Microwave-assisted synthesis and antimicrobial evaluation of 6-[3-aryl-1-phenyl-4′,5′-dihydro[4,5′-bi-1H-pyrazol]-3′-yl]-2H-chromen-5-ols

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Abstract: A new series of 6-[3-aryl-1-phenyl-4′,5′-dihydro[4,5′-bi-1H-pyrazol]-3′-yl]-2H-chromen-5-ol derivatives was synthesized by Michael addition of chalcones 5a–j with hydrazine hydrate in presence of sodium acetate under conventional heating and microwave irradiation. Structural assignment of the products was confirmed based on IR, 1H-NMR, 13C-NMR, MS and analytical data. All the synthesized compounds 6a–j were screened for their antimicrobial activity against various bacterial and fungal strains. Most of the compounds exhibited variable range of antimicrobial activity and compounds 6c–f and 6i showed promising antimicrobial potency.

Keywords: pyrazole; pyrazoline; microwave irradiation; antimicrobial activity.

INTRODUCTION

Heterocyclic compounds containing nitrogen and oxygen play important roles in agrochemical and pharmaceuticals. Heterocyclic compounds have great applicability in pharmaceutics because they have specific chemical reactivity and provide false synthons in biosynthetic processes or block the normal functioning of biological receptors. The interesting biological activities of heterocycles have stimulated considerable research work in recent years, including the synthetic utility. Pyrazoles, an important member of heterocyclic compounds, are widely found as the core structure in a large variety of compounds that possess important agrochemical and pharmaceutical activities. Many pyrazole derivatives are reported to have a broad spectrum of biological activities, such as anti-inflammatory,1,2 antifungal,3 herbicidal,4 insecticidal,5 anti-HIV,6 antiviral,7 anticonvulsant8 and anticancer9 activities. Some of the drugs possessing a pyrazole scaf-
fold, such as celecoxib, rimonabant, deracoxib, and phenylbutazone, exhibiting anti-inflammatory, analgesic and antipyretic activities, are already on the market (Fig. 1).

Pyrazolines are partially reduced form of pyrazoles that contain five-membered ring system with two adjacent nitrogens. Pyrazolines are one of the emerging classes of compounds associated with a broad spectrum of biological activities. Many compounds bearing pyrazoles and their reduced forms pyrazolines constitute an interesting class of heterocycles due to their synthetic versatility and effective biological activities, such as antiviral, anti-inflammatory, antitubercular, anti-amoebic, analgesic, antibacterial, analgesic, antifungal, anti-arthritic, cerebroprotective and antidepressant activities. They are also useful as biodegradable agrochemicals.

A literature survey revealed several synthetic protocols for the synthesis of these compounds and the presence of this core in any molecule plays a key role in enhancing activity. Prompted by the above-mentioned biological properties of pyrazole and pyrazoline incorporated heterocycles, it was contemplated to synthesize a novel series of pyrazole–chromene containing pyrazolines. In continuation to ongoing research, herein, the synthesis of 6-[3-aryl-1-phenyl-4′,5′-dihydro[4,5′-bi-1H-pyrazol]-3′-yl]-2H-chromen-5-ol derivatives in excellent yields is reported.

**EXPERIMENTAL**

**Materials**

All used materials were obtained commercially, mostly from Sigma–Aldrich, and were used without further purification.

**Equipment**

All the microwave irradiation experiments were performed in a CEM Discover microwave system equipped with an IR sensor, with which the reaction temperatures were monitored. All the reactions were monitored on silica gel percolated TLC plates, 60 F254 from Merck and the spots were visualized with UV light. Melting points were determined by the open capillary method and are uncorrected. The 1H-NMR and 13C-NMR spectra were run on a Bruker Avance-400 spectrometer at 400 and 100 MHz, respectively, using tetramethylsilane (TMS) as an internal reference. Mass spectra were recorded on a Shimadzu LCMS 2020 mass spectrometer. Elemental microanalysis was performed on a Perkin Elmer CHN-2400 analyzer.
Spectral and analytical data of the synthesized compounds are given in Supplementary material to this paper.

**General procedure for the synthesis of 6-[3-aryl-1-phenyl-4′,5′-dihydro[4,5′-bi-1H-pyrazol]-3′-yl]-2H-chromen-5-ol derivatives (6a–j)**

**Conventional heating method.** To a solution of 3-(3-aryl-1-phenyl-1H-pyrazol-4-yl)-1-(5-hydroxy-2H-chromen-6-yl)prop-2-en-1-ones 5a–j (0.23 mmol) in DMF (5 mL) containing sodium acetate (0.23 mmol) and hydrazine hydrate (0.23 mmol), few drops of acetic acid was added and the reaction mixture heated at 80–90 °C for 1–3 h. The progress of the reaction was monitored by TLC. After completion of the reaction, ice-cold water was added. The solid product that separated was filtered, washed with water and dried. Recrystallisation from MeOH:CHCl₃ (1:1) afforded 6-[3-aryl-1-phenyl-4′,5′-dihydro[4,5′-bi-1H-pyrazol]-3′-yl]-2H-chromen-5-ols 6a–j. Yield: 57–62 %.

**Microwave irradiation method.** A mixture of 3-(3-aryl-1-phenyl-1H-pyrazol-4-yl)-1-(5-hydroxy-2H-chromen-6-yl)prop-2-en-1-ones 5a–j (0.23 mmol), hydrazine hydrate (0.23 mmol), sodium acetate (0.23 mmol) and few drops of acetic acid in DMF (2 mL) was taken in a glass vessel and then placed into a teflon vial with screw cap and the mixture was subjected to microwave irradiation at 100 W for 1–3 min. After completion of the reaction, indicated by TLC, the vial was cooled and the reaction mixture was poured into ice cold water. The solid product that separated was filtered, washed with water and dried. Recrystallisation from MeOH:CHCl₃ (1:1) furnished 6-[3-aryl-1-phenyl-4′,5′-dihydro[4,5′-bi-1H-pyrazol]-3′-yl]-2H-chromen-5-ol derivatives 6a–j. Yield: 86–96 %.

**Biological assays**

**Antibacterial activity.** The synthesized novel compounds 6a–j were screened for their antibacterial activity against different types of bacterial strains, viz. Gram-positive bacterial strains *Staphylococcus aureus* and *Bacillus subtilis*, and Gram-negative bacterial strains *Escherichia coli* and *Proteus vulgaris* at concentrations of 10 and 20 μg mL⁻¹. The cultures were diluted with 5 % saline, autoclaved, and the final volume was made with a concentration approximately 10⁵–10⁶ CFU mL⁻¹. The synthesized compounds were diluted in acetone for the antibacterial biological assays. For agar disk diffusion method, the solution form of a test compound was allowed to air-dry, such that the disk became completely saturated with the test compound. The saturated chemical disks were introduced onto the upper layer of the medium evenly floated with the bacteria. The disks were dipped in different chemical samples and placed over the evenly spread bacterial nutrient media and incubated at 37 °C for 24–48 h for better inhibition of the bacteria. The zones of inhibition were measured after 24–48 h. All the experiments were performed in triplicate, and the results are expressed as zone of inhibition in mm. The zones of inhibition of synthesized compounds were compared with the zone of inhibition of the standard antibiotic gatifloxacin at concentrations of 10 and 20 μg mL⁻¹.

**Antifungal activity.** The antifungal activities of the synthesized compounds 6a–j were tested against three pathogenic fungi, namely *Fusarium oxysporum*, *Aspergillus niger* and *A. flavus* by the poison plate technique at a concentration of 100 μg/mL. Three kinds of fungi were incubated in potato dextrose agar (PDA) at 25±1 °C for 5 days to obtain new mycelium for the antifungal assay; then the mycelia as disks of approximately 0.45 cm diameter cut from the culture medium were picked up with a sterilized inoculation needle and inoculated in the centre of a PDA plate. The test compounds were dissolved in acetone (10 mL) and then added to the potato dextrose agar medium (PDA, 90 mL). The final concentration of the compounds in the medium was adjusted to 100 μg mL⁻¹. The inoculated plates were incubated at 25±1 °C for 5
days. Acetone was diluted with sterilized distilled water and used as the control, while clotrimazole (100 μg mL\(^{-1}\)) was used as the standard. For each treatment, three replicates of the experiments were performed. The radial growth of the fungal colonies was measured on the sixth day.

RESULTS AND DISCUSSION

The synthetic route for 6-[3-aryl-1-phenyl-4',5'-dihydro[4,5'-bi-1\(H\)-pyrazol]-3'-yl]-2\(H\)-chromen-5-ols is illustrated in Schemes 1 and 2. The synthesis of title compounds involved the preliminary preparation of 1-(5-hydroxy-2\(H\)-chromen-6-yl)ethanone (3). Starting from resacetophenone (1) upon treating with propargyl bromide in the presence of anhydrous K\(_2\)CO\(_3\) in dry acetone yielded 1-(2-hydroxy-4-(prop-2-yn-1-yloxy)phenyl)ethanone (2), which was further refluxed in N,N-dimethylaniline at 180 °C for 3 h to give compound 3\(^{27}\) (Scheme 1). Claisen–Schmidt condensation between 1-(5-hydroxy-2\(H\)-chromen-6-yl)ethanone (3) and substituted pyrazole aldehydes (4a–j) in the presence of powdered KOH under microwave irradiation for 4–7 min (Scheme 2) gave 3-(3-aryl-1-phenyl-1\(H\)-pyrazol-4-yl)-1-(5-hydroxy-2\(H\)-chromen-6-yl)prop-2-en-1-ones\(^{27}\) 5a–j. These chalcones were then cyclised by means of hydrazine hydrate and few drops of glacial acetic acid under conventional heating and microwave irradiation to furnish the title compounds 6a–j in excellent yields.

Scheme 1. Synthesis of 1-(2-hydroxy-4-(prop-2-yn-1-yloxy)phenyl)ethanone (3).

Scheme 2. Synthesis of 6-[3-aryl-1-phenyl-4',5'-dihydro[4,5'-bi-1\(H\)-pyrazol]-3'-yl]-2\(H\)-chromen-5-ols (6a–j).

Preliminarily, the synthesis of compounds 6a–j was carried out under conventional heating method, but this method suffered from poor yields (57–62%).
In order to improve the yields and reduce the reaction time, the synthesis approach was changed to the microwave irradiation method. Microwave-assisted synthesis of title compounds 6a–j is advantageous over conventional method in terms of higher yields in shorter reaction times. A comparison of the yields of the title compounds prepared by the conventional and microwave irradiation methods is demonstrated in Table I.

### TABLE I. Comparison of yields (isolated) of compounds 6a–j under different synthetic conditions

<table>
<thead>
<tr>
<th>Compound</th>
<th>Melting point, °C</th>
<th>Time, h</th>
<th>Yield, %a</th>
<th>Time, min</th>
<th>Yield, %a</th>
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<tbody>
<tr>
<td>6a</td>
<td>98–100</td>
<td>2</td>
<td>61</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>6b</td>
<td>102–104</td>
<td>2</td>
<td>59</td>
<td>1</td>
<td>93</td>
</tr>
<tr>
<td>6c</td>
<td>101–103</td>
<td>2</td>
<td>60</td>
<td>1</td>
<td>94</td>
</tr>
<tr>
<td>6d</td>
<td>94–96</td>
<td>2</td>
<td>60</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>6e</td>
<td>104–106</td>
<td>2</td>
<td>58</td>
<td>2</td>
<td>86</td>
</tr>
<tr>
<td>6f</td>
<td>99–101</td>
<td>2</td>
<td>62</td>
<td>2</td>
<td>96</td>
</tr>
<tr>
<td>6g</td>
<td>108–110</td>
<td>1</td>
<td>62</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>6h</td>
<td>103–105</td>
<td>2</td>
<td>60</td>
<td>2</td>
<td>92</td>
</tr>
<tr>
<td>6i</td>
<td>90–92</td>
<td>2</td>
<td>60</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>6j</td>
<td>136–138</td>
<td>2</td>
<td>57</td>
<td>2</td>
<td>89</td>
</tr>
</tbody>
</table>

*aIsolated yields

Formation of the 6-[3-aryl-1-phenyl-4′,5′-dihydro[4,5′-bi-1H-pyrazol]-3′-yl]-2H-chromen-5-ols (6a–j) were confirmed by IR, 1H-NMR, 13C-NMR, MS and elemental analyses. The IR spectrum of compound 6h showed absorption bands at 3464, 3268 and 1596 cm⁻¹ due to OH, NH and C=N groups, respectively. The 1H-NMR spectrum of 6h displayed two characteristic signals due to the diastereotopic protons (HA, HB). The HA proton, which is cis to HX resonated upfield at δ 3.09 ppm as a doublet of doublets (dd) with J values of 8.87 and 16.24 Hz, while the H9 proton which is trans to HX resonated downfield at δ 3.48 ppm (dd) with J values 10.19, 16.24 Hz. The HX proton which is vicinal to two methylene protons (HA and H9) was observed as doublet of doublets (dd) at δ 5.08 ppm with J values of 8.87 and 10.19 Hz. A triplet appeared at δ 1.43 ppm was due to aliphatic CH3 proton and quartet at δ 4.09 ppm due to Ar–O–CH2. The NH proton appeared at δ 5.90 ppm as a broad singlet. The pyrazole proton appeared as a singlet at δ 7.98 ppm and a broad singlet was observed at δ 11.38 ppm due to the OH proton. In the 13C-NMR spectrum of 6h, CH3, pyrazoline-CH2, pyrazoline CH and Ar-OCH2 carbons appeared at δ 14.8, 40.9, 54.1 and 63.5 ppm, respectively. The mass spectra of 6h showed the molecular ion peak at m/z 479 [M+H]⁺.

### Antimicrobial activity

**Antibacterial activity.** The synthesized compounds were screened in vitro for antibacterial activity against different types of bacterial strains viz. Gram-positive
bacterial strains *Staphylococcus aureus* (ATCC 9144) and *Bacillus subtilis* (ATCC 6633), and Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Proteus vulgaris* at concentrations of 10 and 20 μg mL⁻¹. The inhibitory efficiency of the synthesized compounds was measured through the zone of inhibition (in mm) compared with the standard drug gatifloxacin and the results are presented in Table II. The study of the antibacterial efficiency of the synthesized compounds revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains compared to the standard drug gatifloxacin at concentrations of 10 and 20 μg mL⁻¹. The compounds 6d (Ar = 4-methylphenyl), 6e (Ar = 4-hydroxyphenyl), 6f (Ar = 4-methoxyphenyl) and 6i (Ar = 3,4-dimethoxy-phenyl) showed the equipotent activity through the zone of inhibition (Table II) against *S. aureus*, *B. subtilis*, *E. coli* and *P. vulgaris*, respectively. Compound 6e exhibited the most potent antibacterial activity against Gram-positive and Gram-negative bacterial strains. The remaining compounds showed moderate activity compared to the standard. An analysis of the antibacterial activity results indicated that compound with electron-donating groups, such as methyl, hydroxy and methoxy, on the phenyl ring were more potent as compared to the control drug gatifloxacin.

### TABLE II. Antimicrobial activity of synthesized compounds (zone of inhibition, mm)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
<th>Fungal strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td><em>B. subtilis</em></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>6a</td>
<td>15 26 22 12</td>
<td>15 19 12 9</td>
<td>13.3</td>
</tr>
<tr>
<td>6b</td>
<td>15 25 15 25</td>
<td>13 17 08 14</td>
<td>11.5</td>
</tr>
<tr>
<td>6c</td>
<td>13 23 14 21</td>
<td>10 16 09 11</td>
<td>17.2</td>
</tr>
<tr>
<td>6d</td>
<td>19 31 18 35</td>
<td>16 23 10 17</td>
<td>12.9</td>
</tr>
<tr>
<td>6e</td>
<td>21 34 19 36</td>
<td>18 25 11 18</td>
<td>08.7</td>
</tr>
<tr>
<td>6f</td>
<td>20 32 18 33</td>
<td>16 23 11 17</td>
<td>16.9</td>
</tr>
<tr>
<td>6g</td>
<td>17 28 16 30</td>
<td>16 20 10 16</td>
<td>15.1</td>
</tr>
<tr>
<td>6h</td>
<td>13 21 10 23</td>
<td>12 17 08 12</td>
<td>14.4</td>
</tr>
<tr>
<td>6i</td>
<td>21 33 19 34</td>
<td>17 25 11 16</td>
<td>17.0</td>
</tr>
<tr>
<td>6j</td>
<td>14 21 10 19</td>
<td>09 16 08 12</td>
<td>12.4</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>21 33 20 36</td>
<td>18 25 12 18</td>
<td>–  –  –  –</td>
</tr>
</tbody>
</table>

### Antifungal activity

The antifungal activity of the synthesized compounds was tested against three pathogenic fungi, viz. *Aspergillus flavus*, *A. niger* (ATCC 9029) and * Fusarium oxysporum* by the poison plate technique at a concentration of 100 μg mL⁻¹. Most of the synthesized compounds showed promising antifungal activity compared to standard drug clotrimazole (Table II). The compounds 6c, 6f and 6i
showed equipotent inhibition compared to the standard drug against the tested fungi, whereas the remaining compounds showed moderate activity against the pathogenic fungi.

**CONCLUSION**

In summary, a new series of compounds 6a–j was synthesized under conventional and microwave irradiation conditions. Using the microwave irradiation method, the reactions were completed in short reaction times under mild reaction conditions, in high yields and convenient operation. All the titled compounds were screened for their *in vitro* antimicrobial activity. Compound 6e was found to be the most potent and compounds 6d, 6f and 6i were found to be equipotent compared to the standard drug gatifloxacin against the pathogenic bacteria whereas compounds 6c, 6f and 6i exhibited potent activity against the pathogenic fungi compared to the standard drug clotrimazole at their respective concentrations. The antifungal screening results revealed that compounds 6f and 6i could be considered as promising antifungal drug candidates.

**SUPPLEMENTARY MATERIAL**

Spectral and analytical data of the synthesized compounds are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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