	IC_{50}	–	–	–
	IC_{90}	–	–	–
25	IC_{50}	408.06 (0.63)	–	–
	IC_{90}	–	–	–
26	IC_{50}	–	–	–
	IC_{90}	–	–	–
27	IC_{50}	–	–	–
	IC_{90}	–	–	–
28	IC_{50}	–	–	–
	IC_{90}	–	–	–
Pip.	IC_{50}	315.33 (0.12)	–	–
	IC_{90}	–	–	–
Chl.	IC_{50}	0.975 (276)	0.975 (276)	16.5 (16.3)

Pip. Piplartine, *Chl.* Choramphenicol

[a] Inactive at highest evaluated concentration

acylhydrazides can be exploited as interesting potential antibacterial agents. Phthalic derivative **13** was moderately active at 205 μM (selectivity index of 1.38) and the succinic derivative **25** at 408.06 μM (0.63 selectivity index). All of the compounds were inactive against Gram-negative *E. coli* or *P. aeruginosa* strains up to the highest concentration employed; however, piplartine and some synthetic analogs showed potential against Gram-positive *S. aureus*. Piplartine was the least active and the most toxic compound against this strain, showing IC_{50} value of 315.33 μM and a low selectivity index of 0.12. Again, synthetic analogs showed less toxicity than piplartine. Different from what was noted in the results of antifungal assessment, higher log*P* values contributed to better antibacterial activity of the compounds in each synthesized series (Tables 1 and 4).

Based on the results found in antimicrobial tests it was possible to realize the major structural requirements for antifungal and antibacterial activities among the synthesized piplartine analogs (Fig. 2).

Conclusion

Sixteen piplartine analogs were synthesized and these compounds were assessed for their antimicrobial potential and toxicity. Derivative **24** showed the best results against *C. krusei* (more than twice as potent as fluconazole, four-fold more potent and five-fold less toxic than piplartine). Other analogs had moderate activity against different fungal strains and a direct correlation was seen between the number of aromatic methoxyl groups and antifungal activity. Furthermore, benzoyl hydrazide **17** was more than three-fold more potent than piplartine in antibacterial evaluation against *S. aureus* and five-fold less toxic than piplartine. It was possible to note that an aromatic ring lacking methoxyl groups is important for the antibacterial activity of these compounds. These findings encourage the design and synthesis of new piplartine analogs as potential and safer candidates to new antimicrobial agents.

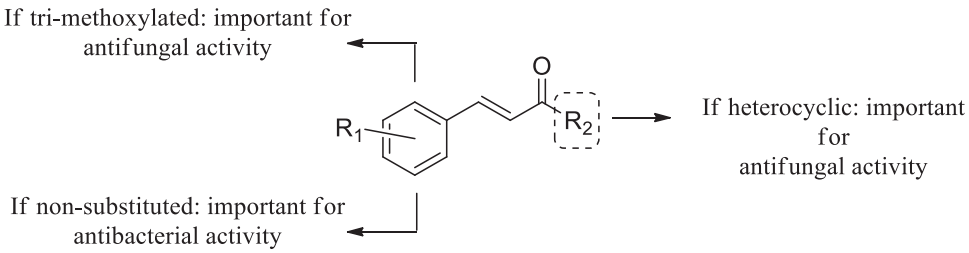


Fig. 2 Structure–antimicrobial activity relationships for piplartine analogs

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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