SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME NOVEL 3,4-DISUBSTITUTED PYRAZOLE DERIVATIVES

GANESH AKULA1*, KAUSHAL K CHANDRUL2, BHIKSHAPATHI DVRN3

1Department of Pharmacy, Mewar University, Chittorgarh, Rajasthan, India. 2Department of Pharmacy, Mewar University, Chittorgarh, Rajasthan, India. 3Department of Pharmacy, Mewar University, Chittorgarh, Rajasthan, India. Email: akulaganesh@gmail.com

ABSTRACT

Objective: The objective of the current work was to synthesize a series of 3,4-substituted pyrazoles from the cyclization of substituted aryl ethanone and hydrazine hydrate in a two-step process and screen the derivatives for their antimicrobial activity.

Methods: The title compounds were derived from the condensation of ethanone intermediate with N,N-Dimethyl formamide-dimethyl acetal and hydrazine hydrate. Ethanone intermediate synthesized from substituted methyl phenylacetate in the presence of potassium t-butoxide with 6-methyl pyridine-2-carboxylic acid methyl ester.

Results: The final products were characterized by detailed spectral analysis using Mass, Nuclear Magnetic Resonance, and Infra Red spectroscopy. All the compounds (4a-4j) showed significant antibacterial properties on both Gram-positive and Gram-negative bacteria. Interestingly, the selected microbes were found to be highly sensitive for compound 4a, 4c, 4d, 4h, and 4i. The molecules are also antifungal in nature, and they have a significant inhibitory effect on the growth of Candida albicans and Aspergillus niger.

Conclusion: The results suggest that the developed derivatives bearing the pyrazole nucleus could be the lead structures for the development of antimicrobial agents for fatal infections.

Keywords: Pyrazoles, Condensation, Antibacterial, Antifungal.

INTRODUCTION

Various substituted pyrazole based drugs are acquiring significance in medicinal and natural products research by virtue of their multifarious properties and broad spectrum of applications for various ailments [1]. Specifically, substituted pyrazoles create a critical position in the pharmaceutical industry as they comprise the important structure for many commercial drugs such as sildenafil, celecoxib, and rimonabant. The abundant texts support the ubiquitous nature of aza-heterocyclic compounds, the pyrazole derivatives. They are documented to possess anticancer [2-4], antibacterial [5], antitubercular [8,9], analgesic [10,11], antimalarial [1,12], antipyretic [14], anticonvulsant [15], antidepressant [16], antangiotensin [17], antidiabetic [18], antiviral [19,20], and anti-inflammatory [21-23] activities.

Since the foundation of the primary synthetic method of pyrazole, i.e., Paal-Knorr synthesis by condensation of 1,3-diketones with hydrazine which yields substituted pyrazoles, it is the subject matter for investigators to create the substituted pyrazoles. Due to the convenience and versatility, Paal-Knorr synthesis had been the principal synthetic method for pyrazole synthesis. There are many customary methodologies for the synthesis of various pyrazole derivatives. A lot of literature in correlation to the variation of Paal-Knorr synthesis with better yields, reported by researchers, by usurping one of the starting material 1,3-diketone with acetylenic and olefinic ketone moieties. Individual substances have been cultivated for producing distinct pyrazole crossbreeds. To overwhelm the severe reaction conditions and to have regioselectivity, a basic and beneficial strategy was utilized for the synthesis of substituted pyrazoles.

EXPERIMENTAL

Methods

For the current work, synthetic grade chemicals were utilized and purchased from Sigma-Aldrich. Merck-pre-coated aluminum thin-layer chromatography plates of silica gel 60 F254 of 0.5 thickness were employed and spots were observed under ultraviolet light. Pure compounds were isolated from the crude mixture by the column chromatography and the pure compounds were recrystallized with ethanol. Remi electronic melting point apparatus was employed for the melting points determination. Infrared (IR) spectra were recorded on Agilent Fourier-transform IR by KBr pellet method. Proton nuclear magnetic resonance (1H NMR) recorded on VARIAN – 400 MHz. The splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. MASS recorded on BRUKER ESI-IT MS. The bacterial and fungal strains were obtained from the Department of Microbiology, Osmania University, Hyderabad. The samples were subcultured and preserved at 4°C.

General procedure for the synthesis of pyrazole derivatives [24]

Synthesis of ethanone intermediate

A solution of substituted methyl phenylacetate (15 mmol) potassium t-butoxide (45 mmol) and 6-methyl pyridine-2-carboxylic acid methyl ester (1.5 mmol) in tetrahydrofuran (75 mL) was heated at 65°C for 48 h. The mixture was concentrated under vacuum and treated carefully with concentrated hydrochloric acid (10 mL). The resulting mixture was heated at 100°C for 12 h. The mixture was cooled to room temperature and the pH adjusted to 9.0 with Na hydroxide solution. The mixture was extracted with ethyl acetate, and the organic layer dried and concentrated under vacuum. The final product was purified by column chromatography with hexane/ethylacetate mobile phase.
Synthesis of pyrazole derivatives

A solution of ethanone intermediate (5 mmol) in tetrahydrofuran (20 mL) was treated with N,N-Dimethyl formamide-dimethyl acetal (50 mmol) and stirred for 40 h. The mixture was concentrated under vacuum, diluted with ethanol (10 mL), and treated with hydrazine monohydrate (150 mmol). The resulting mixture was stirred for 8 h, concentrated, diluted with ethyl acetate, and washed once with water. The combined organic layers concentrated in vacuum and crude product of pyrazole was purified by column chromatography with the mobile phase hexane/ethyl acetate. The complete scheme of synthesis for the title compounds is depicted in Fig. 1.

Antimicrobial screening [25]

Antibacterial activity

Agar disk diffusion method with standard protocols was employed to screen the antibacterial activity of the titled compounds (4a-4j) at a dose of 100 µg/mL by agar plate method using standard protocols. For the antibacterial screening, two Gram-positive (Staphylococcus aureus and Bacillus subtilis) and two Gram-negative bacterial strains (Escherichia coli and Pseudomonas aeruginosa) were selected. Standard neomycin sulfate and dimethyl sulfoxide (DMSO) were served as positive and negative control, respectively. The experiment was done in triplicates and the zone of inhibition measured in mm was taken for the evaluation of the antibacterial activity of the test compounds.

Peptone, meat extract, and sodium chloride were dissolved in distilled water, and pH of the medium was adjusted to 7.2. Agar was dissolved and distributed in 40 mL quantities into 100 mL flasks and was sterilized in an autoclave at 121°C (15 lbs/sq.in) for 20 min. The medium was inoculated at 1% level with 18 h of old cultures of the above-mentioned test organism and transferred into sterile 15 cm diameter Petri dishes.

The plates could set at room temperature for 30 min. For the preparation of cup agar plates, 6 mm diameter holes were made with the help of a sterile borer at the corner of the plate at equal distance. The solution of test compounds was placed in the cups by means of sterile pipettes. In each plate, one cup was used for control with two drops (0.05 mL) of DMSO neomycin sulfate in 10 µg/mL concentration was used as standard. The plates could incubate at room temperature for 1 h to diffuse. Then, the plates were incubated for 24 h at 37°C and zone of inhibition was recorded. The experiments were run in duplicate and the average diameter of the zones of inhibition was recorded and noted.

Antifungal activity

Two fungal strains, Candida albicans and Aspergillus niger were employed to test the antifungal activity of the title compounds in the concentration of 100 µg/mL using the above protocols. The experiments were done in triplicates on A. niger and C. albicans. Standard Nystatin and DMSO were served as positive and negative control, respectively. The zone of inhibition measured in millimeter was taken for the evaluation of an antifungal activity.

Dextrose and agar were added slowly to the filtered solution of peeled potatoes, which were pre-boiled in 200 mL of water for 30 min. Nystatin in 10 µg/mL concentration and DMSO was taken as standard and control, respectively. The diameter of the zones of inhibition was recorded after the incubation of plates at room temperature (30°C) for 48 h.

RESULTS AND DISCUSSION

Synthesis

Synthesized compounds structure and physicochemical data are depicted in Table 1.

[Diagram of synthesis for substituted pyrazoles]
Table 1: Structures and physicochemical data of synthesized compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>M.P (°C)</th>
</tr>
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<tbody>
<tr>
<td>4a</td>
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<td>165–166</td>
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<tr>
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<td>161–163</td>
</tr>
<tr>
<td>4f</td>
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<td>4g</td>
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<td>162–164</td>
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Table 1: (Continued)

<table>
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<th>S. No.</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>M.P (°C)</th>
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<tbody>
<tr>
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<td>4j</td>
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<td>60</td>
<td>166–168</td>
</tr>
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</table>

**Spectral data**

**Compound 4a: 2-(4-phenyl-1H-pyrazol-3-yl)pyridine**

$^1$H NMR: δ 7.23 (1H, ddd, $J = 7.5, 4.8, 1.5$ Hz), 7.37–7.54 (3H, 7.49 (ddd, $J = 7.8, 7.5, 1.9, 0.4$ Hz), 7.42 (tddd, $J = 7.5, 1.7, 1.4$ Hz), 7.81 (2H, dddd, $J = 7.8, 2.8, 1.7, 0.5$ Hz), 7.86–7.99 (2H, 7.96 (ddd, $J = 8.1, 1.5, 0.5$ Hz), 7.91 (ddd, $J = 8.1, 7.5, 1.8$ Hz), 8.58 (1H, s), 8.70 (1H, dddd, $J = 4.8, 1.8, 0.5$ Hz). ESI-MS (m/z): [M+H]$^+$ for C$_{14}$H$_{11}$N$_3$ is 222.6.

**Compound 4b: 2-(4-(4-fluorophenyl)-1H-pyrazol-3-yl)pyridine**

$^1$H NMR: δ 2.19 (3H, s), 7.21–7.34 (3H, 7.30 (ddd, $J = 7.5, 4.7, 1.3$ Hz), 7.24 (ddd, $J = 8.2, 1.3, 0.5$ Hz), 7.81 (2H, dddd, $J = 8.2, 1.7, 0.4$ Hz), 7.83–7.95 (2H, 7.92 (ddd, $J = 7.9, 1.3, 0.5$ Hz), 7.88 (ddd, $J = 7.9, 7.5, 1.8$ Hz), 8.51 (1H, s), 8.66 (1H, dddd, $J = 4.7, 1.8, 0.5$ Hz). ESI-MS (m/z): [M+H]$^+$ for C$_{14}$H$_{10}$FN$_3$ is 240.15.

**Compound 4c: 2-methyl-6-(4-phenyl-1H-pyrazol-3-yl)pyridine**

$^1$H NMR: δ 2.49 (3H, s), 7.31 (1H, dd, $J = 7.8, 1.8$ Hz), 7.39–7.52 (3H, 7.47 (ddd, $J = 7.8, 7.5, 1.5$ Hz), 7.44 (ddd, $J = 7.5, 7.2, 1.4$ Hz), 7.81–7.88 (3H, 7.85 (dd, $J = 8.0, 1.8$ Hz), 7.84 (ddd, $J = 7.8, 2.6, 1.0$, 0.5 Hz Hz), 7.94 (1H, dd, $J = 8.0, 7.8$ Hz), 8.54 (1H, s). ESI-MS (m/z): [M+H]$^+$ for C$_{15}$H$_{13}$N$_3$ is 236.08.

**Compound 4d: 2-(4-(4-fluorophenyl)-1H-pyrazol-3-yl)-6-methylpyridine**

$^1$H NMR: δ 2.38 (3H, s), 7.11 (1H, dd, $J = 7.8, 1.5$ Hz), 7.21 (2H, dddd, $J = 8.9, 1.5, 0.5$ Hz), 7.41 (2H, dddd, $J = 0.9, 1.4, 0.5$ Hz), 7.84 (1H, dd, $J = 7.9, 1.5$ Hz), 7.98 (1H, dd, $J = 7.9, 7.8$ Hz), 8.45 (1H, s). ESI-MS (m/z): [M+H]$^+$ for C$_{15}$H$_{12}$FN$_3$ is 254.25.

**Compound 4e: 2-(4-(4-chlorophenyl)-1H-pyrazol-3-yl)-6-methylpyridine**

$^1$H NMR: δ 2.28 (3H, s), 7.11 (1H, dd, $J = 7.8, 1.5$ Hz), 7.23 (2H, dddd, $J = 8.8, 1.7, 1.0$ Hz), 7.44 (2H, dddd, $J = 8.8, 1.4, 0.5$ Hz), 7.84 (1H, dd, $J = 8.0, 1.5$ Hz), 7.97 (1H, dd, $J = 8.0, 7.8$ Hz), 8.48 (1H, s). ESI-MS (m/z): [M+H]$^+$ for C$_{15}$H$_{12}$ClN$_3$ is 270.6.
Table 2: Antibacterial activity of the compounds against Gram-positive and Gram-negative bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition of the compounds (60 µg/mL) in mm*</th>
<th>Solvent control (DMSO)</th>
<th>Neomycin sulfate (10 µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td>14 12 13 15 12 11 15 15 15 11 3</td>
<td>17</td>
<td></td>
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<tr>
<td><em>S. aureus</em></td>
<td>15 14 13 14 11 10 11 15 14 12 2</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>17 16 18 18 15 13 14 18 18 17 3</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>13 13 13 13 14 12 12 15 14 12 2</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

*Values including diameter of the disc (6.0 mm), are averages of triplicates. *B. subtilis*: Bacillus subtilis, *S. aureus*: Staphylococcus aureus, *E. coli*: Escherichia coli, *P. aeruginosa*: Pseudomonas aeruginosa, DMSO: Dimethyl sulfoxide

Table 3: Antifungal activity of the compounds

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Zone of inhibition of the compounds (60 µg/mL) in mm*</th>
<th>Solvent control (DMSO)</th>
<th>Nystatin (10 µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td>14 12 14 15 12 11 11 15 15 11 3</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>15 13 14 14 11 10 11 15 14 12 2</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

*Values including diameter of the disc (6.0 mm), are averages of triplicates. *A. niger*: Aspergillus niger, *C. albicans*: Candida albicans, DMSO: Dimethyl sulfoxide

Compound 4f: 4-(3-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl)phenol

1H NMR: δ 2.34 (3H, s), 7.09 (1H, dd, J = 7.8, 1.8 Hz), 7.18 (2H, ddd, J = 8.9, 1.5, 0.5 Hz), 7.75 (2H, ddd, J = 8.9, 1.8, 0.5 Hz), 7.82 (1H, dd, J = 7.9, 1.8 Hz). 7.97 (1H, dd, J = 7.9, 1.8 Hz), 8.35 (1H, s). ESI-MS (m/z): [M+H]+ for C18H13N2O is 252.3.

Compound 4g: 4-(3-(4-difluorophenyl)-1H-pyrazol-3-yl)-methylpyridine

1H NMR: δ 2.35 (3H, s), 7.09 (1H, dd, J = 7.8, 1.8 Hz), 7.18 (1H, dd, J = 8.8, 1.8 Hz). 7.51 (1H, dd, J = 8.8, 0.5 Hz), 7.61 (1H, dd, J = 1.8, 0.5 Hz). 7.83 (1H, dd, J = 7.9, 1.8 Hz). 7.97 (1H, dd, J = 7.9, 7.8 Hz), 8.45 (1H, s). ESI-MS (m/z): [M+H]+ for C18H13F2N2 is 272.1.

Compound 4h: 4-(4-(methoxyphenyl)-1H-pyrazol-3-yl)-methylpyridine

1H NMR: δ 2.32 (3H, s), 7.07 (1H, dd, J = 7.8, 1.8 Hz), 7.15 (2H, ddd, J = 8.9, 1.5, 0.5 Hz). 7.64 (2H, ddd, J = 8.9, 1.7, 0.5 Hz). 7.81 (1H, dd, J = 7.9, 1.8 Hz), 7.96 (1H, dd, J = 7.9, 7.8 Hz), 8.87 (1H, s). ESI-MS (m/z): [M+H]+ for C18H14N3O is 266.3.

Compound 4i: 4-(3-(4-methoxyphenyl)-1H-pyrazol-3-yl)-methylpyridine

1H NMR: δ 2.30 (3H, s), 3.69 (3H, s), 3.73 (3H, s), 6.88 (1H, dd, J = 8.8, 0.4 Hz), 7.02 (1H, dd, J = 7.8, 1.7 Hz), 7.23 (1H, dd, J = 1.9, 0.4 Hz). 7.29 (1H, dd, J = 8.8, 1.9 Hz), 7.93 (1H, dd, J = 7.8, 7.8 Hz), 8.02 (1H, dd, J = 7.7, 1.7 Hz). 8.28 (1H, s). ESI-MS (m/z): [M+H]+ for C18H14N3O is 250.2.

Compound 4j: 2-methyl-6-(4-p-tolyl)-1H-pyrazol-3-yl) pyridine

1H NMR: δ 2.18 (3H, s), 2.40 (3H, s), 7.07 (1H, dd, J = 7.8, 1.2 Hz), 7.19 (2H, ddd, J = 8.2, 1.3, 0.5 Hz), 7.76–7.84 (3H, 7.82 (dd, J = 7.9, 1.2 Hz), 7.79 (dd, J = 8.2, 1.6, 0.5 Hz), 7.96 (1H, dd, J = 7.9, 7.8 Hz), 8.46 (1H, s). ESI-MS (m/z): [M+H]+ for C18H13N2O is 296.4.

Antibacterial activity

The antibacterial screening of the compounds (4a-4j) was screened against two Gram-positive, namely, *S. aureus* and *B. subtilis*, two Gram-negative, namely, *Pseudomonas aeruginosa* and *E. coli* using agar plate method. All the synthesized compounds display considerable antibacterial properties. Interestingly, compounds 4a, 4c, 4d, 4h, and 4i are showing comparatively higher inhibition to both Gram-positive and Gram-negative bacteria. The results are tabulated in Table 2.

The results of antifungal screening performed against *C. albicans* and *A. niger* are depicted in Table 3. Results display the antimicrobial potential of the title compounds against selected bacterial and fungal strains. The phenyl ring substituted with both electron-donating and electron-withdrawing groups produced considerable antimicrobial activity. From the above results, the substituted derivatives of pyrazoles are antimicrobial in nature. Remarkably, the compounds 4a, 4c, 4d, 4h, and 4i displayed good antimicrobial potential. The above findings can clear that these pyrazole derivatives can serve as lead molecules to the development of potent antimicrobial agents.

CONCLUSION

Various substituted pyrazole derivatives have been synthesized in a two-step process involving the cyclization of ethenone intermediate with hydrazine moiety. The simple procedure under reflux conditions leads to the synthesis 10 derivatives (4a-4j). The structures were established by detailed spectral analysis. Antimicrobial studies were performed on the synthesized compounds against selected microbial strains and the results revealed the antibacterial, antifungal properties. From the results, it can be concluded that further investigation is needed to understand the exact mechanism of the antimicrobial property in molecular level to develop potent antimicrobial agents to eradicate the pathogenic diseases caused by the bacteria and fungi.

AUTHORS’ CONTRIBUTION

Under the Guidance of the Dr. Kaushal K Chandrul and Dr. DVRN Bhikshapathi, The corresponding author Ganesh Akula contributed majorly in this research work.

CONFLICTS OF INTEREST

All the authors Ganesh Akula, Kaushal K Chandrul and DVRN Bhikshapathi express no conflicts of interest.

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