MICROWAVE-INDUCED, EFFICIENT, CONVENIENT AND RAPID SYNTHESIS OF SUBSTITUTED 2-PYRAZOLINES AS POTENTIALLY ANTIMICROBIAL AGENT

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Keywords: 2-Pyrazolines; 2-methoxyethanol; 2'-hydroxychalcones; microwave irradiation; antimicrobial activity;

Microwave-induced an efficient, rapid and environmentally begin condensation of substituted 2'-hydroxychalcones with hydrazine hydrate in 2-methoxyethanol afford 2-pyrazolines 2a-h in high 88-95% yields. The structure of newly synthesized compounds established on the basis of spectroscopic technique and laboratory chemical test. Further these compounds were screened for antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, Aspergillus flavus and Candida albicans. The most of the compounds shows good to better inhibitory activity.

Introduction

The use of the hazardous reagent, solvent and high loading catalyst in synthetic organic chemistry leads to impact on environment. Due to this varied nature of the chemicals, chemist requires various greener pathways in our quest towards attaining sustainability.1 Microwave (MW) irradiation is one of them rout has been gaining increased popularity as an alternative heat source in green chemistry towards development of organic synthesis.2 This technique has been applied to a variety of reactions resulting in reduction of reaction time, higher yield, greater selectivity, cleaner reaction products, and to reduce tedious job for isolation of product.3 It also provides an opportunity to work with open vessels, thus avoiding the risk of high pressure and hazards of inflammable solvents.4

Among a wide range of biologically active heterocycles, a significant amount of research activity has been directed towards the study of pyrazolines. A number of pyrazoline derivatives have been found to exhibit physiological and pharmacological properties such as antiinflammatory,5-6 antibacterial,7 antineoplastic,8 antiallergic,9 analgesic,10 and hypoglycemic11 activities. Classical route for the preparation of these compounds involve the condensation of α,β-unsaturated carbonyl compounds with hydrazines.12 The various modified methods have been reported for synthesis of 2-pyrazolines using different catalysts such as KHSO4/H2O/SiO2,13 porous calcium hydroxyapatite,14 chlorohaine-T,15 mercuric acetate,16 Bi(NO3)3·5H2O,17 Zn,18 H3PW12O40,19 and Lewis acid/Lewis bases.20 Many of these procedure involve combinations of solvents with various catalyst and long reaction time make these methods environmentally hazardous, economical expensive. Chemist require to improve these methods towards organic synthesis and reaction, increasing attention is being focused on green chemistry using environmentally benign reagents and conditions, particularly non-conventional heating. In view of these observations, it was thought worthwhile to synthesize 2-pyrazoline derivatives from α,β-unsaturated carbonyl compounds in 2-methoxyethanol by using nonconventional tool.

Methods and Materials

Instrumentation

Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer spectrometer. 1H NMR spectra were recorded on a Gemini 300 MHz instrument in DMSO-d6 as solvent and TMS as an internal standard. The mass spectra were recorded on Shimadzu GC/MS spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 CHN elemental analyzer. A multimode microwave oven (2450 MHz, 300 W, Brand LG, India) were used for performing reaction. Reaction progress monitored on TLC using usinghexane/ethyl acetate/petroleum ether combination as the mobile phase.

Typical procedure for synthesis of 2-pyrazolines

A mixture of 3-(3-bromo-4,5-dimethoxyphenyl)-1-(4-bromo-1-hydroxy-naphthalen-2-yl)propanone (10 mmol) and NH2NH2·H2O (50 mmol) dissolved in 2-methoxyethanol (5 ml) in round bottom flask. To this reaction solution 0.001 mmol of glacial acetic acid was added. The resulting reaction mixtures were irradiated in microwave oven for 2-3 minutes with short interval of 20 seconds to avoid the excessive evaporation of solvent. Progress of reaction was monitored on TLC (mixture of hexane, ethyl acetate, petroleum ether). The separated solid was filtered and recrystallized from ethyl alcohol to give 2c. Physical data of synthesized compounds 2a-h are given in Table 1.
Table 1. Synthesis of substituted 2-pyrazolines under microwave irradiation

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Time, min.</th>
<th>Melting point, °C</th>
<th>Yield, %</th>
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<tr>
<td>2a</td>
<td>H</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>4</td>
<td>148-150</td>
<td>91</td>
</tr>
<tr>
<td>2b</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>Br</td>
<td>5-6</td>
<td>161-163</td>
<td>90</td>
</tr>
<tr>
<td>2c</td>
<td>Br</td>
<td>OMe</td>
<td>OMe</td>
<td>Br</td>
<td>2-3</td>
<td>177-179</td>
<td>92</td>
</tr>
<tr>
<td>2d</td>
<td>I</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>3</td>
<td>154</td>
<td>88</td>
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<tr>
<td>2e</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>H</td>
<td>4-5</td>
<td>130</td>
<td>93</td>
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<tr>
<td>2f</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>3-4</td>
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<td>I</td>
<td>OMe</td>
<td>OMe</td>
<td>Br</td>
<td>4</td>
<td>188</td>
<td>89</td>
</tr>
<tr>
<td>2h</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>OMe</td>
<td>5-6</td>
<td>190-192</td>
<td>88</td>
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</tbody>
</table>

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2-[5-(4-Methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]naphthalen-1-ol (2a)

UV/VIS (λ_{max}, nm): 413, 328. IR (KBr pellets): 1590 (C=O), 1474, 1542 (C=C), 1234 (C-N) cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆) δ 12.45 (s, 1H, OH), 7.81-7.64 (m, 10H, Ar-H), 6.82 (s, 1H, NH), 3.26 (dd, J = 5.0, 17.4 Hz, 1H, Hα), δ 3.64 (dd, J = 12.1, 17.5 Hz, 1H, Hδ), δ 4.82 (dd, J = 5.0, 12.1 Hz, 1H, Hδ), 3.77 (s, 3H, OCH₃). ¹³CNMR (DMSO): 160.15 (C of Ar-OCH₃), 154.85 (C of Ar-OH), 152.29 (C of C=N), 138.28 (Ar-C), 137.92 (Ar-C), 136.26 (Ar-C), 135.88 (Ar-C), 134.74 (Ar-C), 128.64 (Ar-C), 128.49 (Ar-C), 127.75 (Ar-C), 127.70 (Ar-C), 126.93 (Ar-C), 126.80 (Ar-C), 124.19 (Ar-C), 122.38 (Ar-C), 117.26 (Ar-C), 56.74 (C of OCH₃), 52.10 (C of CH), 44.75 (C of CH₂). MS (EI, m/z (%): 318(M⁺, 68 %). Anal.Calcd. for C₂₀H₁₉O₂N₂: C, 75.47; H, 5.66. Found: C, 75.55; H, 5.59.

4-Iodo-2-[5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]naphthalen-1-ol (2d)

UV/VIS (λ_{max}, nm): 413, 331. IR (KBr pellets):1590 (C=O), 1479, 1544 (C=C), 1231 (C-N) cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆) δ 12.56 (s, 1H, OH), 7.80-7.36 (m, 9H, Ar-H), 6.85 (s, 1H, NH), 3.28 (dd, J = 5.1, 17.5 Hz, 1H, Hα), δ 3.66 (dd, J = 12.1, 17.5 Hz, 1H, Hδ), δ 4.87 (dd, J = 5.1, 12.1 Hz, 1H, Hδ), 3.74 (s, 3H, OCH₃). ¹³CNMR (DMSO): 159.97 (C of Ar-OCH₃), 155.13 (C of Ar-OH), 152.34 (C of C=N), 137.87 (Ar-C), 137.94 (Ar-C), 134.86 (Ar-C), 136.85 (Ar-C), 135.67 (Ar-C), 128.45 (Ar-C), 128.79 (Ar-C), 127.18 (Ar-C), 126.92 (Ar-C), 126.77 (Ar-C), 124.28 (Ar-C), 123.63 (Ar-C), 108.15 (C of Ar-I), 56.73 (C of OCH₃), 52.17 (C of CH), 44.62 (C of CH₂).MS (EI, m/z (%): 444 (M⁺, 90 %). Anal.Calcd. for C₂₀H₁₉O₂N₂I: C, 54.05; H, 3.82; X (I), 28.60. Found: C, 54.18; H, 3.79; X (I), 28.67.

4-Bromo-2-[5-(3-bromo-4,5-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]naphthalen-1-ol (2e)

UV/VIS (λ_{max}, nm): 411, 329. IR (KBr pellets):1594 (C=O), 1472, 1543 (C=C), 1231 (C-N) cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆) δ 12.61 (s, 1H, OH), 7.89-7.46 (m, 7H, Ar-H), 6.83 (s, 1H, NH), 3.26 (dd, J = 5.1, 17.5 Hz, 1H, Hα), δ 3.65 (dd, J = 12.0, 17.5 Hz, 1H, Hδ), δ 4.88 (dd, J = 5.1, 12.1 Hz, 1H, Hα), 3.86 (s, 3H, OCH₃), 3.78 (s, 3H, OCH₃). ¹³CNMR (DMSO): 155.23 (C of Ar-OH), 152.72 (C of C=N), 152.37 (C of Ar-OCH₃), 149.45 (C of Ar-OCH₃), 139.29 (Ar-C), 137.13 (Ar-C), 129.46 (Ar-C), 128.88 (Ar-C), 127.47 (Ar-C), 126.30 (Ar-C), 124.97 (Ar-C), 121.73 (Ar-C), 118.28 (Ar-C), 116.71 (Ar-C), 116.39 (Ar-C), 115.59 (C of Ar-Br), 109.22 (C of Ar-Br), 57.92 (C of OCH₃), 56.45 (C of OCH₃), 53.27 (C of CH₂), 43.98 (C of CH₂). MS (EI, m/z (%): 506 (M⁺, 78 %). Anal.Calcd. for C₂₁H₁₈O₂N₂Br₂: C, 49.80; H, 3.55; X (Br), 31.62. Found: C, 49.74; H, 3.58; X (Br), 31.68.

2-[5-(4-Fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]4-iodo-naphthalen-1-ol (2e)

UV/VIS (λ_{max}, nm): 410, 330. IR (KBr pellets):1588 (C=O), 1468, 1542 (C=C), 1228 (C-N) cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆) δ 12.48 (s, 1H, OH), 7.88-7.37 (m, 9H, Ar-H), 6.87 (s, 1H, NH), 3.30 (dd, J = 5.2, 17.6 Hz, 1H, Hα), δ 3.70 (dd, J = 12.1, 17.6 Hz, 1H, Hδ), δ 4.89 (dd, J = 5.2, 12.1 Hz, 1H, Hδ). ¹³CNMR (DMSO): 155.23 (C of Ar-OH), 152.48 (C of C=N), 137.18 (Ar-C), 137.82 (Ar-C), 136.25 (Ar-C), 136.96 (Ar-C), 135.27 (Ar-C), 129.31 (Ar-C), 129.57 (Ar-C), 128.62 (Ar-C), 127.19 (Ar-C), 127.91 (Ar-C), 126.73 (Ar-C), 125.57 (Ar-C), 124.85 (Ar-C).
2-[5-(4-Chloro-phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-4-iodonaphthalen-1-ol (2f)

UV/VIS (λmax, nm): 409, 328. IR (KBr pellets): 1590 (C=O), 1475, 1552 (C=O), 1232 (C=N) cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆) δ 12.56 (s, 1H, OH), 7.92-7.39 (m, 9H, Ar-H), 6.90 (s, 1H, NH), 3.28 (dd, J = 5.1, 17.6 Hz, 1H, H₃), δ 3.69 (dd, J = 12.1, 17.6 Hz, 1H, H₅a), δ 4.87 (dd, J = 5.1, 12.1 Hz, 1H, H₅), 13CNMR (DMSO): 156.14 (C of Ar-OH), 152.67 (C of C=N), 138.41 (Ar-C), 138.63 (Ar-C), 136.87 (Ar-C), 135.19 (Ar-C), 135.54 (Ar-C), 133.27 (Ar-C), 131.39 (Ar-C), 128.50 (Ar-Cl), 127.47 (Ar-Cl), 127.98 (Ar-Cl), 126.25 (Ar-Cl), 125.52 (Ar-Cl), 124.90 (Ar-Cl), 122.69 (Ar-Cl), 109.83 (C of Ar-Cl), 53.16 (C of CH₂), 44.65 (C of CH₂), MS (m/z (%): 448 (M⁺, 40 %). Anal.CaCd. for C₁₀H₁₁N₂OCl: C, 50.89; H, 3.12; X (I,Cl), 36.27. Found: C, 50.82; H, 3.15; X (I,Cl), 36.30.

Results and discussion

Synthesis

In continuation of earlier research work on organic heterocyclic synthesis²²-²³ and reported methods for compounds 2a-h using various procedure²⁴-²⁸, we try to investigate herein a convenient route for synthesis of 2-pyrazolines using microwave irradiation. The reaction of 2'-hydroxychalcones with hydrazine in 2-methoxyethanol in presence of glacial acetic acid under MW irradiation gives 2-pyrazolines, Scheme 1.

The combination of 2-methoxyethanol with microwave technique found to be efficient process towards synthesis heterocyclic compounds. Initially, we attempted the condensation of 3-(3-Bromo-4,5-dimethoxy-phenyl)-1-(4-bromo-1-hydroxy-naphthalen-2-yl)-propene (10 mmol) with NH₂NH₂H₂O (50 mmol) using 0.001 mmol of AcOH in 2-methoxyethanol as reaction solvent. The reaction went to completion within 2-3 minutes and corresponding product 2c was obtained in 92% yield. In order to optimize the reaction conditions, we carried out the above reaction in different reaction medium such as ethanol, dichloromethane, dioxane, acetonitrile, DMF and 2-methoxyethanol. Table 2. We found that use of 2-methoxyethanol as an efficient reaction medium in terms of clean reaction conditions, not expensive, higher yields of product and environmentally eco-friendly. As results observed from Table 2, we pay our attention towards various substituted 2'-hydroxy chalcones. In all cases, reaction proceeded efficiently in high yields using 2-methoxyethanol.
The structure characterization of compounds 2a-h was confirmed on the basis of spectral technique, UV, IR, 1H NMR, MS, 13C-NMR and elemental analysis. In IR spectral data of condensed products, compounds 2a-h display disappearance of band at 1625-1635 cm−1 due to C=O of 2'-hydroxychalcone and appearance of a band near 1588 cm−1 due to C=N formed. The N-H stretching band of pyrazoline nucleus appears near 3320 cm−1.

The 1H NMR spectra showing an ABX pattern were observed for Hα, Hb, and Hc proton which appear as pair of doublets near δ 3.28, 3.67, and 4.90 ppm with Jαβ = 17.6 Hz, Jαa = 5.0 Hz, Jαb = 12.1 Hz. The chemical shift for aromatic protons was observed at δ 7.25 ppm and that of N=O stretching found at 1618 cm−1 due to C=N formed. The N=O functionality in the basic structure of pyrazolines respectively.

Antimicrobial activity

The antibacterial activities of the synthesized compounds (2a-h) were determined by agar well diffusion method. Compounds were evaluated for antibacterial activity against Bacillus subtilis [MTCC 2063] and Staphylococcus aureus [MTCC 2901]. The antifungal activity performed against Aspergillus flavus [MTCC 2501] and Candida albicans [MTCC 183] were procured from Institute of Microbial Technology (IMTech), Chandigarh, India. The antibiotic streptomycin (25μ/mL) and fluconazole used as reference drug for antibacterial and antifungal activity, respectively. Dimethyl sulfoxide (1 %, DMSO) used a control without compound.

The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5 °C for 24 h. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85 %) of 105 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of compound solution at fixed concentration 25 μL−1 separately for each bacterial strain. All plates were incubated at 37±0.5 °C for 24 h. Zone of inhibition were noted in mm, Table 2.

The minimum inhibitory concentration values were determined by comparison to standard drugs at 10, 12.5, 25, 50, 100 and 200 μL−1. A lower MIC’s values indicate that less drug is required for inhibiting growth of the organism, therefore, drug with lower MIC scores more effective antimicrobial agent.

Table 2. Optimization of solvent effect on model reaction.a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time, min</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol (10 ml)</td>
<td>12</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>Dichloromethane (10 ml)</td>
<td>18</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>Dioxane (15 ml)</td>
<td>15</td>
<td>68</td>
</tr>
<tr>
<td>4</td>
<td>Acetonitrile (15 ml)</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>DMF (10 ml)</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>2-Methoxyethanol (5 ml)</td>
<td>2-3</td>
<td>92</td>
</tr>
</tbody>
</table>

aReaction of 3-(3-Bromo-4,5-dimethoxyphenyl)-1-(4-bromo-1-hydroxynaphthalen-2-yl)propone (10 mmol) with NH₂NH₂·H₂O (50 mmol) using 0.001 mmol of AcOH under microwave irradiation.

The culture strains of bacteria were maintained on nutrient agar slant at 37 ±0.5 °C for 24-48 hr, until sporulation. Spore of strains were transferred into 5 mL of sterile distilled water containing 1 % Tween-80 (to suspend the spore properly). The spores were counted by haemocytometer (106 CFU/mL). Sterile PDA plate was prepared containing 2 % agar; 0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27±0.2 °C for 7 days except Candida albicans. After incubation, zone of inhibition of compounds were measured in mm along with standard, Table 3.

For antifungal activity, all culture strains of fungi maintained on potato dextrose agar (PDA) slant at 27 ± 0.2 °C for 24-48 hr, until sporulation. Spore of strains were transferred into 5 mL of sterile distilled water containing 1 % Tween-80 (to suspend the spore properly). The spores were counted by haemocytometer (106 CFU/mL). Sterile PDA plate was prepared containing 2 % agar; 0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27±0.2 °C for 7 days except Candida albicans. After incubation, zone of inhibition of compounds were measured in mm along with standard, Table 3.

Table 3. Antimicrobial data of 2-pyrazolines.

| Compound | Zone of inhibition in mm and minimum inhibitory concentration, µg mL−1 |
|----------|------------------------------------------------|------------------|
|          | A | B | C | D |
| 2a       | 23 (50) | 14 (<100) | 14 (100) | 16 (>100) |
| 2b       | 21 (50) | 17 (<100) | 12 (<200) | 10 (>200) |
| 2c       | 24 (25) | 28 (15.5) | 25 (50) | 26 (50) |
| 2d       | 20 (<100) | 21 (100) | 19 (<100) | 19 (<100) |
| 2e       | 23 (50) | 22 (<50) | 21 (<100) | 24 (<50) |
| 2f       | 26 (12.5) | 21 (100) | 10 (200) | 16 (100) |
| 2g       | 28 (10.5) | 30 (<10) | 26 (<50) | 29 (10) |
| 2h       | 25 (12.5) | 26 (50) | 24 (50) | 27 (12.5) |
| Fluconazole | 26 (12.5) | 28 (10) | -- | -- |
| Streptomyacin | -- | -- | 30 (25) | 28 (10.5) |

A: B. subtilis B: S. aureus C: A. flavus D: C. albicans

Conclusion

In summary we developed new methodology towards the synthesis of substituted 2'-pyrazolines from 2'-hydroxy chalcones using 2-methoxyethanol in presence of slightly acidic medium. The combination of microwave with 2-methoxyethanol found to be excellent and convenient reaction route in terms of simple reaction procedure, quick reaction time giving percent yield of product. The preliminary in vitro antimicrobial screening of this series revealed that compounds 2c, 2g and 2h showed potent activity when compared with standard drug. Therefore, the present study is useful drug in medicinal investigation against bacterial and fungal diseases.
Microwave-induced synthesis of 2-pyrazolines as antimicrobial agents

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References


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