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Artigo

In Vitro Antifungal Activity Against *Trychophyton Rubrum* of *p*-Aminochalcones and 3'-Methoxy-4'-Hydroxy Chalcone

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Atividade Antifúngica *In Vitro* Contra *Trychophyton Rubrum* de *P*-Aminochalcones e 3'-Metoxi-4'-Hidroxi Chalcona

Resumo: O presente estudo relata as atividades antifúngicas avaliadas *in vitro* de 10 chalconas derivadas da *p*-aminoacetofenona e da 3'-metoxi-4'-hidroxiacetofenona contra quatro estirpes dos dermatófitos *Trychophyton rubrum* (CEMM 0201 e 0202, LABMIC 0203 e 0204). As *p*-aminochalonas e 3'-metoxi-4'-hidroxi-calconas foram sintetizadas pela reação de condensação do Claisen-Schmidt em meio básico. A CIM foi definida pela menor fração de teste capaz de inibir o crescimento fúngico visualmente detectado. A concentração mínima de fungos (CFM) correspondeu à menor concentração, o que não resultou em crescimento fúngico após 2 dias. As chalconas foram efetivas contra o *T. rubrum* (CIM de 0.015 to 1.25 µg/mL).

Palavras-chave: Acetofenona; chalconas; dermatófitos; atividade antifúngica.

Abstract

This study evaluates the *in vitro* antifungal activities of 10 chalcones derived from *p*-aminoacetophenone and 3'-methoxy-4'-hydroxyacetophenone against four strains of the dermatophytes of *Trychophyton rubrum* (CEMM 0201 and 0202, LABMIC 0203 and 0204). The *p*-aminochalcone and 3'-methoxy-4'-hydroxy chalcone derivatives were synthesized via the Claisen-Schmidt condensation reaction conducted under basic conditions. The minimum inhibitory concentration (MIC) was defined as the lowest concentration capable of inhibiting fungal growth. The minimum fungicidal concentration (MFC) corresponds to the lowest concentration that results in no fungal growth after 2 days. The chalcones were effective against *T. rubrum (*MIC = 0.015–1.25 µg/mL).

Keywords: Acetophenone; chalcones; dermatophytes; anfifungical activity.

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In Vitro Antifungal Activity Against *Trychophyton Rubrum* of *p*-Aminochalcones and 3'-Methoxy-4'-Hydroxy Chalcone

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1. Introduction

Chalcones have an open chain flavonoid structure in which the two aromatic rings are joined by a three carbon α,β-unsaturated carbonyl linker (Figure 1). They can be obtained from natural sources or by synthesis, and are widely distributed in fruits, vegetables, and tea.[1,2] Their antimicrobial activity and, in particular, antifungal action have been largely attributed to the reactive enone moiety.[3-6]

Dermatophytes are fungi that can cause infections (known as tinea) of the skin, hair, and nails

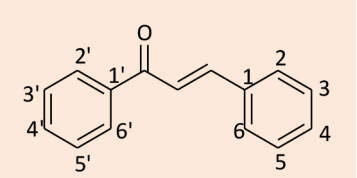

Figure 1. Fundamental structure of chalcones

because of their ability to use keratin. Superficial mycoses probably represent the most prevalent infectious disease found worldwide.[7] The fungus colonizes keratin tissues and causes inflammation as a result of the host's response to its metabolic by-products. The dermatophytes of *Trichophyton rubrum* are distributed over the world.[8] *T. rubrum* is seldom isolated from animals and is rarely found in soil. The infections caused by dermatophytes may have different manifestations depending on the site of the infection. The inflammatory reaction caused by *T. rubrum* can cause severe damage to the skin and in particular, the nails. Recently, sequential pulse therapy with itraconazole (a triazole drug) and terbinafine (an allylamine drug) has been used to treat onmychomycosis of the fingernail.[9-11] The objective of this study was to evaluate the antifungal activity of chalcones derived from p-aminoacetophenone and 3'-methoxy-4'-hydroxyacetophenone against four strains of the dermatophytes of *Trychophyton rubrum*.

2. Materials and Methods

2.1. Synthesis and characterization of chalcones

The *p*-aminochalcone and 3'-methoxy-4'-hydroxy chalcone derivatives used in this study were synthesized via Claisen-Schmidt condensation conducted under basic conditions.[12] A solution of *p*-aminoacetophenone (2 mmol) and 3-methoxy-4-hydroxyacetophenone (2 mmol) in ethanol (5 mL) was added the to a solution of benzaldehyde (2 mmol) in ethanol (5 mL) containing 10 drops of 50 % v/v sodium hydroxide, and the resulting mixture was stirred for 48 h. The mixture was filtered under vacuum, washed with cold water to pH 7.0, and analyzed by TLC (Figure 2 and 3).

The chemical reagents were from obtained Sigma-Aldrich. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker Spectrometer, model Avance DPX - 300 and model Avance DRX-500 operating at a frequency of 300 MHz and 500 MHz for hydrogen, 75 MHz and 125 MHz for carbon respectively. The spectra were measured in CD_3OD, CD_3COCD_3 and $CDCl_3$ solvents and chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0.00) as internal standard. The mass spectra were obtained with a Shimadzu QP201 GC-MS (Gas Chromatography coupled to Mass Spectrometry) using RTX-5MS capillary column (30.0m x 0.25mm x 0.30mm) for compounds with in the literature record and UPLC-QTOF-MS performed in an ACQUITY UPLC BEH column (150 × 2.1 mm, 1.7 um; Waters Co.) on a Waters Acquity UPLC system. The column temperature was set at 40 °C. The binary gradient

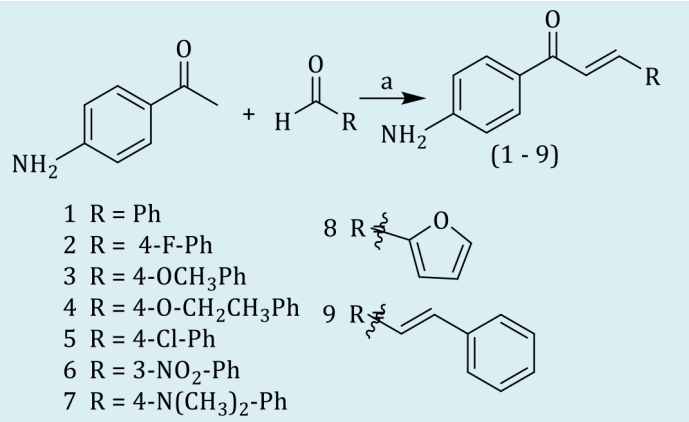

Figure 2. Preparation of chalcones (**1 – 9**) a) NaOH 50 % w /v, ethanol, r.t., 48 h

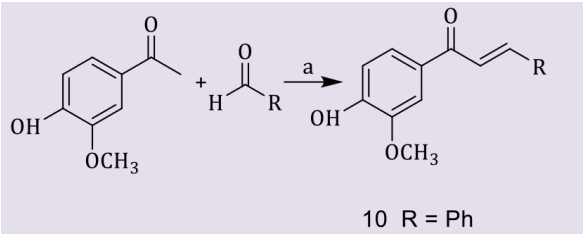

Figure 3. Preparation of chalcone (**10**) a) NaOH 50 % w /v, ethanol, r.t., 48 h

elution system consisted of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B), with linear gradient from 2 to 95 % B (0-15 min), with a flow rate of 0.4 mL.min^{-1} for the new compounds (**6a**) and (**11**). Infrared spectra were determined on a Perkin Elmer FT/IR 1000 spectrophotometer and are reported in wave number (cm^{-1}). The melting point was done in the apparatus MQAPF-302 (microchemistry) with heating rate 3.0 °C/min.

2.2. Fungal strains

Experiments were conducted using *T. rub*rum LABMIC 0208, 0210, 0204, and 0209. The fungal strains were obtained from potato agar stock at –20 °C, gently yielded by the Laboratory of Microbiology of the Vale do Acaraú State University (UVA) and the Center Specialist in Medical Mycology (CEMM), Department of Pathology and Legal Medicine of the Federal University of Ceara. The fungal samples were peeled into tubes containing potato dextrose agar. (Difco, Detroit, MI, USA) and incubated at 28 °C for 10 days in order to verify the presence of colony growth.[13,14]

2.3. Preparation of the inoculum used for the antifungal sensitivity tests

After confirming the viability of the fungal strains, the cultures were covered with 5 mL of saline solution or sterile water with subsequent scraping of the surface of the colonies in order to obtain a suspension that was free of fragments in the culture medium. The solution containing conídia and hyphal filaments was diluted in a 1:5 proportion using RPMI 1640 medium with L-glutamine without sodium bicarbonate buffered at pH 7.0 with (3-(*N*-morpholino)propane sulfonic acid (MPOS; 0.165 M solution), resulting in concentrations of 5.0×10^4 CFU mL^{-1}.[15].

2.4. Antifungal activity

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the compounds against the dermatophytes were determined using the microdilution method in broth with 96-well plates according to CLSI M27A2. The compounds were prepared in (DMSO) at a concentration of 10 mg mL–1, diluted in 100 µL of RPMI 1640 medium (Sigma), and tested over a concentration range of 625–10000 µg mL–1. Thereafter, 100 µL of the inoculum was added to the 96-well plate. Ketoconazole, an antifungal drug, was used as a positive control in a concentration range of 0.07–16 µg mL–1. The microplates were incubated at 37 °C and read visually after 5 days. The MIC was defined as the lowest concentration capable of inhibiting fungal growth. In turn, the minimum fungicidal concentration (MFC) was determined after transferring 100 mL of the contents of the well without turbidity into tubes containing potato agar at 28 °C. The MFC against the various dermatophytes was calculated according to the fungal growth in the culture medium after 5 days, whereas for the yeasts it was determined after 24 h. *Miroxylon peruiferum* L. F. exhibits an MIC of 625 µg mL–1 and CFM of 1250 µg mL–1 for the two strains of *T. rubrum*, while *Astronium fraxinifolium* Schot exhibits a MIC of 2500 µg mL–1 and MFC of 5000 µg mL–1 against the two strains tested.

3. Results and Discussions

3.1. Analytical data

(2*E*)-1-(4'-aminophenyl)-3-(phenyl)-prop-2-en-1-one (1*)*

Yellow solid (Yield: 25.60 %), m.p. 109.3 – 109.9°C; FT-IR (KBr, ν_{cm}^{-1}): 3522, 3434, 1623, 1578, 1554. ^{1}H-NMR (CD_3OD, 300 MHz) δ: 7.40 – 7.42 (m, H-3/H-5, H-4), 7.93 (d, H-2'/H-6', *J* = 8.7 Hz), 6.73 (d, H-3'/H-5', *J* = 8.7 Hz), 7.69 – 7.72 (m, H-2/H-6, H-α, H-β). ^{13}C-NMR (CD_3OD, 75 MHz) δ: C-1 136.8, C-2/C-6 129.6 C-3/C-5 130.1, C-4 131.4, C-1'128.2, C-2'/C-6' 132.6, C-3'/C-5' 115.1, C-4' 154.8, C-α 123.3, C-β 144.4, C=O 190.3. MS (EI) m/z ($M^{+\cdot}$ 223), calcd for $C_{15}H_{13}NO$/223.

(2*E*)-1-(4'-aminophenyl)-3-(4-fluophenyl)-prop-2-en-1-one (2)

Yellow solid (Yield: 28 %), m.p. 161.5 – 162.5 9°C; FT-IR (KBr, ν_{cm}^{-1}): 3600, 1660, 1590, 1570. ^{1}H-NMR (CD_3OD, 300 MHz) δ: 7.76 (dd, H-2/H-6, *J* = 8.8/2.1 Hz), 7.14 (t, H-3/H-5, *J* = 8,8 Hz), 7.91 (d, H-2'/H-6', *J* = 6.93 Hz), 6.73 (d, H-3'/H-5', *J* = 8.8 Hz), 7.67 (d, H-α, *J* = 12.1 Hz), 7.74 (d, H-β, *J* = 14,2 Hz). ^{13}C-NMR (CD_3OD, 75 MHz) δ: C-1 132.6, C-2/C/6 132.6, C-3/C-5 116.1, C-4 163.8, C-1' 128.1, C-2'/C-6' 131.8, C-3'/C-5' 114.9, C-4' 155.0, C-α 123.2, C-β 142.9, C=O 190.1. MS (EI) m/z ($M^{+\cdot}$ 241), calcd for $C_{15}H_{12}NOF$/241.

(2*E*)-1-(4'-aminophenyl)-3-(4-methoxyphenyl)-prop-2-en-1-one (3)

Yellow solid (Yield: 38 %), m.p.148 - 148,5°C; FT-IR (KBr, ν_{cm}^{-1}): 1628, 1570, 1560 1555, 1480, 1240. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.92 (d, H-2/H-6, *J* = 8.61 Hz), 6.70 (d, H-3/H-5, *J* = 8.58 Hz), 7.59 (d, H-2'/H-6', *J* = 8.64 Hz), 6.93 (d, H-3'/H-5', *J* = 8.67 Hz), 7.42 (d, H-α, *J* = 15.57 Hz), 7,76 (d, H-β, *J* = 15.39 Hz), 3.89 (s, OCH_3). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ C-1 128.3, C-2/C-6 131.1, C-3/C-5 114.2, C-4 161.5, C-1' 127.8, C-2'/C-6' 130.2, C-3'/C-5' 114.5, C-4' 151.1, C-α 120.1, C-β 143.2, C=O 188.4, OCH_3 55.8). MS (EI) m/z ($M^{+\cdot}$ 253), calcd for $C_{16}H_{15}NO_2$/253.

(2*E*)-1-(4'-aminophenyl)-3-(4-ethoxyphenyl)-prop-2-en-1-one (4)

Yellow solid (Yield: 33.89 %), m.p. 140 - 140,8°C; FT-IR (KBr, ν_{cm}^{-1}): 1634, 1600, 1588, 1575, 1480, 1167. ^{1}H-NMR (CD_3OD, 300 MHz): δ 7.90 (d, H-2/H-6, *J* = 8.70 Hz), 6.69 (d, H-3/H-5, *J* = 8.73 Hz), 7.63 (d, H-2'/H-6', *J* = 7,98 Hz), 6.94 (d, H-3'/H-5', *J* = 8.70 Hz), 7.58 (d, H-α, *J* = 15.57 Hz), 7.65 (d, H-β, *J* = 15.06 Hz), 4,08 (q, CH_2, *J*= 6.96 Hz), 1.39 (t, CH_3, *J* = 6.99 Hz). ^{13}C-NMR (CD_3OD, 75 MHz): δ C-1 129.3, C-2/C-6 132.5, C-3/C-5 114.6, C-4 155.5, C-1' 127.9, C-2'/C-6' 131.4, C-3'/C-5' 116.1, C-4' 162.6, C-α 120.7, C-β 144.4, C=O 199.3, CH_2 64.8; CH_3 15.2. MS (EI) m/z ($M^{+\cdot}$ 267), calcd for: $C_{17}H_{17}NO_2$/267.

(2*E*)-1-(4'-aminophenyl)-3-(4-clorophenyl)-prop-2-en-1-one (5)

Yellow solid (Yield: 53.59 %), m.p. 162,9 - 163,3 °C; FT-IR (KBr, ν_{cm}^{-1}): 3555, 3350, 1621, 1570, 1550, 1490. ^{1}H-NMR (CD_3OD, 300 MHz): δ 7.91 (d, H-2/H-6, *J* = 8.73 Hz), 7.69 (d, H-3/H-5, *J* = 8.79 Hz), 7.42 (d, H-2'/H-6', *J* = 8.46 Hz), 6.68 (d, H-3'/H-5', *J* Hz), 7.70 (d, H-α, *J* = 14.28 Hz), 7.74 (d, H-β, *J* = 15.60 Hz). ^{13}C-NMR (CD_3OD, 75 MHz): δ C-1 135.6, C-2/C-6 132.7, C-3/C-5 130.9, C-4 137.5, C-1' 127.6, C-2'/C-6' 130.8, C-3'/C-5' 114.6, C-4' 155.8, C-α 124.3, C-β 142.9, C=O 189.9. MS (EI) m/z ($M^{+\cdot}$ 257.5), calcd for: $C_{15}H_{12}NOCl$/257.5.

(2*E*)-1-(4'-aminophenyl) -3- (3-nitrophenyl) prop-2-en-1-one (6)

Orange solid (Yield: 69.85 %), m.p. 208 - 208,7°C; FT-IR (KBr, ν_{cm}^{-1}): 3400, 1640, 1600, 980. ^{1}H-NMR (CD_3COCD_3, 300 MHz): δ 8.26 (d, H-2, *J* = 1.95 Hz), 7.98 - 8.26 (m, H-4/H-5), 7.75 (d, H-2'/H-6', *J* = 7.89 Hz), 6.74 (d, H-3'/H-5', *J* = 8.67 Hz), 7.72 (d, H-α, *J* = 15.96 Hz), 7.80 (d, H-β, *J* = 17.34 Hz). ^{13}C-NMR (CD_3COCD_3, 75 MHz): δ C-1 138.6, C-2 124.9, C-3 149.9, C-4 126.3, C-5 131.2, C-6 135.2, C-1' 127.6, C-2'/C-6' 132.3 C-3'/C-5' 114.2, C-4' 154.7, C-α 123.9, C-β 140.0, C=O 186.9. MS (EI) m/z ($M^{+\cdot}$ 268), calcd for $C_{15}H_{12}N_2O_3$/268.

(2*E*)-1-(4'-aminophenyl) -3-(4-dimethyaminophenyl) -prop-2-en-1-one (7)

Orange solid (Yield: 35.66 %), m.p. 169 - 170°C; FT-IR (KBr, ν_{cm}^{-1}): 3500, 3460, 1610, 1570, 1350, 1160, 980. ^{1}H-NMR (CD_3OD, 300 MHz): δ 7.57 (d, H-2/H-6, *J* = 8.85 Hz), 6.69 (d, H-3/H-5, *J* = 8.76 Hz), 7.89 (d, H-2'/H-6', *J* = 8.73 Hz), 6.76 (d, H-3'/H-5', *J* = 8.88 Hz), 7.46 (d, H-α, *J* = 15.39 Hz), 7.68 (d, H-β, *J* = 15.39 Hz), 3.02 (s, $2CH_3$). ^{13}C-NMR (CD_3OD, 75 MHz): δ C-1 124.7, C-2/C-6 131.5, C-3/C-5 113.3, C-4 153.8, $2CH_3$ 40.4, C-1' 128.4, C-2'/C-6' 132.3, C-3'/C-5' 114.6, C-4' 155.2, C-α 117.6, C-β 145.8, C=O 190.8. MS (EI) m/z ($M^{+\cdot}$ 266), calcd for $C_{17}H_{18}N_2O$/266.

(2*E*)-1-(4'-aminophenyl) -3- furan-2-yl-prop-2-en-1-one (8)

Dark orange solid (Yield: 69,85 %), m.p. 118 - 118,3°C; FT-IR (KBr, ν_{cm}^{-1}): 3447, 3434, 1640, 1584, 1545, 1173. ^{1}H-NMR (CD_3OD, 300 MHz): δ 6.78 (d, H-3, *J* = 3.36 Hz), 6.55 (m, H-4); 7.62 (s, H-5), 7,85 (d, H-2`/H-6`, *J* = 8.73 Hz), 6.68 (d, H-3`/H-5`, *J* = 8.73 Hz), 7.50 – 7.62 (s broad, H-α, H-β). ^{13}C-NMR (CD_3OD, 75 MHz): δ C-2 153.4, C-3 113.7, C-4 116.5, C-5 146.3, C-1` 127.6, C-2`/C-6` 132.4, C-3`/C-5` 114.6, C-4` 155.5, C-2 153.4, C-3 113.7, C-α 130.5, C-β 120.5 , C=O 189.7. MS (EI) m/z ($M^{+\cdot}$ 213), calcd for $C_{13}H_{11}NO_2$ / 213.

(2*E*,4*E*)-1-(4-aminophenyl)-5-phenylpenta-2,4-dien-1-one (9)

Dark orange solid (Yield: 34 %), m.p. 151,8 - 152°C; FT-IR (KBr, ν_{cm}^{-1}): 3458, 3376, 1635, 1611, 1576 1564. ^{1}H-NMR (CD_3OD, 300 MHz): δ 7.26 – 7.35 (m, Ar), 7.84 (d, H-2`/H-6`, *J* = 8.79 Hz). 6.67 (d, H-3`/H-5`, *J* = 8.76 Hz). 7.00 – 7.25 (m, H-α, H-7, H-8), 7.38 (ddd, H-β, *J* = 15.96, 8.43, 1.65 Hz). ^{13}C-NMR (CD_3OD, 75 MHz): δ C-1 138.0, C-2/C-6 128.7, C-3/C-5 130.0, C-4 128.4, C-7 126.8, C-8 142.6, C-1` 127.7, C-2`/C-6` 132.5, C-3`/C-5` 114.6, C-4`155.6, C-α 130.5, C-β 144.6, C=O 190.3. MS (EI) m/z ($M^{+\cdot}$ 253), calcd for $C_{17}H_{15}NO$/253.

(2*E*) -1- (3'-methoxy-4'-hydroxyphenyl) -3-(3-nitrophenyl) prop-2-en-1-one (10)

Yellow solid (Yield: 46,33 %), m.p. 179.4 - 180°C; FT-IR (KBr, ν_{cm}^{-1}): 3425, 1621, 1575, 1350, 990. ^{1}H-NMR (CD_3COCD_3, 300 MHz): δ 6.96 – 8.26 (m, Ar),

8.65 (s, H-2'), 7.73 (m, H-5'), 8.23 (d, H-6', *J* = 6.36 Hz), 7.82 (d, H-α, *J* = 15.35 Hz), 7.87 (d, H-β, *J* = 15.35 Hz), 3.90 (s, OCH_3) ^{13}C-NMR (CD_3COCD_3, 75 MHz): δ C-1 131.1, C-2 124.9, C-3 152.9, C-4 125.1, C-5 131.2, C-6 135,4, C-1` 138.3, C-2` 124.9, C-3` 148.8, C-4` 149.9, C-5` 115.6, C-6` 125.8, C-α 123.5, C-β 141.1, OCH_3 56.5, C=O 187.8. HRESIMS, m/z: 300.0867 ($C_{16}H_{13}NO_3$) $[M + H]^+$ (calcd. 300.0872).

3.2. Chemistry

The synthesis of chalcones in this work was carried out by a Claisen-Schmith condensation reaction in basic medium[11]. According to the product formed, the base acting as a catalyst removes an α hydrogen from the *p*-aminoacetophenone, transforming it into a nucleophile, which then reacts with the carbonyl carbon of the aldehyde that acts as an electrophile. The reaction precedes the release of a water molecule and formation of the chalcone with the α, β-unsaturated conjugate system. The reaction yield ranges from 25.60 - 69.85 %. The variation in yield is dependent on the nature of the group attached to ring B. The presence of electron-withdrawing groups leads to greater yield. The presence of electron removal groups in the ring produces a lower yield.

The structures of the *p*-aminochalcone and 3'-methoxy-4'-hydroxy chalcone derivatives were determined using nuclear magnetic resonance (NMR) and infra-red (IR) spectroscopies, and mass spectrometry (MS). The ^{1}H NMR spectra indicate that only the *E*-isomers were formed in the Claisen-Schmidt condensation reaction. In the ^{1}H NMR spectra obtained for the chalcone derivatives, the signal observed at δ = 4.81 ppm corresponds to the hydrogen atoms in the amino group. The doublets observed at δ = 7.75 and 7.94 ppm (J = 15.6 Hz) were attributed to the α,β-unsaturated hydrogen atoms, whose coupling constants (J) confirmed the *E* geometry of the double bond. For ring B in the chalcone derivatives, three signals were observed between δ = 7.42–7.60, a doublet of doublets corresponding to the hydrogen atoms at C2/C6, and two multiplets attributed to the hydrogen atoms at C3/C5 and C4. For ring B in chalcones **2** and **3**, two doublets were observed between δ = 7.38–7.65 ppm corresponding to the hydrogen atoms at C2/C6 and C3/C5. For ring B in chalcone **4**, four signals were observed at δ = 8.45 corresponding to the hydrogen atom at C2, a doublet at δ = 7.59 attributed to the hydrogen atom at C5, and two doublet peaks at δ = 8.22 (d, J = 8, 13 Hz) and 7.86 (d, J = 7.60 Hz) assigned to the hydrogens atoms at C4 and C6, respectively. In the ^{13}C NMR spectra obtained for the synthesized chalcone derivatives, it was possible to observe the peak corresponding to the α,β-unsaturated carbonyl atom for δ = 192.7–193.5 ppm. The olefinic α and β carbon atoms were observed at δ = 127.4 and 142.8 ppm, respectively.

The infrared data corroborated the confirmation of the structure, affirming the presence of stretch bands characteristic of C = O with values of system conjugated to C-sp2 and stretch bands of the trans type demonstrating the formation of the double bond C = C . Stretch bands C = C, characteristic of mono, meta and substituted aromatic rings, are also observed. These and other values are found in tables 1 and 2.

The structures of the synthesized and

Table 1. Infrared data for 4'-aminochalcones (1-9)

Main vibrations	Chalcones								
	1	2	3	4	5	6	7	8	9
ν N-H	3452/3415	3460/3340	3450/3400	-	3455-3345	3400/3380	3500/3460	3425/3390	3460/3380
ν C=O	1662	1630	1630	1660	1622	1640	1610	1630	1615
ν C=C	1600-1420	1600-1550	1600-1500	1600-1520	1660- 1478	1600-1540	1570-1500	160-1470	1600-1530
ν C-N	1334	1340	1310	1320	1345	1330	1350/1310	1315	1340
ν C-O	-	-	1180	1170	1180	-	1160	1180	-
δ $C-H_{Trans}$	970	970	975	960	975	980	950	980	985
δ $C-H_{Mono}$	790/750	-	-	-	-	-	-	-	-
δ $C-H_{Meta}$	-	-	-	-	-	680/650/590	-	-	-
δ $C-H_{Para}$	820	820	820	850	820	840	820	810	815
δ C(C=O)C	1220	1220	1230	1260	1250	1220	1230	1225	1250
δ C-F	-	1140	-	-	-	-	-	-	-
δ C-Cl	-	-	-	-	1088	-	-	-	-

Table 2. Infrared data for (2*E*)-1- (3'-methoxy-4'-hydroxyphenyl) -3- (3-nitrophenyl) prop-2-en-1-one (**10**)

Main vibrations	Chalcone
	10
ν O-H	3425
ν C=O	1621
ν C=C	1575-1465
ν C-N	1350
ν C-O	1160
δ $C-H_{Trans}$	990
δ $C-H_{Mono}$	-
δ $C-H_{Meta}$	785/750/660
δ $C-H_{Para}$	-
δ C(C=O)C	1190
δ C-Cl	-

acetylated chalcones were also confirmed through the analysis of the mass spectra in a fragmentation proposal, whose ions formed come from an α segmentation, which from there, there is the loss of a CO molecule by inductive segmentation, generating a second fragment ion. The mass spectra revealed peaks of the M^+ molecular ion. Justifying the molecular formulas of the synthesized and acetylated chalcones, in addition to the base peak characteristic of the general process of fragmentation of chalcones. The data were compared with the literature of chalcones of similar structures. (Figures 4 and 5).

3.3. Antifungal activity

The microdilution assays conducted against the dermatophytes using the chalcone derivatives are summarized in Table 3 and show MIC values ranging from 0.015 to 1.25 μg mL^{-1} against *T. rubrum* LABMIC 0208, 0210, 0204, and 0209. However, (2E)-1-(4'-aminophenyl)-3-(phenyl)-prop-2-en-1-one (1), (2E)-1-(4'-aminophenyl)-3-(4-fluorophenyl)-prop-2-en-1-one (**2**), (2*E*)-1-(4'-aminophenyl)-3-(4-chlorophenyl)-prop-2-en-1-one (5), (2*E*)-1-(4'-aminophenyl)-3- furan-2-yl-prop-2-en-1-one (**8**), and (2E)-1-(3'-methoxy-4'-hydroxyphenyl)-3-(3-nitrophenyl) prop-2-en-1-one (**10**) show activity against *T. rubrum* LABMIC 0208 (MIC = 0.07 μg mL^{-1}) and LABMIC 0204 (MIC = 0.07 μg mL^{-1}).

The results revealed that *p*-aminochalcone derivatives bearing a phenyl group and electron-withdrawing substituents, such as fluorine and chlorine, as well as the presence of a heterocyclic ring show enhanced activity when compared to those with electron-donating substituents. In addition, the presence of the hydroxy and methoxy groups in ring A of 3'-methoxy-4'-hydroxy chalcone as well as a nitro group on ring B appears to be important for activity against the dermatophytes studied.

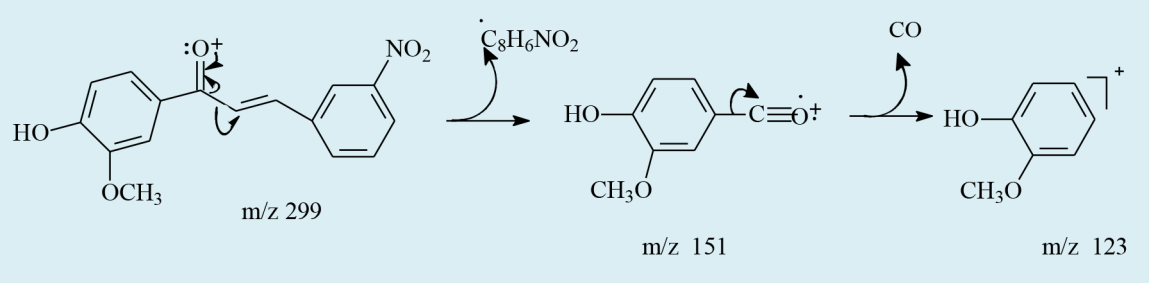

Figure 4. General fragmentation mechanism justifying the fundamental peaks of 3'-methoxy-4'-hydroxy chalcone (**10**)

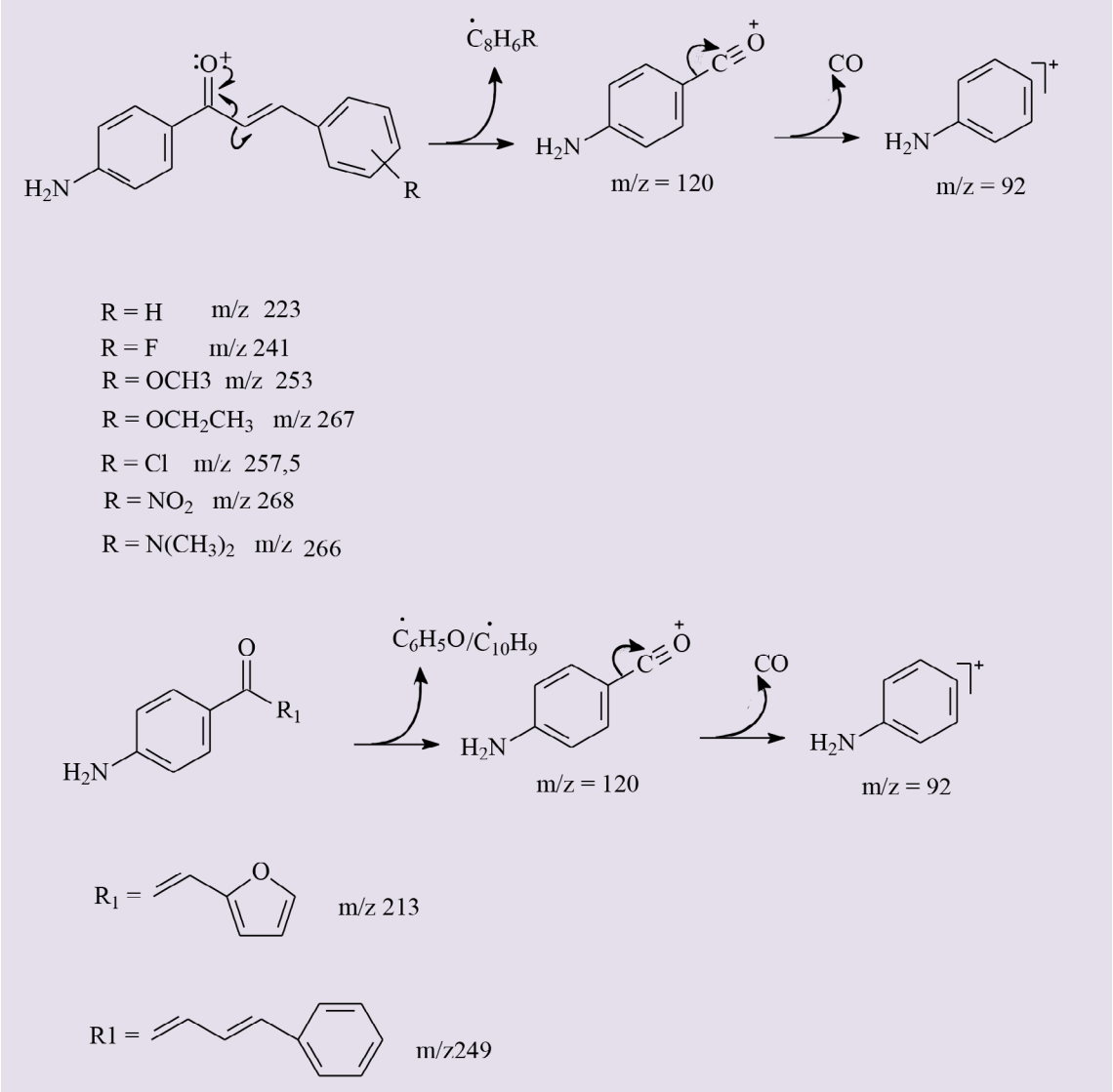

Figure 5. General fragmentation mechanism justifying the fundamental peaks of 4'-aminochalcones (1 – 9)

Table 3. *In vitro* inhibitory concentration of chalcones

Chalcones	Fungal strains							
	***T. rubrum* LAMBIC 0208**		***T. rubrum* LAMBIC 0210**		***T. rubrum* LAMBIC 0204**		***T. rubrum* LAMBIC 0209**	
	CIM	**CFM**	**CIM**	**CFM**	**CIM**	**CFM**	**CIM**	**CFM**
1	0.07	0.015	0.03	0.07	0.07	0.15	0.15	0.31
2	0.15	0.31	0.07	0.015	0.07	0.15	0.015	0.031
3	0.31	0.62	0.62	1.25	0.31	0.62	0.15	0.31
4	0.31	0.62	0.31	0.62	0.15	0.31	0.31	0.62
5	0.07	0.15	0.07	0.15	0.15	0.31	0.07	0.15
6	0.62	1.25	0.62	1.25	0.62	1.25	0.62	1.25
7	0.62	1.25	0.62	1.25	0.62	1.25	0.62	1.25
8	0.07	0.15	0.07	0.15	0.31	0.62	0.07	0.15
9	0.31	0.62	0.31	0.62	0.62	1.25	0.15	0.30
10	0.07	0.15	0.07	0.15	0.07	0.15	0.15	0.31

CIM and CFM (µg/mL)

4. Conclusion

A series of ten chalcones was synthesized via the Claisen-Schmidt condensation reaction including nine 4'-aminochalcone derivatives and 3'-methoxy-4'-hydroxychalcone. A microdilution assay against the dermatophytes of *T. rubrum* showed that the *p*-aminochalcone derivatives bearing a phenyl group and electron-withdrawing substituents such as fluorine and chlorine atoms as well as the presence of a heterocyclic ring, have been shown to be more active than those with electron-donating substituents. In addition, the presence of the hydroxy and methoxy groups on ring A in 3'-methoxy-4'-hydroxy chalcone as well as a nitro group on ring B appears to be important for activity against the dermatophytes studied.

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References

[1] Abbas, A.; Naseer, M. M.; Hasan, A.; Hadda, T. B. Synthesis and Cytotoxicity of 4-Alkoxychalcones as New Antitumor Agents. *Journal Materials & Environmental Science* **2014**, *5*, 281. [Link]

[2] Di Carlo, G.; Mascolo. N.; Izzo, A. A.; Capasso, F. Flavonoids: Old and New aspects of a class of natural therapeutic drugs. *Life Science.* **1999**, *65*, 337. [CrossRef] [PubMed]

[3] Boeck, P.; Leal, P. C.; Yunes, R. A.; Filho, V. C.; Lopez, S.; Sortino, M.; Escalante, A.; Furlan, R. L. E.; Zacchino S. Antifungal Activity and Studies on Mode of Action of Novel Xanthoxiline-drived Chalcones. *Archiv der Pharmazie* **2005**, *338*, 87. [CrossRef] [PubMed]

[4] Lopez, S. N.; Castelli, M. V.; Zacchino, S. A.; Dominguez, J. N.; Lobo, C.; Charris-Charris, J.; Cortes, J. C. G.; Ribas, J. C.; Devia, C.; Rodriguez, A. M.; Entriz, R. D. In Vitro Antifungal Evaluation and Structure–Activity Relationships of a New Series of Chalcone Derivatives and Synthetic Analogues, with Inhibitory Properties Against Polymers of the Fungal Cell Wall. *Bioorganic Medicinal Chemistry* **2001**, *9*, 1999. [CrossRef] [PubMed]

[5] Asif, M. A review on recent Advances and potential pharmacological activities of versatile chalcone molecule. *Chemistry International* **2016**, *2*, 1. [Link]

[6] Bag, S.; Ramar, S.; Degani, M. S. Synthesis and biological evaluation of α,β-unsaturated ketone as potential antifungal agents. *Medicinal Chemistry Research* **2009**, *18*, 309. [CrossRef]

[7] Smijs, T. G. M.; Schuitmaker, H. J. Photodynamic Inactivation of the Dermatophyte *Trichophyton rubrum. Phytochemistry and Photobiology* **2003**, *77*, 556. [CrossRef]

[8] Aly, R. Ecology and epidemiology of dermatophyte infections. *Journal American Academy Dermatology* **1994**, *31*, 21. [CrossRef]

[9] Hart, R.; Bell-Syer, S. E. M.; Crawford, F.; Torgerson, D. J.; Young, P.; Russel, I. Systematic review of topical treatments for fungal infections of the skin and nails of the feet. Br. *Medical Journal* **1999**, *19*, 79. [CrossRef] [PubMed]

[10] Tosti, A.; Piraccini, B. M.; Stinchi, C.; Venturo, N.; Bardazzi, F. M. Colombo, M. D. Treatment of dermatophyte nail infections: an open randomised study comparing intermittent terbinafine treatment and intermittent itraconazole therapy. *Journal American Academy Dermatology* **1996**, *34*, 595. [CrossRef]

[11] Faergemann, J.; Gupta, A. K.; Al Mofadi, A.; Abanami, A.; Shareaah, A. A, Marynissen, G. Efficacy of itraconazole in the prophylactic treatment of pityriasis (tinea) versicolor. *archives of dermatology* **2002**, *138*, 69. [CrossRef] [PubMed]

[12] Bhat, B. A.; Dhar, K. L.; Puri, S. C.; Saxena, A.K.; Shanmugavel, M.; Qazi, G. N. Synthesis and biological evolution of chalcones and their derived pyrazoles as potential cytotoxic agents. *Bioorganic Medicinal Chemistry Letters* **2005**, *15* , 3177. [CrossRef]

[13] Fontenelle, R.O.S.; Morais, S.M.B.; Mendonça, E.H.S.; Mendonça, M.R.; Brilhante K.; Cordeiro, R. A.; Nascimento, N. R. F.; Sidrim, J. J. C.; Rocha, M. F. G. Chemical composition, toxicological aspects and antifungal activity of essential oil from *Lippia sidoides* Cham. *Journal of Antimicrobial Chemotherapy* 2007, *59*, 934. [CrossRef] [PubMed]

[14] Fontenelle, R. O. S.; Morais, S. M.; Brito. E. H. S.; Brilhante, R. S. N.; Cordeiro, R. A.; Nascimento, N. R. F.; Kerntopf, M. R.; Sidrim, J. J. C.; Rocha, M. F. G. Antifungal activity of essential oils of *Croton* species from the Brazilian Caatinga biome. *Journal Applied Microbiology* **2008,** *104*, 1383. [CrossRef] [PubMed]

[15] Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Surviral: Application to Proliferation and Cytotoxicity Assays. *Journal Immunology Methods* **1983**, *65*, 55. [PubMed]