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Synthesis and anti-candidal activity of some new pyrazoline derivatives.

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Abstract

New pyrazoline derivatives were synthesized via the reaction of chalcones with cyclohexyl/phenyl thiosemicarbazide in presence of ethanol and acetic acid. All the compounds were confirmed by FT IR, ^{1}H NMR, ^{13}C NMR and MS spectral data. The compounds (1-11) were evaluated for antifungal activity against various strains of Candida species and compared with standard drug Itraconazole. MIC_{50} values were found to be within the range of 41.099-127.895 µg/ml and MIC_{90} values were found to be within the range of 62.121-240.955 µg/ml. Compound 7 was found to be most potent antifungal agent against Candida strains. It appears that para-methoxy substitution at one phenyl ring and meta-methoxy substitution at other phenyl ring of pyrazoline moiety made a significant contribution to the anti-Candidal activity in this series of pyrazolines.

Keywords: Pyrazoline, Chalcones, Anti-candidal activity, Thiosemicarbazides.

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Introduction

Invasive Fungal Infections (IFIs) are the opportunistic infections in patients especially with weak immune function [1]. *Candida* spp. is responsible for majority of these infections [2]. Azoles are currently used as antifungal agents. These drugs are acting by inhibiting cytochrome 450 dependent 14 α-demethylase. Imidazoles and triazoles are the commonly found moieties in azole drugs. Ketaconazole, an imidazole derivative, is used as antifungal agent. Pyrazoles are the structural isomers of imidazole and pyrazolines are the reduced forms of the pyrazoles. Pyrazoline derivatives have been reported to possess biological activities, which include anti-tumor [3,4], anti-inflammatory [5-7], anti-parasitary [8], anticonvulsant [9], antimicrobial [10-14] and antifungal [15,16]. Method for the synthesis of pyrazoline compounds from α, β-unsaturated carbonyl compounds (chalcones) is by the cyclization with hydrazine hydrate/substituted hydrazine. Based on molecular modelling and docking studies, the possible target for antifungal activity of arylthiosemicarbazides is the N-myristoyltransferase (NMT) enzyme. High electron density around sulphur atom and geometry of N-N-C (=S)-NH pharmacophore is responsible for ligand recognition [17]. Based on the literature survey and in continuation of our work on the synthesis of anti-Candidal agents [18,19], we present herein the syntheses of new pyrazolines which have embedded thiosemicarbazide pharmacophore and are found to possess an interesting anti-Candidal activity.

Material and Methods

Experimental

Chemistry: All the solvents were procured from Merck. The compounds were checked by TLC performed on silica gel G coated plates (Merck). Visualization of TLC spots was done in iodine chamber. The FT-IR spectra were recorded in KBr pellets on a (Spectrum BX) Perkin Elmer FT-IR spectrophotometer. Melting points were recorded on a Gallenkamp melting point apparatus, and are uncorrected. NMR Spectra were scanned in DMSO-d6 on a Bruker NMR spectrophotometer (500 MHz) for ^{1}H and (125 MHz) for ^{13}C at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. The molecular masses of compounds were determined by UPLC/TQMS.

General procedure for the synthesis of chalcone thiosemicarbazones: A mixture of appropriate substituted acetophenones (10 mmol), substituted benzaldehydes (10 mmol) and 60% aqueous NaOH solution (10 ml) in ethanol (20 ml) was stirred at 0°C for 4 hours. The reaction mixture was kept at room temperature for 48 hours and then water (50 ml) was added, 2 N HCl (50 ml) was used for neutralization and the mixture was extracted with EtOAc (3 × 200 ml). The organic layer was separated, washed with water several times, dried over anhydrous $MgSO_4$ and concentrated over under reduced pressure. It was purified by column chromatography eluted with mixtures of hexane/EtOAc (70/30) to give pure chalcone derivatives.

General procedure for synthesis of pyrazoline derivatives of chalcone: To a mixture Chalcone (0.05 mmol) and thiosemicarbazide (0.05 mmol) in ethanol (50 ml), glacial acetic acid (250 µl) was added as a catalyst and stirred at 80°C for 24 h. The reaction mixture was concentrated by rotavapour. Purification of compounds was done by column chromatography on silica gel with mixtures of hexane/EtOAc (70/30), to yield pure pyrazoline derivative.

5-[4-(dimethylamino) phenyl]-3-(4-methoxyphenyl)-N-cyclohexyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (1)

IR (KBr) cm^{-1}: 3419 (NH str.), 2922 (=CH str.), 1603 (C=C str.), 1119 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 1.1-1.8 (^{10}H, s, 5 × -CH_2), 2.5 (^{1}H, s, -CH), 2.9 (^{6}H, s, 2 × -NCH_3), 3.3 (^{3}H, s, -OCH_3), 4.1 (^{1}H, dd, J=5.0 Hz, HA), 6.7 (^{1}H, dd, J=5.0 Hz, HB), 6.71 (^{1}H, dd, J=7.5 Hz, Hx), 7.5-7.9 (^{8}H, m, Ar-H), 11.1 (^{1}H, s, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 25.3, 25.6, 49.0, 52.7, 112.1, 121.7, 129.0, 143.6, 151.8, 175.4; MS (ESI) m/z 436 [M]$^{+}$.

5-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-N-cyclohexyl-4, 5-dihydro-1H-pyrazole-1-carbothioamide (2)

IR (KBr) cm^{-1}: 3413 (NH str.), 2929 (=CH str.), 1604 (C=C str.), 1035 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 1.1-1.7 (^{10}H, s, 5 × -CH_2), 2.5 (^{1}H, s, -CH), 3.3 (^{1}H, dd, J=7 Hz, HA), 3.8 (^{3}H, s, -OCH_3), 4.1 (^{1}H, dd, J=7.5 Hz, HB), 5.8 (^{1}H, dd, J=11.0 Hz, HX), 6.6-8.1 (^{8}H, m, Ar-H), 9.3 (^{1}H, s, -OH), 11.1 (^{1}H, s, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 15.6, 25.4, 32.5, 42.4, 49.0, 53.2, 55.8, 63.0, 65.4, 70.5, 114.3, 114.5, 115.5, 116.0, 116.2, 118.9, 123.9, 126.4, 127.1, 129.3, 131.1, 134.0, 144.0, 154.9, 156.7, 160.4, 161.6, 163.4, 174.2, 187.7; MS (ESI) m/z 409 [M]$^{+}$.

5-(4-ethoxyphenyl)-3-(3-methoxyphenyl)-N-cyclohexyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (3)

IR (KBr) cm^{-1}: 3467 (NH str.), 2933 (=CH str.), 1599 (C=C str.), 1047 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 1.3 (^{3}H, t, J=6 Hz, CH_3), 1.3-1.8 (^{10}H, s, 5 × -CH_2), 2.5 (^{2}H, s, -CH), 3.3 (^{1}H, dd, J=7.5 Hz, HA) 3.8 (^{2}H, q, J=5 Hz, -OCH_2), 4.0 (^{3}H, s, -OCH_3), 4.2 (^{1}H, dd, J=7.0 Hz, HB), 6.9 (^{1}H, dd, J=7.5 Hz, HX), 7.6-8.1 (^{8}H, m, Ar-H), 11.3 (^{1}H, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 15.0, 25.4, 25.6, 26.8, 32.3, 49.0, 52.9, 55.9, 63.6, 114.2, 115.2, 119.8, 126.9, 127.7, 129.3, 130.9, 131.1, 132.2, 142.5, 143.6, 160.4, 160.9, 163.5, 175.9, 187.7; MS (ESI) m/z 437 [M]$^{+}$.

5-(3, 4-dimethoxyphenyl)-3-(3-methoxyphenyl)-N-cyclohexyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (4)

IR (KBr) cm^{-1}: 3337 (NH str.), 2925 (=CH str.), 1601 (C=C str.), 1025 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 1.1-1.8 (^{10}H, s, 5 × -CH_2), 2.5 (^{2}H, s, -CH), 3.1 (^{1}H, dd, J=5.0 Hz, HA), 3.8 (^{9}H, s, 3 × -OCH_3), 4.1 (^{1}H, dd, J=7.5 Hz, HB), 6.9 (^{1}H, dd, J=11.5 Hz, HX), 7.0-7.8 (^{7}H, m, Ar-H), 10.7 (^{1}H, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 25.1, 25.3, 25.5, 49.0, 52.7, 53.6, 55.7, 55.9, 56.0, 56.2, 110.0, 111.1, 111.4, 112.0, 112.1, 114.2, 114.4, 115.6, 117.1, 120.0, 120.9, 122.2, 122.6, 124.1, 126.9, 129.1, 129.2, 129.8, 130.2, 131.0, 131.2, 137.4, 140.5, 144.1, 149.1, 149.3, 149.4, 150.0, 150.6, 151.4, 160.5, 175.5, 176.6; MS (ESI) m/z 453 [M]$^{+}$.

3-(4-methoxyphenyl)-N-cyclohexyl-5-phenyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (5)

IR (KBr) cm^{-1}: 3321 (NH str.), 2935 (=CH str.), 1605 (C=C str.), 1108 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 1.1-1.8 (^{10}H, s, 5 × -CH_2), 2.5 (H, s, -CH), 3.1 (^{1}H, dd, J=5 Hz, HA), 3.8 (^{3}H, s, -OCH_3), 4.1 (^{1}H, dd, J=5.0 Hz, HB), 6.5 (^{1}H, dd, J=7.0 Hz, H), 6.9-8.3 (^{9}H, m, Ar-H), 10.9 (^{1}H, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 25.2, 25.3, 25.5, 32.1, 32.2, 49.0, 52.7, 53.7, 55.7, 112.4, 112.9, 113.0, 113.6, 114.2, 114.5, 115.6, 117.1, 122.2, 124.1, 126.8, 130.2, 130.9, 131.1, 144.6, 145.2, 150.3, 152.2, 160.5, 160.3, 176.0; MS (ESI) m/z 391 [M]$^{+}$.

5-[4-(dimethylamino) phenyl]-3-(3-methoxyphenyl)-N-cyclohexyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (6)

IR (KBr) cm^{-1}: 3352 (NH str.), 2927 (=CH str.), 1615 (C=C str.), 1037 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 1.1-1.9 (^{10}H, s, 5 × -CH_2), 2.5 (H, s, -CH), 2.8 (^{6}H, s, 2 × -NCH_3), 3.3 (^{1}H, dd, J=5.0 Hz, HA), 3.8 (^{3}H, s, -OCH_3), 4.1 (^{1}H, dd, J=5.0 Hz, HB), 5.8 (^{1}H, dd, J=9.5 Hz, HX), 6.6-7.9 (^{8}H, m, Ar-H), 11.0 (^{1}H, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 25.4, 25.6, 32.4, 42.4, 49.0, 53.3, 55.7, 63.2, 112.8, 116.5, 120.0, 126.7, 130.2, 131.2, 132.8, 149.9, 154.9, 159.9, 174.4; MS (ESI) m/z 436 [M]$^{+}$.

3, 5-bis (4-methoxyphenyl)-N-phenyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (7)

IR (KBr) cm^{-1}: 3333 (NH str.), 2935 (=CH str.), 1594 (C=C str.), 1021 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 3.3 (^{1}H, dd, J=5.0 Hz, HA), 3.8 (^{6}H, s, 2 × -OCH_3), 3.88 (^{1}H, dd, J=5.0 Hz, HB), 6.5 (^{1}H, dd, J=5.0 Hz, HX), 6.8-7.6 (^{13}H, m, Ar-H), 11.2 (^{1}H, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 31.1, 55.6, 55.7, 56.0, 112.1, 113.6, 113.8, 114.2, 114.5, 115.5, 115.6, 116.9, 119.8, 121.0, 122.0, 122.8, 125.3, 125.6, 125.7, 125.8, 128.5, 128.6, 128.8, 129.4, 129.5, 130.1, 130.3, 130.3, 131.2, 131.4, 137.2, 137.6, 137.7, 139.2, 139.4, 140.4, 143.6, 149.2, 160.0, 160.1, 160.7, 176.9, 206.9; MS (ESI) m/z 417 [M]$^{+}$.

5-[4-(dimethylamino) phenyl]-3-(4-methoxyphenyl)-N-phenyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (8)

IR (KBr) cm^{-1}: 3414 (NH str.), 2903 (=CH str.), 1601 (C=C str.), 1023 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 2.8

(6H, s, 2 × -NCH_3), 2.9 (^{1}H, dd, J=5.0 Hz, HA) 3.8 (^{3}H, s, -OCH_3), 3.85 (^{1}H, dd, J=5.0 Hz, HB) 6.0 (^{1}H, dd, J=5.0 Hz, HX), 6.7-8.0 (^{13}H, m, Ar-H), 11.5 (^{1}H, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 14.7, 26.8, 31.1, 55.6, 55.8, 55.9, 63.2, 111.1, 112.1, 112.2, 114.1, 114.3, 114.6, 116.4, 117.2, 121.5, 122.5, 123.7, 125.1, 125.5, 125.7, 125.8, 126.0, 126.8, 128.4, 128.5, 128.8, 129.3, 129.5, 129.6, 130.2, 130.7, 130.9, 131.0, 131.5, 139.6, 140.0, 144.6, 144.8, 149.5, 150.0, 152.0, 152.3, 156.0, 161.8, 163.2, 163.5, 173.5; MS (ESI) m/z 429 $[M-1]^+$.

5-(4-ethoxyphenyl)-3-(3-methoxyphenyl)-N-phenyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (9)

IR (KBr) cm^{-1}: 3325 (NH str.), 2927 (=CH str.), 1598 (C=C str.), 1035 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 1.3 (^{3}H, t, J=4.5 Hz, CH_3), 2.5 (^{2}H, q, CH_2), 3.1 (^{1}H, dd, J=5.0 Hz, HA), 3.83 (^{3}H, s, -OCH_3), 3.9 (^{1}H, dd, J=6.5 Hz, HB), 6.0 (^{1}H, dd, J=5.0 Hz, HX), 6.8-7.5 (^{13}H, m, Ar-H), 10.1 (^{1}H, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 15.1, 31.1, 42.6, 49.0, 55.8, 63.3, 63.4, 112.5, 114.8, 117.3, 120.5, 125.4, 126.0, 127.2, 128.4, 130.2, 132.6, 135.0, 140.0, 155.8, 158.0, 159.9, 174.2, 206.9; MS (ESI) m/z 431 $[M]^+$.

5-(3, 4-dimethoxyphenyl)-3-(3-methoxyphenyl)-N-phenyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (10)

IR (KBr) cm^{-1}: 3314 (NH str.), 2949 (=CH str.), 1598 (C=C str.), 1019 (C=S str.); 1H NMR (DMSO-d6, 500 MHz) δ 3.1 (^{1}H, dd, J=5.0 Hz, HA), 3.7 (^{9}H, s, 3 × -OCH_3), 3.8 (^{1}H, dd, J=6.5 Hz, HB), 6.7 (^{1}H, dd, J=5.0 Hz, HX), 6.9-7.6 (^{12}H, m, Ar-H), 11.1 (^{1}H, NH, D_2O exchg.); ^{13}C NMR (DMSO-d6, 125 MHz) δ 31.1, 49.0, 55.7, 55.8, 55.9, 56.0, 56.2, 110.1, 111.2, 111.5, 112.1, 112.2, 114.1, 114.4, 115.6, 117.1, 121.0, 122.2, 122.7, 124.1, 125.3, 125.6, 125.8, 126.9, 128.5, 128.6, 128.9, 129.1, 129.2, 129.6, 130.0, 131.2, 137.7, 139.2, 139.4, 140.9, 144.1, 149.3, 149.4, 149.5, 150.0, 150.1, 150.7, 160.6, 176.7, 187.8, 207.0; MS (ESI) m/z 445 $[M-2]^+$.

5-(3, 4-dimethoxyphenyl)-3-(4-methoxyphenyl)-N-cyclohexyl-4, 5-dihydro-^{1}H-pyrazole-1-carbothioamide (11)

IR (KBr) cm^{-1}: 3314 (NH str.), 2949 (=CH str.), 1598 (C=C str.), 1019 (C=S str.); ^{1}H NMR (DMSO-d6, 500 MHz) δ 1.2-1.8 (^{10}H, s, 5 × -CH_2), 2.3 (^{1}H, s, CH), 3.1 (^{1}H, dd, J=5.0 Hz, HA) 3.8 (^{9}H, s, 3 × -OCH_3), 4.2 (^{1}H, dd, J=5.0 Hz, HB), 5.9 (^{1}H, dd, J=4.5 Hz, HX), 6.6-8.0 (^{7}H, m, Ar-H), 11.2 (^{1}H, NH, D_2O exchg.); MS (ESI) m/z 447 $[M]^+$.

Anti-Candidal activity

Microorganisms: *Candida albicans* (ATCC® 90028™) strains were procured from American Type Culture Collection (ATCC) and another clinical strain of *Candida* was collected from King Khalid University Hospital, Riyadh. Both the strains were maintained on Mueller Hinton Agar plates during the experiment.

Experimental protocol: The log phase growth of *Candida* was transferred to Mueller Hinton Broth for incubation at 37°C. The turbidity of growth was adjusted to 0.5 McFarland turbidity standards. This microbial concentration was used for further experiment.

Initial screening of compounds: The compounds were dissolved in DMSO and were tested against *Candida* strains using disc diffusion method. The Mueller Hinton Agar was inoculated with test *Candida albicans* using a sterile disposable swab to make a lawn. The filter paper disks were placed on the lawn and added with 20 µL of each 500 µg/ml adjusted compounds. The plates were incubated at 37°C for 24 hours. Next day the compound showing zone of inhibition were selected for broth microdilution based MIC determination.

Minimum Inhibitory Concentration determination: The compounds were dissolved in Mueller Hinton Broth with DMSO as co-solvent. The 250 µg/ml highest concentration of each compound was taken in 96 well microtiter plate and serially diluted half fold to achieve 250, 125, 62.5, 31.25, 15.625, 7.81, 3.90, and 0 µg/ml concentration of compounds respectively. The 100 µL McFarland standard adjusted culture was added and plates were incubated at 37°C for overnight incubation. The growth was recorded by measurement of OD_{570} through Biotech Synergy HT plate reader. The percentage survival of Candida was plotted against compound concentration and minimum inhibitory concentration was determined for 50 and 90% inhibition while assuming 100% growth in media without any inhibitory compounds. Itraconazole was used as positive control while DMSO was used as internal standard.

Results and Discussion

The synthesis of pyrazoline derivatives (1-11) was carried out in two step reaction as outlined in (Scheme 1). These compounds were synthesized from the reaction of chalcones and cyclohexyl/phenyl thiosemicarbazides. Chalcones and thiosemicarbazides were reacted in a mixture of ethanol and acetic acid at 80°C for 24 hours. Compounds were obtained with yields of 60-70% (Table 1). Elemental analysis and TLC was used to check the purity of compounds. Compounds (1-11) were confirmed by FT IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. All the compounds gave satisfactory analytical and spectral data.

In the FT IR spectra of compounds (1-11), band in the region of 3467-3314 cm^{-1} was observed due to NH stretching vibrations. The band of =CH, C=C and C=S stretching vibrations of the compounds appeared in the region of 2949-2903 cm^{-1}, 1605-1598 cm^{-1} and 1119-1019 cm^{-1} respectively. In the ^{1}H NMR spectra of compounds (1-11), protons of CH_2 group of pyrazoline ring resonated at δ=2.9-4.1 and 3.8-6.7 ppm as pair of doublets of doublets. The CH proton appeared at δ=5.8-6.7 ppm as doublet of doublets due to vicinal coupling of CH_2 group of pyrazoline ring (JAB=5.0-7.0 Hz, JAX=5.0-7.5 Hz, JBX=4.5-11.5 Hz). All other aromatic and aliphatic protons were observed at expected positions. The

mass spectral data were also consistent with expected structures. Elemental analysis of all the compounds gave satisfactory results.

All the compounds of pyrazoline series (1-11) were screened for anti-Candidal activity by disc diffusion method. *Candida* 12775 and *Candida albicans* ATCC 90028 strains were used for screening of the compounds (1-11). MIC_{50} and MIC_{90} were recorded as minimum concentration of compound that inhibited 50% and 90% growth of the tested organism respectively. Compounds showing significant zone of inhibition were selected for calculation of MIC_{50} and MIC_{90} values. Compounds 1, 2, 4, 7 and 8 were selected for MIC_{50} and MIC_{90} determination. Itraconazole was used as positive control while DMSO was used as internal standard (Table 2). MIC_{50} values were found to be within the range of 41.099-127.895 µg/ml and MIC_{90} values were found to be within the range of 62.121-240.955 µg/ml. Comparing MIC_{50} values with itraconazole, all the five compounds were effective against *Candida albicans* ATCC 90028 and *Candida* 12775, especially compound 7 showed strong activity, similar level to the activity of itraconazole. MIC_{90} results of compounds also presented compound 7 as highly active against *Candida albicans* ATCC 90028 and *Candida* 12775. Heat map of *Candida albicans* ATCC 90028 growths incubated with different concentration of each compound (Figure 1) and heat map of *Candida* 12775 growth incubated with different concentration of each compound (Figure 2). The heat maps represent growth of respective *Candida* strains with certain concentration of each compound. The 100% growth which was considered as growth without addition of any inhibitory compounds was shown in green color while no growth is represented as black color. The respective color range and their percentage growth are given with the heat maps. The zero concentration of compound is showing same growth due to no inhibition, while further increase in compound concentrations led to change in growth sometime complete inhibition of growth (indicated by black color). The same graph of growth was used to calculate MIC_{50} and MIC_{90} of individual compound. This heat map gives better representation of growth inhibition of *Candida* strain with different concentration of compounds in comparison to simple MIC determination.

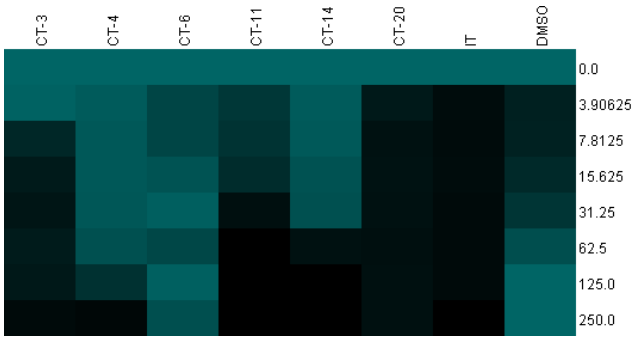


Figure 1. *Heat map of Candida albicans ATCC 90028 growth incubated with different concentration of each compound.*

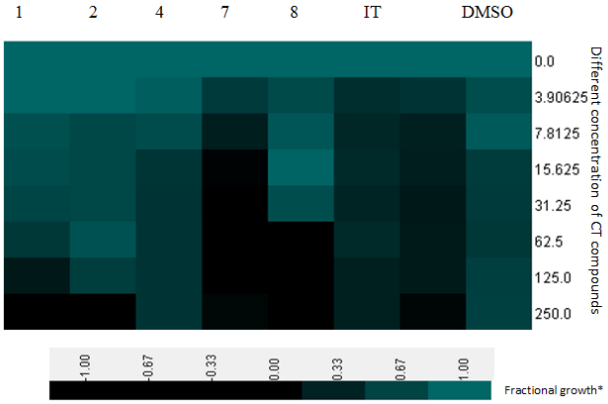
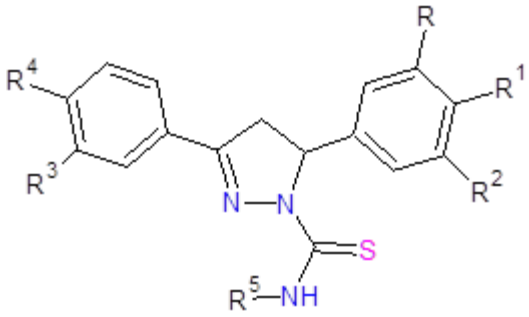


Figure 2. *Heat map of Candida 12775 growth incubated with different concentration of each compound. *Growth of Candida was indicated by heat map. The color scale is represented below and fractional growth value 1 indicates 100% Candida growth and inhibition is represented by change in color as per the scale. Fractional growth=OD_{570} with no compounds/OD_{570} with certain concentration of compounds.*



Scheme 1. *Synthesis of pyrazoline derivatives.*

Table 1. *Physical data of synthesized compounds (1-11).*

Compd.	R	R^1	R^2	R^3	R^4	R^5	Yield (%)	Mp (°C)
1	H	N (CH_3)	H	H	OCH_3	C_6H_{11}	70	163-165

2	H	OH	H	H	OCH_3	C_6H_{11}	60	123-125
3	H	OC_2H_5	H	OCH_3	H	C_6H_{11}	65	120-122
4	OCH_3	OCH_3	H	OCH_3	H	C_6H_{11}	60	173-175
5	H	H	H	H	OCH_3	C_6H_{11}	70	198-200
6	H	N (CH_3)	H	OCH_3	H	C_6H_{11}	65	185-187
7	OCH_3	H	H	H	OCH_3	C_6H_5	70	123-125
8	H	N (CH_3)	H	H	OCH_3	C_6H_5	60	138-140
9	H	OC_2H_5	H	OCH_3	H	C_6H_5	60	118-120
10	OCH_3	OCH3	H	OCH_3	H	C_6H_5	70	140-142
11	OCH_3	OCH3	H	H	OCH_3	C_6H_{11}	70	138-140

Table 2. *MIC_{50} and MIC_{90} values of selected pyrazoline compounds against Candida.*

Test compounds	*Candida* **12775**		*Candida albicans* **ATCC 90028**	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
1	91.815	185.107	52.05	108.818
2	108.26	203.532	127.895	240.955
4	97.84	187.488	58.0145	--
7	41.099	62.121	31.67	94.742
8	50.17	89.17	67.935	131.083
Itraconazole	38.18	111.204	31.634	73.63
DMSO	105.64	191.42	--	--

*--MIC was not able to determine as growth observed was variable due to either no inhibition (In case of DMSO with *Candida albicans* ATCC 90028) or due to color of compounds.

Conclusion

Considering all the results obtained from the anti-candidal screening, in comparison with the reference drug itraconazole, it can be concluded that Compound 7 was found to be most potent compound of the series. The heat map represent that low concentration of this compound is sufficient to inhibit growth of *Candida* as indicated by black color. Based on the evaluation of compounds for anti-Candidal activity, it appears that para-methoxy substitution at one phenyl ring and meta-methoxy substitution at other phenyl ring of pyrazoline moiety made a significant contribution to the anti-Candidal activity in this series of pyrazolines. Substitutional changes in cyclohexyl/ phenyl rings on the basic structure did not affect the activity.

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