http://dx.doi.org/10.5935/0103-5053.20150084

J. Braz. Chem. Soc., Vol. 26, No. 6, 1201-1210, 2015.
Printed in Brazil - ©2015 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Green Synthesis of Chalcones and Microbiological Evaluation

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A green method was developed for the synthesis of chalcones using glycerin as solvent. Subsequently, the potential microbiology activity of these molecules was evaluated by testing them against the Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) ATCC 19095 and *Enterococcus faecalis* (*E. faecalis*) ATCC 4083, the Gram-negative bacteria *Escherichia coli* (*E. coli*) ATCC 29214 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 9027, and the fungus *Candida albicans* (*C. albicans*), which includes ATCC 62342 and three clinical strains of *C. albicans* from human oral cavities. The results showed that some chalcones exhibited moderate inhibitory activity, the most prominent being those acting against the fluconazole-resistant strains of *C. albicans*.

Keywords: chalcones, glycerin, microbial evaluation

Introduction

Major problems in public health are associated with fungal and bacterial infections, mainly when these microorganisms are resistant. This situation is becoming increasingly common, even with the wide variety of antimicrobial drugs and their medicinal-chemical evolution, owing to the indiscriminate use of these drugs by people. Consequently, medicines used cannot combat pathogenic organisms, thereby placing limitations on options for treatment.[1] Studies show that more than 70% of pathogenic bacteria develop resistance to at least one antibiotic available for clinical use.[2]

To circumvent this, it is necessary to obtain substances that are able to be used as prototypes of drugs, with pharmacological activity similar to or greater than the original ones and which have an effect on resistant organisms.[3,4] Advances in the development of analytical techniques purification and organic synthesis can contribute to this process, making it possible to obtain a variety of molecules with highest purity.[5]

One of the most studied classes of molecules are the chalcones, which are α,β-unsaturated ketones with two aromatic rings. Many studies were performed with these molecules and a range of activities were reported, such as anti-inflammatory,[6] antifungal, antibacterial,[7] antileishmanial, antiparasitic,[8] antitubercular[9] and antioxidant activity.[10] Due to their structure, these molecules are very versatile, as they may contain different aromatic rings, fused or not, with heteroatoms in their structure and different substituents.[11] These molecules can be obtained from natural products or by synthesis.[6] The Claisen-Schmidt reaction, which is the condensation of an aromatic ketone with an aromatic aldehyde in presence of a catalyst, such as NaOH, $Ba(OH)_2$, KOH, $AlCl_3$ or HCl,[7] is the most widely used method for the synthesis of chalcones. Furthermore, chalcones can be used as intermediates in different organic reactions to obtain pyrazoles,[12,13] isoxazoles,[14] and thiazoles.[15]

Among several lines of study, the search for methodologies that include a green process has been attracting attention

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in the scientific community in order to be environment friendly,[16] as the investment in techniques that reduce the demand for chemical waste using alternative technologies, such as sonochemistry[13,15,17] and microwave technology,[18,19] reusable catalysts,[20] renewable solvents or even solvent-free reactions.[21] An example of a renewable solvent is glycerin, a byproduct of biodiesel production. With a significant increase in the demand for this fuel, the amount of glycerin produced also increased, becoming an inconvenience. Aimed at alleviating this situation, it became necessary to find applications for the same.[22] An alternative is to increase its industrial value by employing glycerin as a raw material for the production of glycerol carbonate, which is used as a polar solvent, emulsifier for cosmetics, and a source of new polymeric materials and fuel additives.[23,24] In synthetic chemistry, glycerin can be used as a solvent in reactions, and several articles have shown this application.[25-28]

In this context, our group developed a methodology for the synthesis of chalcones using glycerin as a solvent, and subsequently, these molecules were tested for *in vitro* antimicrobial activity against two representatives of Gram-positive bacteria, *Staphylococcus aureus* (*S. aureus*) ATCC 19095 and *Enterococcus faecalis (E. faecalis*) ATCC 4083; two Gram-negative bacteria, *Escherichia coli* (*E. coli*) ATCC 29214 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 9027; and, four strains of yeasts: *Candida albicans* (*C. albicans*) ATCC 62342 and three clinical strains from the human oral cavity by the broth microdilution method according to the guidelines of the National Committee for Clinical and Laboratory Standards (NCCLS) for yeasts (M27-A3) and bacteria (M7-A7).

Experimental

All solvents and reagents used in the synthesis were obtained from Sigma-Aldrich Co., St. Louis, MO, USA, and used without further purification. Reagents for the microbial assays included Mueller-Hinton broth (BD, Sparks, MD, USA) and RPMI-1640 (Sigma, St Louis, MO, USA). Progress of the reactions was monitored on a Shimadzu 2010 gas chromatograph by flame ionization (GC-FID) using a split/splitless injector and a HP-1 30 m × 0.32 mm × 0.25 μm column. Analyses by Fourier transform infrared spectroscopy (FTIR) were obtained using attenuated total reflection (ATR) on an Agilent Technologies Cary 600 series equipment, and mass spectra were obtained on a Shimadzu GC-MS-QP 2010SE mass spectrometer-coupled gas chromatograph (GC-MS) with an AOC-20i automatic injector and Rtx-5MS 30 m × 0.25 mm × 0.25 μm column. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DPX 400 spectrometer (400.13 MHz for ^{1}H and 100.48 MHz for ^{13}C) at 300 K and the melting points (mp) were determined on apparatus Fisatom 430 with mercury thermometer, using capillary tubes to the samples.

The biological assays were performed at the School of Dentistry, and chemical analyzes at the Center of Chemical, Pharmaceutical and Food Sciences, both at the Federal University of Pelotas, except the NMR experiments, conducted at the Chemical Institute at the Federal University of Santa Maria (Brazil).

General procedure for chalcones **3a-n**

A solution of 5 mmol of acetophenone or 2-acetylthiophene **1**, 5 mmol of aldehyde **2** and 6 mmol of sodium hydroxide (NaOH) in 5 mL of glycerin was stirred overnight, neutralized with 0.5% HCl and filtered. The solid residue was recrystallized from ethanol to obtain the purity product.

Antimicrobial screening

The *in vitro* antimicrobial activity of each compound and the standard drugs were determined against two representatives of Gram-positive bacteria, *S. aureus* ATCC 19095 and *E. faecalis* ATCC 4083; two Gram-negative bacteria *E. coli* ATCC 29214 and *P. aeruginosa* ATCC 9027; and, four strains of yeasts: *C. albicans* ATCC 62342 and three clinical strains from the human oral cavity by the broth microdilution method according to the guidelines of NCCLS for yeasts (M27-A3) and bacteria (M7-A7).

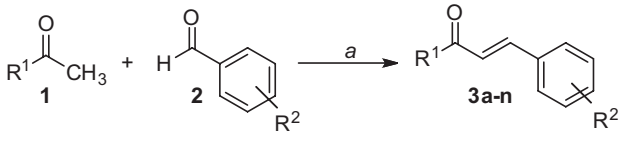
Microbial strains were primarily inoculated for overnight growth. An aliquot of the colonies was directly suspended in saline solution until the turbidity matched of the 0.5 McFarland standard and adjusted to give 5×10^5 CFU mL^{-1} of final microorganisms. Mueller-Hinton broth (BD, Sparks, MD, USA) was used as a nutrient medium to grow and dilute the compound suspension for the test bacteria and Roswell Park Memorial Institute medium (RPMI) 1640 (Sigma, St. Louis, MO, USA) buffered to pH 7.0 with 3-(*N*-morpholino) propanesulfonic acid (MOPS) was used for fungal nutrition. Dilutions of tetracycline and fluconazole were used as the reference compounds for comparing the data between independent experiments and as indicators for assessing the relative level of inhibition for the samples tested. Dimethyl sulfoxide (DMSO) was used as the diluent/vehicle to obtain the desired concentrations of the compounds and standard drugs.

The diluted samples were serially transferred in duplicate to 96-well microplates, and 100 μL microbial inoculums were added to achieve a final volume of 200 μL and concentrations ranging from 1 to 500 μg mL^{-1}. The final concentration of DMSO in the assay did not exceed 0.5%.

Controls for microbial viability, sterility of the medium and sterility of the extract were also carried out. The plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi. Plates were read at 630 nm for bacteria or 590 nm for fungi, prior to and after incubation. The antimicrobial effect was characterized by half maximal inhibitory concentration (IC_{50}) values, the concentration that affords 50% inhibition of bacterial/fungal growth relative to the growth control, and minimal inhibitory concentration (MIC) values, the minimal concentration of a substance that completely inhibits the bacterial or fungal growth. IC_{50} values were determined from logarithmic graphs of growth inhibition *versus* concentration. After determining the MIC, the minimal microbicidal concentration (MMC) was determined. Aliquots of 20 μL from the wells were plated on Mueller-Hinton agar for bacteria or Sabouraud dextrose agar for fungi and incubated at 37 °C for 24 h. MMC was defined as the lowest concentration of each compound that resulted in no cell growth on the surface of the plates.

Results and Discussion

The chalcones **3a**-**n** were synthesized from an equimolar mixture of acetophenone or 2-acetylthiophene and one of a series of eight aldehydes in glycerin using NaOH as a catalyst with stirring at room temperature, as shown in Scheme 1.



R^1 = Phenyl or 2-thiophen

R^2 = H; 4-OCH_3; 4-F; 4-Br; 4-Cl; 4-CH_3; 3-OCH_3

Scheme 1. (a) NaOH, glycerin, 20 °C.

Our group has been working with chalcones[10,29,30] because they are very versatile molecules and present activities against different microorganisms as described previously. The molecules chosen started with two aryl ketones, acetophenone and 2-acetylthiophene, and seven substituted benzaldehydes, where most are substituted in the 4-position to compare the influence of the groups on the activity of each compound. Furthermore, we wanted to evaluate the influence of the thiophenyl group, compared with the phenyl group, on the antimicrobial activities. Although the structure of these chalcones are published, we think it is important that their activity be evaluated against the bacteria *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa* and the fungi *C. albicans* and three clinical strains of this fungi from human oral cavities.

Generally, chalcones are obtained using an alcohol (methanol[36-38] or ethanol[10,39,40]) as the solvent. Glycerin was employed as a solvent in this work because it had similar properties to the cited solvents, such as polarity and organic functionality.

The yields of the synthesized chalcones **3a**-**n** are listed in Table 1, as well as their experimentally determined melting point (mp) and those found in the literature. Chalcones were obtained in good yields and with high purity, which is important for subsequent tests. All compounds exhibited purity up to 95% by GC-FID analysis and had their respective molecular ions identified by GC-MS.

We present advantages observed over other earlier published methods, such as that the reaction occurs at room temperature[36,37] and the solvent used is less toxic.[10,39,40] The improved mild and convenient protocol described here is an economical and efficient method for carrying out the Claisen-Schmidt reaction, resulting in high purity molecules in good yields (73-94%). The main advantage of this method is the employment of glycerin, a byproduct of biodiesel, often discarded as a consequence of its high production. The biofuel industries are unable to absorb the amount of glycerin for the preparation of chalcones, molecules of pharmaceutical interest.

To confirm the structures of the chalcones, they were analyzed by GC-MS, FTIR and ^{1}H and ^{13}C NMR spectroscopy. All compounds showed a molecular ion corresponding to the molecular mass, and both fragments were consistent with the chalcones structures. Analyses by FTIR identified the carbonyl group (band at 1640-1480 cm^{-1}), the double bond and aromatic bonds (bands at 1720-1640 cm^{-1} and 840-700 cm^{-1}, respectively). Generally, the carbonyl group is identified by a band at 1820-1600 cm^{-1}; however, conjugation with the aromatic rings caused a decrease in the absorption wavelength. The ^{1}H NMR spectrum showed the isomer with the *E* configuration in these molecules owing to the presence of two doublets with *J* ca. 15 Hz at approximately 7.35-7.99 ppm, indicating that the Cα–Cβ hydrogens are in the *trans* configuration.

Despite all the molecules having been identified and having their structures confirmed, some melting points did not agree with those previously determined. We think that this may have occurred due to different form crystallization of chalcones during the purification process.

Data of chalcones **3a**-**n**

(*E*)-1,3-Diphenylprop-2-en-1-one (**3a**)

mp 53-54 °C; IR (KBr) ν_{max} / cm^{-1} 3061.73, 1662.20, 1604.43, 1574.35, 1447.59, 1336.59, 1214.26, 1013.90,

Table 1. Synthesized chalcones **3a**-**n**

Compound	R^1	R^2	Exact mass[a] / (g mol^{-1})	Ion[b] / (g mol^{-1})	Yield / %	mp exp.[c] / °C	mp lit.[d] / °C
3a	C_6H_5	H	208.09	208.10	80	53-54	55-56[31]
3b	C_6H_5	4-OCH_3	238.28	238.25	80	78-79	75-76[31]
3c	C_6H_5	4-F	226.08	226.10	90	82-83	83[32]
3d	C_6H_5	4-Br	286.00	288.00[M + 2]	94	109-110	123[32]
3e	C_6H_5	4-Cl	242.05	243.10[M + 1]	92	100-102	111-112[31]
3f	C_6H_5	4-CH3	222.10	222.15	90	85-86	93[32]
3g	C_6H_5	3-OCH_3	238.10	238.15	88	58-59	58-59[31]
3h	C_4H_3S	H	214.05	214.10	78	145-146	144-146[33]
3i	C_4H_3S	4-OCH_3	244.06	244.10	80	70-72	70[34]
3j	C_4H_3S	4-F	232.04	232.10	92	119-120	123-124[35]
3k	C_4H_3S	4-Br	291.96	294.00[M + 2]	96	132-133	131-133[35]
3l	C_4H_3S	4-Cl	248.01	249.00[M + 1]	91	125-126	118-120[33]
3m	C_4H_3S	4-CH_3	228.06	228.10	90	101-102	116-117[35]
3n	C_4H_3S	3-OCH_3	244.06	244.10	89	58-59	not found

[a]Mass calculated; [b]experimental mass values for the molecular ion; [c]experimentally obtained melting point; [d]literature melting point.

745.86, 686.36; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.38 (m, 3H, Ph-H), 7.44-748 (m, 2H, Ph-H), 7.50 (d, 1H, *J* 15.73 Hz, Hα), 7.54-7.60 (m, 3H, Ar), 7,79 (d, 1H, *J* 15.72 Hz, Hβ), 8.00-799 (m, 2H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 121.61, 127.98, 128.03, 128.15, 130.06, 132.30, 134.40, 137.73, 144.30, 189.95; GC-MS *m/z*, observed: 208.10; $C_{15}H_{12}O$ $[M]^+$ requires: 208.09.

(*E*)-3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one (**3b**)

mp 78-79 °C; IR (KBr) ν_{max} / cm^{-1} 3089.58, 3016.86, 2955.13, 2902.07, 2842.33, 1656.32, 1593.93, 1509.34, 1261.24, 1209.29, 1015.39, 984.24, 824.33, 778.69, 688.02; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (s, 3H, CH_3), 6.90 (d, 2H, *J* 8.69 Hz, Ar), 7.38 (d, 1H, *J* 15.64 Hz, Hα), 7.44-757 (m, 5H, Ph-H), 7.76 (d, 1H, *J* 15.63 Hz, Hβ), 7.98 (d, 2H, *J* 7.29 Hz, Ph-H); ^{13}C NMR (100 MHz, CDCl-d_6) δ 54.88, 113.96, 119.26, 127.10, 127.90, 128.05, 129.72, 132.04, 138.00, 144.15, 161.19, 189.98; GC-MS *m/z*, observed: 238.25; $C_{16}H_{14}O_2$ $[M]^+$ requires: 238.28.

(*E*)-3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (**3c**)

mp 82-83 °C; IR (KBr) ν_{max} / cm^{-1} 3067.35, 1658.87, 1587.75, 1505.32, 1211.34, 1158.18, 1011.27, 835.69, 776.39, 687.22; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, 2H, Ar), 7.45 (d, 1H, *J* 15.42 Hz, Hα), 7.49-7.63 (m, 5H, Ph-H), 7.76 (d, 1H, *J* 15.70 Hz, Hβ), 8.01 (d, 2H, *J* 8.21 Hz, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 115.59 (d, 2C, J^2_{CF} 21.93 Hz, Ph-F), 121.28, 127.96, 127.96, 128.13, 129.83 (d, 2C, J^3_{CF} 8.50 Hz, Ph-F), 130.64 (d, 1C, J^4_{CF} 3.32 Hz, Ph-F), 132.32, 137.61, 142.92, 164.52 (d, 1C, J^1_{CF} 251.75 Hz, Ph-F), 189.71; GC-MS *m/z*, observed: 227.10 [M + 1], 226.10; $C_{15}H_{11}FO$ $[M]^+$ requires: 226.08.

(*E*)-3-(4-Bromophenyl)-1-phenylprop-2-en-1-one (**3d**)

mp 109-110 °C; IR (KBr) ν_{max} / cm^{-1} 3058.21, 1655.76, 1605.49, 1580.84, 1560.70, 1216.77, 978.22, 819.40, 772.18, 868.46; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.56 (m, 7H, Ph-H), 7.47 (d, 1H, *J* 15.94 Hz, Hα), 7.69 (d, 1H, *J* 15.71 Hz, Hβ), 7.96-798 (m, 2H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 122.44, 124.66, 128.38, 128.57, 129.68, 132.07, 132.82, 133.69, 137.88, 143.18, 189.99; GC-MS *m/z*, observed: 288.36 [M + 2], 286.00; $C_{15}H_{11}BrO$ $[M]^+$ requires: 286.00.

(*E*)-3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (**3e**)

mp 100-102 °C; IR (KBr) ν_{max} / cm^{-1} 3059.83, 1656.96, 1600.19, 1486.72, 1310.86, 1214.12, 1010.51, 981.74, 820.20, 773.14, 683.46; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, 2H, *J* 8.48 Hz, Ph-H), 7.44-7.57 (m, 5H, Ph-H), δ 7.46 (d, 1H, *J* 15.61 Hz, Hα), 7.71 (d, 1H, *J* 15.71 Hz, Hβ), 7.98 (d, 2H, *J* 7.15 Hz, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 122.36, 128.39, 128.56, 129.12, 129.48, 132.81, 133.28, 136.29, 137.91, 143.28, 190.02; GC-MS *m/z*, observed: 243.10 [M + 1], 242.10; $C_{15}H_{11}ClO$ $[M]^+$ requires: 242.05.

(*E*)-1-Phenyl-3-p-tolylprop-2-en-1-one (**3f**)

mp 85-86 °C; IR (KBr) ν_{max} / cm^{-1} 3051.59, 3023.95, 1654.66, 1595.56, 1567.44, 1333.61, 1221.72, 1017.23, 983.31, 816.59, 692.25; ^{1}H NMR (400 MHz, $CDCl_3$) δ

2.36 (s, 3H, CH_3), 7.19 (d, 2H, *J* 7.95 Hz, Ph-H), 7.47 (d, 1H, *J* 15.47 Hz, Hα), 7.45-7.57 (m, 5H, Ph-H), 7.78 (d, 1H, *J* 15.70 Hz, Hβ), 8.00 (d, 2H, *J* 7.04 Hz, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.41, 120.98, 128.35, 128.37, 128.47, 129.59, 132.05, 132.54, 138.25, 140.95, 144.78, 190.44; GC-MS *m/z*, observed: 222.15; $C_{16}H_{14}O$ $[M]^+$ requires: 222.10.

(*E*)-3-(3-Methoxyphenyl)-1-phenylprop-2-en-1-one (**3g**)

mp 58-59 °C; IR (KBr) ν_{max} / cm^{-1} 3061.19, 2832.76, 1655.48, 1599.98, 1574.53, 1262.62, 1214.35, 1047.77, 988.92, 859.30, 786.32, 665.83; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 3H, CH_3), 6.91-6.94 (m, 1H, Ph-H), 7.12-7.13 (m, 1H, Ph-H), 7.19-7.21 (m, 1H, Ph-H), 7.30 (t, 1H, Ph-H), 7.44-7.56 (m, 3H, Ph-H), 7.48 (d, 1H, *J* 15.56 Hz, Hα), 7.74 (d, 1H, *J* 15.70 Hz, Hβ), 7.99 (d, 2H, *J* 7.02 Hz, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.19, 113.35, 116.17, 120.95, 122.24, 128.37, 128.49, 129.81, 132.66, 136.13, 136.13, 138.04, 144.58, 159.83, 190.32; GC-MS *m/z*, observed: 238.15; $C_{16}H_{14}O_2$ $[M]^+$ requires: 238.10.

(*E*)-3-Phenyl-1-(thiophen-2-yl)prop-2-en-1-one (**3h**)

mp 145-146 °C; IR (KBr) ν_{max} / cm^{-1} 3086.75, 3027.96, 1645.31, 1590.43, 1412.46, 1334.76, 1219.70, 974.81, 856.29, 760.05, 720.08, 681.37; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13-7.15 (m, 1H, Ph-H), δ 7.36-7.42 (m, 3H, Ph-H), δ 7.40 (d, 1H, *J* 15.56 Hz, Hα), 7.59-7.64 (m, 3H, Ph-H), 7.82 (d, 1H, *J* 14.78 Hz, Hβ), 7.13-7.15 (m, 1H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 121.48, 128.13, 128.33, 128.80, 130.44, 131.73, 133.76, 134.52, 143.84, 145.36, 181.85; GC-MS *m/z*, observed: 214.10; $C_{13}H_{10}OS$ $[M]^+$ requires: 214.05.

(*E*)-3-(4-Methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**3i**)

mp 70-72 °C; IR (KBr) ν_{max} / cm^{-1} 3080.00, 3008.71, 2937.84, 2837.38, 1647.77, 1583.91, 1509.54, 1409.50, 979.14, 815.70, 727.16; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 3H, CH_3), 6.89-6.91 (m, 2H, Ph-H), 7.13-7.15 (m, 1H, Ph-H), 7.23 (d, 1H, *J* 15.51 Hz, Hα), 7.55-7.63 (m, 3H, Ph-H), 7.79 (d, 1H, *J* 15.59 Hz, Hβ), 7.81-7.83 (m, 1H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.33, 114.36, 119.22, 127.35, 128.10, 130.19, 131.40, 133.41, 119.22, 143.78, 145.70, 161.65, 181.98; GC-MS *m/z*, observed: 244.10; $C_{14}H_{12}O_2S$ $[M]^+$ requires: 244.06.

(*E*)-3-(4-Fluorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**3j**)

mp 119-120 °C; IR (KBr) ν_{max} / cm^{-1} 3118.67, 3074.19, 2979.79, 1649.47, 1586.03, 1506.06, 1417.05, 1203.22, 1157.06, 987.00, 825.56, 724.58; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12-7.14 (m, 2H, Ph-H), 7.04-7.08 (m, 1H, Ph-H), 7.31 (d, 1H, *J* 15.56 Hz, Hα), 7.56-7.64 (m, 3H, Ph-H), 7.76 (d, 1H, *J* 15.58 Hz, Hβ), 7.82-7.83 (m, 1H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 116.00 (d, 2C, J^2_{CF} 21.94 Hz, Ph-F), 121.24 (d, 1C, J^4_{CF} 2.45 Hz, Ph-F), 128.18, 130.24, 130.33, 131.75, 133.85, 130.28 (d, 2C, $J^3_{C\text{-}F}$ 8.61 Hz, Ph-F), 142.58, 145.32, 163.94 (d, 1C, $J^1_{C\text{-}F}$ 251.82 Hz, Ph-F), 181.70, 190.79; GC-MS *m/z*, observed: 232.10; $C_{13}H_9FOS$ $[M]^+$ requires: 232.04.

(*E*)-3-(4-Bromophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**3k**)

mp 132-133 °C; IR (KBr) ν_{max} / cm^{-1} 3077.47, 3012.70, 1642.38, 1581.06, 1560.81, 1411.93, 1219.22, 1066.14, 970.05, 808.85, 763.75, 707.21; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12-7.14 (m, 1H, Ph-H), 7.35 (d, 1H, *J* 15.59 Hz, Hα), 7.42-7.50 (m, 4H, Ph-H), 7.63-765 (m, 1H, Ph-H), 7.71 (d, 1H, *J* 15.59 Hz, Hβ), 7.81-7.83 (m, 1H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 122.03, 124.72, 128.21, 129.71, 131.87, 132.04, 133.48, 134.02, 142.45, 145.22, 181.58; GC-MS *m/z*, observed: 294.00 [M + 2], 292.00; $C_{13}H_9BrOS$ $[M]^+$ requires: 291.96.

(*E*)-3-(4-Chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**3l**)

mp 125-126 °C; IR (KBr) ν_{max} / cm^{-1} 3102.49, 3070.67, 3023.01, 1646.44, 1597.47, 1556.92, 1413.99, 1216.56, 1088.37, 988.04, 809.38, 767.31, 708.35; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.12-7.14 (m, 1H, Ph-H), 7.31-7.36 (m, 2H, Ph-H), 7.34 (d, 1H, *J* 15.77 Hz, Hα), 7.49-7.64 (m, 3H, Ph-H), 7.72 (d, 1H, *J* 15.58 Hz, Hβ), 7.82 (m, 1H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 121.91, 128.19, 129.08, 129.49, 131.84, 133.04, 133.98, 136.30, 142.35, 145.22, 181.57; GC-MS *m/z*, observed: 249.00 [M + 1], 248.05; $C_{13}H_9ClOS$ $[M]^+$ requires: 248.01.

(*E*)-1-(Thiophen-2-yl)-3-p-tolylprop-2-en-1-one (**3m**)

mp 101-102 °C; IR (KBr) ν_{max} / cm^{-1} 3084.06, 1645.79, 1586.25, 1566.37, 1421.33, 1207.60, 984.67, 813.02, 721.37; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 7.13 (t, 1H, Ph-H), 7.18 (d, 2H, *J* 7.91 Hz, Ph-H), 7.35 (d, 1H, *J* 15.56 Hz, Hα), 7.50 (d, 2H, *J* 8.04 Hz, Ph-H), 7.62 (d, 1H, *J* 4.95 Hz, Ph-H), 7.80 (d, 1H, *J* 15.92 Hz, Hβ), 7.82 (d, 1H, *J* 4.19 Hz, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.38, 120.45, 128.08, 128.37, 129.56, 131.56, 131.80, 133.55, 140.98, 143.92, 145.51, 181.93; GC-MS *m/z*, observed: 228.10; $C_{14}H_{12}OS$ $[M]^+$ requires: 228.06.

(*E*)-3-(3-Methoxyphenyl)-1-(thiophen-2-yl) prop-2-en-1-one (**3n**)

mp 58-59 °C; IR (KBr) ν_{max} / cm^{-1} 3082.62, 3005.22, 2669.88, 2935.14, 2835.13, 1650.28, 1595.11, 1412.12,

1249.86, 1033.03, 968.99, 792.73, 714.50, 678.37; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 3H, CH_3), 6.91-6.93 (m, 1H, Ph-H), 7.11-7.14 (m, 2H, Ph-H), 7.19 (d, *J* 7.53 Hz, 1H, Ph-H), 7.29 (t, 1H, Ph-H), 7.36 (d, 1H, *J* 15.58 Hz, Hα), 7.62-7.64 (m, 1H, Ph-H), 7.77 (d, 1H, *J* 15.57 Hz, Hβ), 7.82-7.83 (m, 1H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.20, 113.46, 116.15, 120.94, 121.78, 128.14, 129.81, 131.76, 133.80, 135.92, 143.78, 145.35, 159.80, 181.84; GC-MS *m/z*, observed: 244.10; $C_{14}H_{12}O_2S$ $[M]^+$ requires: 244.06.

The synthesized chalcones were tested against the Gram-positive bacteria *S. aureus* ATCC 19095 and *E. faecalis* ATCC 4083, Gram-negative bacteria *E. coli* ATCC 29214 and *P. aeruginosa* ATCC 9027, and four strains of the yeast *C. albicans* ATCC 62342 and three clinical strains from the human oral cavity and were compared with the reference drugs fluconazole, chloramphenicol and tetracycline. The results for the microbial assays are listed in Table 2.

Analyzing the results obtained, the IC_{50}, the concentration of product which inhibits 50% of the growth of microorganisms compared with positive control was determined, which was calculated by nonlinear regression using GraphPad Prism®. The MIC was also determined, corresponding to the minimal concentration able to inhibit 100% of microorganism growth, and the MMC. Chalcones which showed no inhibition at a concentration higher than 500 μg mL^{-1} have a negative sign (–) in the Table 2, and they were not evaluated for MMC.

Generally, the fungi were more susceptible to the chalcones tested. Antifungal assays determined that (*E*)-1,3-diphenylprop-2-en-1-one **3a** in contact with *C. albicans* and the strains *C. albicans* 1, 2 and 3 showed IC_{50} of 76.6 μg mL^{-1}, 29.5 μg mL^{-1}, 141.4 μg mL^{-1} and 134.3 μg mL^{-1}, respectively. Despite the fact that all values were lower than that for the reference drug fluconazole (IC_{50} 21.2 μg mL^{-1}), chalcone **3a** showed a MIC of 250 μg mL^{-1} against *C. albicans* 1, whereas fluconazole had a value greater than 500 μg mL^{-1}. This is a positive result taking into account that this strain was resistant to fluconazole.

(*E*)-3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one **3b** also showed activity against *C. albicans* and *C. albicans* 1, with IC_{50} of 41.2 μg mL^{-1} and 14.8 μg mL^{-1}, respectively, whereas the value corresponding to the resistant strain was smaller than that shown for fluconazole. The strain *C. albicans* 1 was also susceptible to (*E*)-3-(4-fluorophenyl)-1-phenylprop-2-en-1-one **3c**, with an IC_{50} of 63. μg mL^{-1} and MIC of 500 μg mL^{-1}, and (*E*)-3-(4-bromophenyl)-1-phenylprop-2-en-1-one **3d** exhibited an IC_{50} of 122.6 μg mL^{-1} against *C. albicans* 1. (*E*)-3-(3-Methoxyphenyl)-1-phenylprop-2-en-1-one **3g** exhibited activity against *C. albicans* and *C. albicans* 1 and 2, with IC_{50} of 373.4 μg mL^{-1}, 76.8 μg mL^{-1} and 202.3 μg mL^{-1}, respectively.

Analyzing the results, it can be observed that the *p*-methoxy group (chalcone **3b**) was more effective against *C. albicans* than the analog with a *m*-methoxy group (chalcone **3g**). Furthermore, molecules with smaller atoms in the aryl ring, i.e., the hydrogen in chalcone **3a** and the *p*-fluoro in chalcone **3c**, showed better results when compared with other chalcones without activity, which had a *p*-bromo, *p*-chloro or *p*-methyl group in the structure.

With regard to the chalcones synthesized from acetylthiophene, (*E*)-3-phenyl-1-(thiophen-2-yl)prop-2-en-1-one **3h** exhibited activity against all strains of *C. albicans*, with an IC_{50} of 471.2 μg mL^{-1} against *C. albicans*, 209.1 μg mL^{-1} against *C. albicans* 1, 223.3 μg mL^{-1} against *C. albicans* 2 and 111.3 μg mL^{-1} against *C. albicans* 3. These values were higher than those shown for chalcone **3a**, except against strain *C. albicans* 3, showing that the aryl-aryl compound had a better IC_{50} than the thiophenyl-aryl compound. Chalcone **3h** exhibited MIC of 500 μg mL^{-1} against the resistant strains *C. albicans* 1, 2 and 3, wherein the analog with the aryl group (**3a**) showed MIC of 250 μg mL^{-1} against the resistant strains *C. albicans* 1 and 3. Chalcone **3a** is more potent that **3h**, but **3h** showed a minimal microbicidal concentration against more strains of *C. albicans*.

(*E*)-3-(4-Methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one **3i** did not show activity against any strain of *C. albicans*, as opposed to analog **3b** with the aryl group. Chalcones (*E*)-3-(4-fluorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one **3j**, (*E*)-3-(4-bromophenyl)-1-(thiophen-2-yl)prop-2-en-1-one **3k** and (*E*)-3-(4-chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one **3l** exhibited activity against *C. albicans*, with IC_{50} of 14.8, 21.0 and 272.5 μg mL^{-1}, respectively. Again, molecules with smaller atoms in the structure showed better results against *C. albicans*.

(*E*)-1-(Thiophen-2-yl)-3-*p*-tolylprop-2-en-1-one **3m** exhibited activity against *C. albicans* 1, with IC_{50} of 28.8 μg mL^{-1} and MIC of 62.5 μg mL^{-1}. It appears that the change of the aryl group to the thiophenyl group increased activity for this chalcone. Similarly, (*E*)-3-(3-methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one **3n** also showed better activity against the tested strains, with an IC_{50} of 204.9 μg mL^{-1} against *C. albicans,* an IC_{50} of 163.0 μg mL^{-1} against *C. albicans* 1, an IC_{50} of 54.6 μg mL^{-1} against *C. albicans* 2 and an IC_{50} of 41.4 μg mL^{-1} against *C. albicans* 3.

The tested chalcones exhibited IC_{50} higher than fluconazole against strains of *C. albicans*, but some

Table 2. *In vitro* antimicrobial activities of compounds and reference substances against human pathogens

Compound	Assay / (μg mL^{-1})	Microbial strain							
		Ca	Ca1	Ca2	Ca3	Sa	Ec	Pa	Ef
3a	IC_{50}	76.6	29.5	141.4	134.3	–	–	171.8	–
	MIC	–	250.0	–	250.0	–	–	–	–
	MMC	nd	–	nd	–	nd	nd	nd	nd
3b	IC_{50}	41.2	14.8	–	–	–	–	–	–
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd
3c	IC_{50}	–	63.0	–	–	–	–	–	–
	MIC	–	500.0	–	–	–	–	–	–
	MMC	nd	–	nd	nd	nd	nd	nd	nd
3d	IC_{50}	–	–	–	122.6	–	–	–	–
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd
3e	IC_{50}	–	–	–	–	–	–	–	–
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd
3f	IC_{50}	–	–	–	–	–	–	–	–
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd
3g	IC_{50}	376.4	76.8	202.3	–	–	–	–	425.9
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd
3h	IC_{50}	471.2	209.1	223.3	111.3	420.5	–	–	471.0
	MIC	–	500.0	500.0	500.0	–	–	–	–
	MMC	nd	–	–	–	nd	nd	nd	nd
3i	IC_{50}	–	–	–	–	–	–	–	–
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd
3j	IC_{50}	14.8	–	–	–	–	–	–	30.2
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd
3k	IC_{50}	21.0	–	–	–	–	–	–	13.9
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd
3l	IC_{50}	272.5	–	–	–	–	–	–	–
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd
3m	IC_{50}	–	24.8	–	–	–	–	–	–
	MIC	–	62.5	–	–	–	–	–	–
	MMC	nd	–	nd	nd	nd	nd	nd	nd
3n	IC_{50}	204.9	163.0	54.6	41.4	219.1	–	441.9	338.5
	MIC	–	–	–	–	–	–	–	–
	MMC	nd	nd	nd	nd	nd	nd	nd	nd

Table 2. *In vitro* antimicrobial activities of compounds and reference substances against human pathogens (cont.)

Reference drug	Assay / (μg mL^{-1})	Microbial strain							
		Ca	Ca1	Ca2	Ca3	Sa	Ec	Pa	Ef
Fluconazole	IC_{50}	5.3	21.2	< 1	< 1	nt	nt	nt	nt
	MIC	–	–	1.9	31.2	nt	nt	nt	nt
	MMC	nd	nd	62.5	31.2	nt	nt	nt	nt
Chloramphenicol	IC_{50}	nt	nt	nt	nt	2.1	148.7	1.9	1.9
	MIC	nt	nt	nt	nt	3.9	250.0	3.9	3.9
	MMC	nt	nt	nt	nt	62.5	500.0	31.25	31.2
Tetracycline	IC_{50}	nt	nt	nt	nt	< 1	36.4	< 1	< 1
	MIC	nt	nt	nt	nt	< 1	62.5	< 1	< 1
	MMC	nt	nt	nt	nt	3.9	500.0	3.9	15.6

(–): > 500 μg mL^{-1}; nd: not determined as MIC was greater than 500 μg mL^{-1}; nt: not tested. Microbial strains: Ca: *Candida albicans* ATCC 62342; Ca1: *Candida albicans* clinical isolate 1; Ca2: *Candida albicans* clinical isolate 2; Ca3: *Candida albicans* clinical isolate 3; Sa: *Staphylococcus aureus* ATCC 19095; Ec: *Escherichia coli* ATCC 29214; Pa: *Pseudomonas aeruginosa* ATCC 9027; Ef: *Enterococcus faecalis* ATCC 4083.

chalcones showed a minimal microbicidal concentration against the resistant strain *C. albicans* 2.

Apparently, more electronically stable molecules showed better results against fungi strains. In chalcones **3a**-**g**, with an aryl moiety corresponding to the ketone used in the synthesis, compounds **3a**-**c** proved to be the most promising. Chalcone **3a** has two aryl groups lacking substituents in its structure, which allows resonance between the double bonds without interference. An activating group, such as methoxy, in the *para* position in chalcone **3b** increased the electronic density and stability of the ring, which may have been responsible for the decrease in IC_{50} values. The methoxy group in the *meta* position increased the IC_{50} values, most likely because there is no uniform electronic distribution in the ring, and the molecule was less stable.

In particular, in chalcones **3h**-**n**, with a thiophenyl moiety corresponding to the ketone used in the synthesis, the sulfur atom in a five-membered ring changes the properties of the molecules. Chalcone **3i**, for example, with a methoxy group in the *para* position, did not exhibit activity against fungi, unlike its analog with an aryl moiety, chalcone **3b**. A similar situation appeared in chalcones with halogens and a methoxy group in the *para* position, which exhibited better activity compared with analogs with an aryl moiety. The presence of such leads to a less stable molecule, and atoms with high electronic density can counterbalance this loss of stability.

Recently, Shakil *et al.*,[41] showed some analogs similar to those described in our work and evaluated their fungicidal activity against two phytopathogenic fungi: *Rhizoctonia solani* and *Sclerotium rolfsii*. The results suggested that chalcones with a *p*-fluoro in the aryl ring corresponding to the precursor ketone were highly effective as fungicides against *R. solani*. Chalcones **3c** and **3j** have a *p*-fluoro substituent in their structure, but in the ring corresponding to the benzaldehyde precursor.

Another study, reported by Sivakumar *et al.*,[36] obtained a series of chalcones that was tested for bacterial adhesion and slime production on coated polymeric biomaterials against *S. aureus*, *E. coli* and *P. aeruginosa*. The results showed that bacterial adhesion decreased considerably on all the coated surfaces with 3-hydroxy-4-methoxychalcone. As with the chalcone **3b** mentioned in our study, the *p*-methoxy substituent showed better results against fungi strains.

With respect to antibacterial assays, the best result for IC_{50} was exhibited with compound **3k**, with a value of 13.9 μg mL^{-1} against *E. faecalis*, however, a value higher than chloramphenicol (IC_{50} 3.9 μg mL^{-1}) and tetracycline (IC_{50} 1 μg mL^{-1}). Chalcone **3n** was the compound that exhibited activity against the major bacteria, with an IC_{50} of 219.1 μg mL^{-1} against *S. aureus*, 441.9 μg mL^{-1} against *P. aeruginosa* and 338.5 μg mL^{-1} against *E. faecalis*. All values are higher than those for the reference drugs.

The *E. faecalis* strain of bacteria was the most susceptible to the chalcones tested, where compound **3g** showed an IC_{50} of 425.9 μg mL^{-1}, **3h** showed an IC_{50} of 471 μg mL^{-1}, **3j** showed an IC_{50} of 30.2 μg mL^{-1} and **3n** showed an IC_{50} of 338.5 μg mL^{-1}, as well as chalcone **3k**, which showed an aforementioned IC_{50} of 13.9 μg mL^{-1}.

Chalcones **3h**-**n** exhibited better results than chalcones **3a**-**g** against bacteria, most likely because of the presence of a thiophenyl group in their structure. Among them, the chalcones with *p*-fluoro and *p*-bromo substituents showed

better IC_{50} values, and these atoms can be responsible for the improved results. Two previous studies comment about the better results with chalcones containing thiophene.

Molecules containing a 5-chlorothiophene moiety and a halogen-substituted phenyl ring were synthesized by Kumar *et al.*[42] Results indicated that the tested compounds exhibited a range of inhibition values against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and the Gram-negative bacteria *Xanthomonas campestris* and *Escherichia coli*. The authors attribute the elevated antimicrobial activity to the *p*-fluoro substituent on the phenyl ring.

Rizvi *et al.*[43] reported the synthesis of chalcones from 2-chloro-6-methyl-3-formylquinoline, 2-chloro-6-methoxy-3-formylquinoline and a series of aryl aldehydes. All compounds were screened for antimicrobial activity and the authors observed that compounds with substituted heteroaryl groups, such as 2,5-dichloro-3-methylthiophene, showed antibacterial activities almost equivalent to the standard chloramphenicol against three bacterial strains, *Escherichia coli*, *Micrococcus luteus* and *Staphylococcus aureus*.

To continue the work, the chalcones that exhibited promising results will be tested for their cytotoxicity to determine the concentrations at which the exhibited activity will not be toxic. Fluconazole is toxic at a concentration of > 69 μg mL^{-1}. A cell viability study will illustrate whether chalcones are toxic at concentrations that inhibit microbial growth.

Conclusions

According to the results presented, the method to synthesize chalcones using glycerin as a renewable solvent was efficient, because the products were obtained in good yields and with excellent purity. In microbiological assays, chalcone **3b** showed the lowest IC_{50} value of 14.8 μg mL^{-1} against *C. albicans* 1, whereas fluconazole presents an IC_{50} of 21.21 μg mL^{-1}. Chalcone **3n** showed activity against the largest number of bacteria, with an IC_{50} of 219.1 μg mL^{-1} against *S. aureus*, 441.9 μg mL^{-1} against *P. Aeruginosa* and 338.5 μg mL^{-1} against *E. faecalis*; however, all these values are higher than that for the reference drug. Ultimately, chalcone **3a** had MIC of 250 μg mL^{-1} against *C. albicans* 1, whereas fluconazole showed MIC of > 500 μg mL^{-1}; therefore, this compound may be regarded as a promising agent against this strain, which is resistant to fluconazole. Subsequently, cytotoxicity tests will be conducted to determine cell viability with these molecules, because fluconazole is cytotoxic at concentrations above 69 μg mL^{-1}.

Supplementary Information

Supplementary data (GC-MS, FTIR, ^{1}H and ^{13}C NMR) are available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgements

The authors are grateful to the FAPERGS, CAPES and CNPq for financial and fellowship support and the Federal University of Pelotas for physical and intellectual support.

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Submitted: February 4, 2015
Published online: April 7, 2015

FAPERGS has sponsored the publication of this article.