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ORIGINAL ARTICLE

Synthesis of 2-pyrazolines from pyridine based chalcone by conventional and microwave techniques: Their comparison and antimicrobial studies

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Abstract 2-Pyrazolines from pyridine based chalcones by conventional and microwave techniques have been synthesized and their antimicrobial activity as well as a comparison study between conventional and microwave techniques has been done. Antimicrobial activity was carried out according to the broth micro dilution method and it was observed that compound **2d** was found to be most active against gram negative bacteria and fungus *Candida albicans*. Microwave technique has been found superior over the conventional method in view of reaction time and energy requirement. Compounds have been characterized by ^{1}H NMR, ^{13}C NMR, IR and Mass spectral analyses.

1. Introduction

Synthesis from microwave irradiation was first carried out in 1986 (Gedye et al., 1986). This pioneer work ignited the scope of microwave assisted synthesis. A number of articles were published on the microwave assisted synthesis and its comparison with conventional methods. This revealed that the microwave irradiated synthesis proved to be superior to that of conventional ones in terms of time and energy consumption. Microwave synthesis has been important in the medicinal chemistry which is useful for the rapid synthesis of a library of large number of compounds.

Chalcones act as precursor for biologically important heterocyclic compounds. It can be prepared by easy synthetic approaches and can be readily cyclized to a variety of compounds including 2-pyrazolines. 2-Pyrazolines are biologically active scaffolds with a variety of biological activities like antimicrobial (Patel et al., 2012), antitubercular (Taj et al., 2011), anti-inflammatory (Bano et al., 2011), anticancer (Lee et al., 2011), antitumor (Bai et al., 2012), anticonvulsant (Aboul-Enein et al., 2012), and anti-HIV (Ali et al., 2007). 2-Pyrazolines have been

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synthesized from cyclization of chalcones using conventional heating (Sridhar and Rajendraprasad, 2012; Koduru et al., 2012; Santos et al., 2012) as well as from microwave irradiation (Chawla et al., 2010; Azarifar and Ghasemnejad, 2003).

In this regard, we have also reported the cyclization of chalcones from conventional methods (Patel and Patel, 2010, 2012). Considering the above facts, here in this study, we are reporting the synthesis of 2-pyrazolines from chalcones by conventional and microwave techniques and a study of their comparison in terms of reaction time and yield as well as antimicrobial activity.

2. Experimental

2.1. General

Laboratory Chemicals were supplied by Rankem India Ltd. and Ficher Scientific Ltd. Melting points were determined by the open tube capillary method and are uncorrected. IR absorption spectra were recorded on a Thermo scientific Nicolet iS10 FT-IR spectrometer using KBr pellet, ^{1}H NMR spectra were recorded in a $CDCl_3$/DMSO-d_6 Bruker Avance II 400 NMR (400 MHz FT NMR) instrument (chemical shifts in δ ppm), ^{13}C NMR spectra were recorded in a Bruker Avance II 400 NMR (100 MHz FT NMR) instrument (chemical shifts in δ ppm) and Mass spectra recorded on a micromass Q-T of micro (TOF MS ES$^+$). Elemental analyses were performed on a Heraeus Carlo Erba 1180 CHN analyzer. The purity of products was routinely checked by TLC using silica gel in toluene:methanol solvents. The microwave assisted reactions were conducted in a ''QPro-M Microwave Synthesis System'' manufactured by Questron Technologies Corporation, Ontario L4Z 2E9 Canada, whereby microwaves are generated by magnetron at a frequency of 2450 MHz having an output energy range of 100–500 Wand with an individual sensor for temperature control (fiber optic is used as a individual sensor for temperature control) with attachment of reflux condenser with constant stirring (thus avoiding the risk of high pressure development) and synthesis on preparative scales.

3-(4-(2-(5-Ethylpyridin-2-yl)ethoxy)phenyl)-1-phenylprop-2-en-1-ones **1a**–**j** have been synthesized from reported methods (Patel and Patel, 2009).

2.2. Synthesis of 5-Ethyl-2-{2-[4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy]ethyl} pyridine(**2a**)

Conventional method:

A mixture of 3-(4-(2-(5-Ethylpyridin-2-yl)ethoxy)phenyl)-1-phenylprop-2-en-1-ones **1a**–**j** (0.003 mol) and hydrazine hydrate (0.0045 mol) was refluxed for 5–6 h in acetic acid (50 mL). Progress of the reaction was checked by TLC using toluene:methanol (7.5:2.5) as mobile phase. After completion of reaction, the mixture was cooled and poured into crushed ice with continuous stirring. The resulting solid thus obtained was collected by filtration, washed well with cold water, dried and recrystallized from methanol.

Microwave method:

A mixture of 3-(4-(2-(5-Ethylpyridin-2-yl)ethoxy)phenyl)-1-phenylprop-2-en-1-ones **1a**–**j** (0.003 mol) and hydrazine hydrate (0.0045 mol) was irradiated under microwave in solvent-free conditions. All these reactions were carried out by microwave irradiation for 3–7 min at the power level 700 W and at the temperature of 80–85 °C which was recorded by the temperature probe of the microwave. Progress of the reaction was checked after a regular interval of one minute till the completion of reaction by TLC using toluene:methanol (7.5:2.5) as mobile. Product was collected by the same way as in conventional methods.

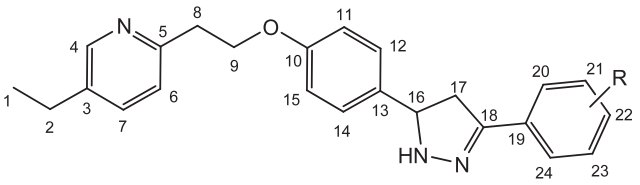


Fig. 1 2-Pyrazolines **2a**–**j**.

Compounds **2(b–j)** have also been synthesized by using the similar method. (Fig. 1)

2.2.1. 5-Ethyl-2-{2-[4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy]ethyl}pyridine (**2a**)

m.p. 127–129 °C; IR spectrum (cm^{-1}): 3268 (N–H), 2923, 2815 (C–H, asym, sym), 1617 (>C=N), 1220, 1034 (C–O–C). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.13 (t, 3H, –CH_3), 2.54 (q, 2H, –CH_2–), 3.17 (t, 2H, –CH_2–), 3.29–3.34 (dd, 1H, pyrazoline), 3.65–3.72 (dd, 1H, pyrazoline), 4.33 (t, 2H, –CH_2–O), 5.87–5.91 (dd, 1H, pyrazoline), 6.98–7.70 (m, 9H, Ar–H), 8.23 (s, 1H, –NH), 7.18–8.32 (m, 3H, Pyridine-H). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ: 18.72 (C-1), 25.90 (C-2), 35.61 (C-8), 46.76 (C-17), 55.20 (C-16), 69.20 (C-9), 114.80 (C-11,15), 123.92 (C-6), 125.61 (C-12,14), 127.93 (C-20,24), 128.71 (C-21,23), 131.97 (C-22), 134.71 (C-7), 135.94 (C-3), 136.73 (C-13), 137.86 (C-19), 148.22 (C-4), 153.40 (C-18), 159.23 (C-5), 161.28 (C-10). MS: *m*/*z* 371.18 (M$^+$). Elemental analyses data of $C_{24}H_{25}N_3O$: calculated, %: C, 77.60; H, 6.68; N, 11.31; found, %: C, 77.62; H, 6.66; N, 11.29.

2.2.2. 5-Ethyl-2-{2-[4-(3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy]ethyl} pyridine (**2b**)

m.p. 133–135 °C; IR spectrum (cm^{-1}): 3273 (N–H), 2927, 2832 (C–H, asym, sym), 1624 (>C=N), 1218, 1041 (C–O–C). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.15 (t, 3H, –CH_3), 2.57 (q, 2H, –CH_2–), 3.12 (t, 2H, –CH_2–), 3.27–3.31 (dd, 1H, pyrazoline), 3.67–3.74 (dd, 1H, pyrazoline), 3.80 (s, 3H, –OCH_3), 4.37 (t, 2H, –CH_2–O), 5.89–5.93 (dd, 1H, pyrazoline), 7.16–8.27 (m, 3H, Pyridine-H), 7.05–7.82 (m, 8H, Ar–H), 8.21 (s, 1H, –NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ: 18.68 (C-1), 25.79 (C-2), 35.56 (C-8), 46.71 (C-17), 52.55 (-OCH_3), 55.23 (C-16), 69.23 (C-9), 114.83 (C-11,15), 115.72 (C-21,23), 123.92 (C-6), 125.67 (C-12,14), 128.12 (C-20,24), 129.76 (C-19), 134.76 (C-7), 135.82 (C-3), 136.65 (C-13), 147.25 (C-4), 153.42 (C-18), 159.18 (C-5), 161.18 (C-10), 164.93 (C-22). MS: *m*/*z* 401.15 (M$^+$). Elemental analyses data of $C_{25}H_{27}N_3O_2$; calculated, %: C, 74.79; H, 6.78; N, 10.47; found, %: C, 74.77; H, 6.75; N, 10.45.

2.2.3. 5-Ethyl-2-{2-[4-(3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy]ethyl} pyridine (**2c**)

m.p. 112–114 °C; IR spectrum (cm^{-1}): 3265 (N–H), 2930, 2835 (C–H, asym, sym), 1618 (>C=N), 1221, 1035 (C–O–C), 1212

(C–F). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.16 (t, 3H, –CH_3), 2.54 (q, 2H, –CH_2–), 3.10 (t, 2H, –CH_2–), 3.25–3.29 (dd, 1H, pyrazoline), 3.69–3.76 (dd, 1H, pyrazoline), 4.35 (t, 2H, –CH_2–O), 5.87–5.91 (dd, 1H, pyrazoline), 7.05–7.79 (m, 8H, Ar–H), 7.19–8.31 (m, 3H, Pyridine-H), 8.27 (s, 1H, –NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ: 18.68 (C-1), 25.79 (C-2), 35.56 (C-8), 46.71 (C-17), 55.23 (C-16), 69.23 (C-9), 114.83 (C-11,15), 116.43 (C-21,23), 123.92 (C-6), 125.67 (C-12,14), 130.32 (C-20,24), 132.16 (C-19), 134.76 (C-7), 135.82 (C-3), 136.65 (C-13), 147.25 (C-4), 153.42 (C-18), 159.18 (C-5), 161.18 (C-10), 167.91 (C-22). MS: m/z 389.19 (M^+). Elemental analyses data of $C_{24}H_{24}FN_3O$: calculated, %: C, 74.01; H, 6.21; N, 10.79; found, %: C, 74.03; H, 6.19; N, 10.81.

*2.2.4. 5-Ethyl-2-{2-[4-(3-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy]ethyl} pyridine (**2d**)*

m.p. 145–147 °C; IR spectrum (cm^{-1}): 3264 (N–H), 2927, 2836 (C–H, asym, sym), 1621 (>C=N), 1215, 1030 (C–O–C), 760 (C–Cl). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.12 (t, 3H, –CH_3), 2.57 (q, 2H, –CH_2–), 3.12 (t, 2H, –CH_2–), 3.29–3.33 (dd, 1H, pyrazoline), 3.61–3.70 (dd, 1H, pyrazoline), 4.31 (t, 2H, –CH_2–O), 5.82–5.87 (dd, 1H, pyrazoline), 7.00–7.76 (m, 7H, Ar–H), 7.21–8.21 (m, 3H, Pyridine-H), 8.29 (s, 1H, –NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ: 18.62 (C-1), 25.76 (C-2), 35.45 (C-8), 46.69 (C-17), 55.22 (C-16), 69.24 (C-9), 114.72 (C-11,15), 123.88 (C-6), 125.58 (C-12,14), 126.68 (C-20), 128.91 (C-21), 131.98 (C-22), 133.35 (C-23), 134.71 (C-7), 135.18 (C-24), 135.76 (C-3), 136.62 (C-13), 137.74 (C-19), 147.21 (C-4), 153.34 (C-18), 159.11 (C-5), 161.10 (C-10). MS: m/z 439.12 (M^+), 441.15 ($M^+ + 2$), 443.17 ($M^+ + 4$). Elemental analyses data of $C_{24}H_{23}Cl_2N_3O$: calculated, %: C, 65.46; H, 5.26; N, 9.54; found, %: C, 65.44; H, 5.28; N, 9.56.

*2.2.5. 5-Ethyl-2-{2-[4-(3-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy]ethyl} pyridine (**2e**)*

m.p. 137–139 °C; IR spectrum (cm^{-1}): 3325 (O–H), 3261 (N–H), 2921, 2841 (C–H, asym, sym), 1612 (>C=N), 1204, 1018 (C–O–C). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.12 (t, 3H, –CH_3), 2.59 (q, 2H, –CH_2–), 3.15 (t, 2H, –CH_2–), 3.25–3.29 (dd, 1H, pyrazoline), 3.65–3.74 (dd, 1H, pyrazoline), 4.28 (t, 2H, –CH_2–O), 5.76–5.81 (dd, 1H, pyrazoline), 7.04–7.69 (m, 8H, Ar–H), 7.15–8.24 (m, 3H, Pyridine-H), 8.71 (s, 1H, –NH), 9.01 (s, 1H, –OH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ: 18.58 (C-1), 25.71 (C-2), 35.43 (C-8), 46.62 (C-17), 55.18 (C-16), 69.21 (C-9), 114.65 (C-11,15), 118.65 (C-21,23), 123.82 (C-6), 125.48 (C-12,14), 128.65 (C-19), 129.62 (C-20,24), 134.65 (C-7), 135.71 (C-3), 136.68 (C-13), 147.12 (C-4), 153.38 (C-18), 159.21 (C-5), 161.28 (C-10), 163.85 (C-22). MS: m/z 387.19 (M^+). Elemental analyses data of $C_{24}H_{25}N_3O_2$: calculated, %: C, 74.39; H, 6.50; N, 10.84; found, %: C, 74.36; H, 6.47; N, 10.83.

*2.2.6. 5-Ethyl-2-{2-[4-(3-(3,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy]ethyl} pyridine (**2f**)*

m.p. 156–158 °C; IR spectrum (cm^{-1}): 3272 (N–H), 2928, 2832 (C–H, asym, sym), 1622 (>C=N), 1212, 1021 (C–O–C), 765 (C–Cl). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.11 (t, 3H, –CH_3), 2.61 (q, 2H, –CH_2–), 3.12 (t, 2H, –CH_2–), 3.27–3.31 (dd, 1H, pyrazoline), 3.61–3.70 (dd, 1H, pyrazoline), 4.31 (t, 2H, –CH_2–O), 5.78–5.83 (dd, 1H, pyrazoline), 6.96–7.71 (m, 7H, Ar–H), 7.18–8.25 (m, 3H, Pyridine-H), 8.67 (s, 1H, –NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ: 18.62 (C-1), 25.65 (C-2), 35.53 (C-8), 46.61 (C-17), 55.23 (C-16), 69.25 (C-9), 114.69 (C-11,15), 123.85 (C-6), 125.52 (C-12,14), 126.18 (C-24), 130.63 (C-20), 130.85 (C-23), 132.92 (C-21), 133.71 (C-19), 134.58 (C-7), 135.67 (C-3), 136.12 (C-22), 136.78 (C-13), 147.18 (C-4), 153.45 (C-18), 159.31 (C-5), 161.29 (C-10). MS: m/z 439.15 (M^+), 441.17 ($M^+ + 2$), 444.16 ($M^+ + 4$). Elemental analyses data of $C_{24}H_{23}Cl_2N_3O$: calculated, %: C, 65.46; H, 5.26; N, 9.54; found, %: C, 65.48; H, 5.26; N, 9.52.

*2.2.7. 5-Ethyl-2-{2-[4-(3-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl) phenoxy]ethyl} pyridine (**2g**)*

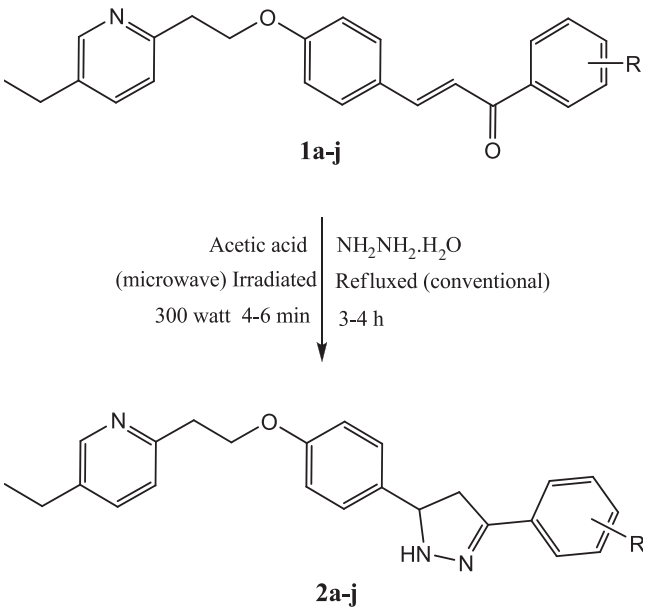
m.p. 161–163 °C; IR spectrum (cm^{-1}): 3262 (N–H), 2927, 2821 (C–H, asym, sym), 1618 (>C=N), 1212, 1028 (C–O–C). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.18 (t, 3H, –CH_3), 2.58 (q, 2H, –CH_2–), 3.18 (t, 2H, –CH_2–), 3.26–3.30 (dd, 1H, pyrazoline), 3.63–3.73 (dd, 1H, pyrazoline), 3.82 (s, 3H, –OCH_3), 4.26 (t, 2H, –CH_2–O), 5.76–5.81 (dd, 1H, pyrazoline), 7.04–7.72 (m, 8H, Ar–H), 7.12–8.29 (m, 3H, Pyridine-H), 8.56 (s, 1H, –NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ: 18.67 (C-1), 25.69 (C-2), 35.51 (C-8), 46.58 (C-17), 55.21 (C-16), 69.28 (C-9), 112.59 (C-20), 114.62 (C-11,15), 116.78 (C-22), 120.59 (C-24), 123.81 (C-6), 125.48 (C-12,14), 130.17 (C-23), 134.51 (C-7), 135.62 (C-3), 136.71 (C-13), 137.79 (C-19), 147.25 (C-4), 153.48 (C-18), 159.21 (C-5), 161.35 (C-10), 162.86 (C-21). MS: m/z 401.17 (M^+). Elemental analyses data of $C_{25}H_{27}N_3O_2$: calculated, %: C, 74.79; H, 6.78; N, 10.47; found, %: C, 74.75; H, 6.81; N, 10.50.

*2.2.8. 5-Ethyl-2-{2-[4-(3-(3-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy]ethyl} pyridine (**2h**)*

m.p. 168–169 °C; IR spectrum (cm^{-1}): 3238 (N–H), 2931, 2829 (C–H, asym, sym), 1618 (>C=N), 1212, 1022 (C–O–C), 1225 (C–F). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.11 (t, 3H, –CH_3), 2.61 (q, 2H, –CH_2–), 3.23 (t, 2H, –CH_2–), 3.22–3.26 (dd, 1H, pyrazoline), 3.68–3.77 (dd, 1H, pyrazoline), 4.31 (t, 2H, –CH_2–O), 5.72–5.77 (dd, 1H, pyrazoline), 7.02–7.65 (m, 8H, Ar–H), 7.08–8.30 (m, 3H, Pyridine-H), 8.51 (s, 1H, –NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ: 18.57 (C-1), 25.64 (C-2), 35.45 (C-8), 46.52 (C-17), 55.27 (C-16), 69.21 (C-9), 113.56 (C-20), 114.68 (C-11,15), 117.72 (C-22), 123.78 (C-6), 124.12 (C-24), 125.42 (C-12,14), 129.92 (C-23), 134.45 (C-7), 135.58 (C-3), 136.67 (C-13), 137.74 (C-19), 147.21 (C-4), 153.43 (C-18), 159.17 (C-5), 161.28 (C-10), 163.69 (C-21). MS: m/z 389.15 (M^+). Elemental analyses data of $C_{24}H_{24}FN_3O$: calculated, %: C, 74.01; H, 6.21; N, 10.79; found, %: C, 74.05; H, 6.24; N, 10.76.

*2.2.9. 5-Ethyl-2-{2-[4-(3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy]ethyl} pyridine (**2i**)*

m.p. 118–119 °C; IR spectrum (cm^{-1}): 3242 (N–H), 2928, 2834 (C–H, asym, sym), 1612 (>C=N), 1202, 1018 (C–O–C), 766 (C–Cl). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.15 (t, 3H, –CH_3), 2.57 (q, 2H, –CH_2–), 3.27 (t, 2H, –CH_2–), 3.21–3.25 (dd, 1H, pyrazoline), 3.70–3.78 (dd, 1H, pyrazoline), 4.25 (t, 2H, –CH_2–O), 5.68–5.73 (dd, 1H, pyrazoline), 6.94–7.64 (m, 8H, Ar–H), 7.12–8.20 (m, 3H, Pyridine-H), 8.49 (s, 1H, –NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ:



Scheme 1 Synthesis of 2-pyrazolines from chalcones via conventional and microwave irradiated techniques.

18.62 (C-1), 25.61 (C-2), 35.42 (C-8), 46.48 (C-17), 55.31 (C-16), 69.27 (C-9), 114.65 (C-11,15), 123.74 (C-6), 125.35 (C-12,14), 127.45 (C-20,24), 128.61 (C-21,23), 134.32 (C-7), 135.48 (C-3), 136.57 (C-13), 137.72 (C-19), 138.65 (C-22), 147.18 (C-4), 153.46 (C-18), 159.21 (C-5), 161.31 (C-10). MS: *m/z* 405.16 (M^+), 407.18 ($M^+ + 2$). Elemental analyses data of $C_{24}H_{24}ClN_3O$: calculated, %: C, 71.01; H, 5.96; N, 10.35; found, %: C, 71.03; H, 5.94; N, 10.37.

*2.2.10. 5-Ethyl-2-{2-[4-(3-(2,4-difluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy] ethyl} pyridine (**2j**)*

m.p. 149–151 °C; IR spectrum (cm^{-1}): 3232 (N–H), 2912, 2831 (C–H, asym, sym), 1622 (>C=N), 1201, 1023 (C–O–C), 1212 (C–F). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS), δ: 1.18 (t, 3H, $–CH_3$), 2.48 (q, 2H, $–CH_2–$), 3.29 (t, 2H, $–CH_2–$), 3.23–3.27 (dd, 1H, pyrazoline), 3.67–3.75 (dd, 1H, pyrazoline), 4.28 (t, 2H, $–CH_2–O$), 5.66–5.71 (dd, 1H, pyrazoline), 6.98–7.79 (m, 7H, Ar–H), 7.10–8.20 (m, 3H, Pyridine-H), 8.32 (s, 1H, –NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS), δ: 18.54 (C-1), 25.52 (C-2), 35.37 (C-8), 46.41 (C-17), 55.28 (C-16), 69.25 (C-9), 111.51 (C-23), 112.68 (C-21), 113.68 (C-19), 114.71 (C-11,15), 123.72 (C-6), 125.21 (C-12,14), 132.34 (C-24), 134.26 (C-7), 135.38 (C-3), 136.47 (C-13), 147.21 (C-4), 153.36 (C-18), 159.11 (C-5), 161.23 (C-10), 162.34 (C-20), 165.15 (C-22), MS: *m/z* 407.18 (M^+). Elemental analyses data of $C_{24}H_{23}F_2N_3O$: calculated, %: C, 70.75; H, 5.69; N, 10.31; found, %: C, 70.73; H, 5.71; N, 10.33.

3. Result and discussion

3.1. Chemistry

Cyclization of chalcone with hydrazine hydrate in conventional and microwave methods has been described in Scheme 1. Comparison of conventional and microwave techniques, in terms of time and yields has been described in Table 1. Spectral and elemental analyses data conformed the structure of compounds **2a**–**j**. In IR spectra an absorption band at 3232 cm^{-1} was observed for N–H stretching. Absorption band at 2928, 2812 cm^{-1} was observed for the methylene group. Absorption bands for functional groups like 1552, 1330 cm^{-1} ($–NO_2$ asym, sym), 1220 cm^{-1} (C–F), 1215, 1028 cm^{-1} (C–O–C), 767 cm^{-1} (C–Cl) were observed. In ^{1}H NMR spectra of **2a**–**j** three double doublets observed at δ 3.23–3.27, 3.67–3.75 and 5.66–5.71 conformed the cyclization of chalcones to 2-pyrazolines. Singlets at δ 3.78 for methoxy and 9.1 for hydroxy group were also observed. ^{13}C NMR spectra for compounds **2a**–**j** were in accordance with expected shifts. Mass spectra of compounds **2a**–**j** were collected and the molecular ion peak was found correct according to the molecular formula.

3.2. Antibacterial and antifungal activities

Antimicrobial activity of synthesized compounds is reported in Table 2. Compounds were screened for *in vitro* against two Gram positive (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442) and two Gram negative (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 741) bacteria for antibacterial and three fungal species (*Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323) for antifungal activity respectively using the broth microdilution method (Rattan, 2005). Minimum inhibitory concentration (MIC) was determined and compared with standard drugs ampicillin for antibacterial activity while greseofulvin and nystatin for antifungal activity.

Compounds **2b** (R = 4-OCH_3), **2d** (R = 2,4-di-Cl) and **2j** (R = 2,4-di-F) showed good to comparable activity

Table 1 Comparison of conventional and microwave techniques.

Comp. No.	R	Irradiation in Microwave		Conventional Refluxed	
		Time (min)	Yield %	Time (hour)	Yield %
2a	-H	4.2	87	4.4	76
2b	4-OCH_3	4.8	84	3.8	78
2c	4-F	6.5	89	5.1	75
2d	2,4-di-Cl	6.2	86	5.8	72
2e	4-OH	3.8	85	5.2	79
2f	3,4-di-Cl	4.5	84	4.6	73
2g	3-OCH_3	5.6	83	4.9	74
2h	3-F	4.0	82	4.2	76
2i	4-Cl	5.2	89	5.1	73
2j	2,4-di-F	5.7	85	5.5	76

Table 2 Antimicrobial data of compounds **2a–j**.

Compd. No.	R	Minimum Inhibition Concentration in μg/ml						
		Gram negative		Gram negative		Fungal species		
		E. coli	*P. aeruginosa*	*S. aureus*	*S. pyogenes*	*C. albicans*	*A. niger*	*A. clavatus*
2a	-H	500	500	200	200	>1000	>1000	>1000
2b	4-OCH_3	100	200	250	250	>1000	200	200
2c	4-F	200	200	500	500	>1000	500	500
2d	2,4-di-Cl	100	62.5	500	500	250	1000	1000
2e	4-OH	200	100	250	250	500	1000	1000
2f	3,4-di-Cl	200	200	250	250	100	500	500
2g	3-OCH_3	250	250	500	500	250	500	500
2h	3-F	250	250	200	200	200	500	500
2i	4-Cl	200	200	200	200	500	500	500
2j	2,4-di-F	125	250	100	100	500	1000	1000
Ampicillin		100	100	250	100	–	–	–
Griseofulvin		–	–	–		500	100	100
Nystatin		–	–	–		100	100	100

(MIC = 100–125 μg/ml) whereas **2c** (R = 4-F), **2e** (R = 4-OH), **2f** (R = 3,4-di-Cl) and **2i** (R = 4-Cl) showed a moderate activity (MIC = 200 μg/ml) against *E. coli*. Compounds **2d** (R = 2,4-di-Cl) and **2e** (R = 4-OH) exhibited good activity (MIC = 62.5–100 μg/ml) whereas **2b** (R = 4-OCH_3), **2c** (R = 4-F), **2f** (R = 3,4-di-Cl) and **2i** (R = 4-Cl) showed a moderate activity (MIC = 200 μg/ml) against *P. aeruginosa*. Compounds **2a** (R = -H), **2b** (R = 4-OCH_3), **2e** (R = 4-OH), **2f** (R = 3,4-di-Cl), **2h** (R = 3-F), **2i** (R = 4-Cl) and **2j** (R = 2,4-di-F) showed good to very good activity (MIC = 100–250 μg/ml) against *S. aureus*. None of the compounds except **2j** (R = 2,4-di-F) showed good activity (MIC = 100 μg/ml) against *S. pyogenes*.

Compounds **2d** (R = 2,4-di-Cl), **2e** (4-OH), **2f** (R = 3,4-di-Cl), **2g** (3-OCH_3), **2h** (R = 3-F), **2i** (R = 4-Cl) and **2j** (R = 2,4-di-F) displayed very good to good activity (MIC = 100–500 μg/ml) against *C. albicans*, when compared with standard drug griseofulvin. Compound **2f** (3,4-di-Cl) showed good to comparable activity (MIC = 100–125 μg/ml) against *C. albicans* when compared with nystatin. None of the synthesized compounds showed good activity against other two fungal species *A. niger* and *A. clavatus*.

4. Conclusion

A series of 2-pyrazolines have been synthesized from cyclization of chalcones by a conventional heating approach as well as non-conventional microwave irradiated solvent free conditions and evaluated as antibacterial and antifungal agents. Most of the compounds were found active against *S. aureus* as antibacterial while against *C. albicans* as antifungal agents. The non-conventional protocol offers several advantages such as simple procedure, fast reaction rate, mild reaction conditions and improved yields compared to conventional methods.

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