Synthesis and Anti-microbial Evaluation of Some 3, 4-Dihydro Pyrimidine-2-one Derivatives

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Abstract: 4-phenyl-5-carboxyethyl-6-methyl-1,2,3,4-tetrahydropyrimidin-2-ones have been synthesized using the principle of Biginelli condensation from easily available starting materials. The carboxethoxy group at the C5 position of the pyrimidine ring is converted to corresponding hydrazide which in turn is condensed with cyclizing agents such as aromatic aldehyde, CS2, to give fused heterocycles. The fused heterocycles are then subjected to substitution to give N-aryl/alkylpyrimido-heterocycles in excellent yields. The compounds were tested for antimicrobial action relative to Norfloxacin against Gram positive and Gram negative bacteria using serial dilution technique.

Key words: Biginelli synthesis, dihydro pyrimidine, thiazololo, triazolo, antibacterial, serial dilution method

INTRODUCTION

Pyrimidines are of great importance in fundamental metabolism, being an integral part of DNA and RNA, found in the three bases uracil, thymine and cytosine of the six present in the nucleotides (Joulé and Smith, 1979). They are found to possess diverse biological properties like bactericide, fungicide, viricide, insecticide and mecticide (Cheng, 1969). Many derivatives of pyrimidines have been used as other therapeutic agents (Carg and Prakash, 1971). Several triazolo and pyrazolopyrimidine derivatives are found to possess antifungal and antileishmanial activity (Ran, 1989). Certain pyrimidine derivatives are known to display antimalarial (Howells et al., 1981) and antifilarial activities (Fulco et al., 1951). In the recent years, a lot of attention has been drawn by the pyrimidine derivatives due to their diverse range of activities, especially calcium channel blocker property (Atwal et al., 1991). Certain pyrimidine derivatives are also found to be potent inhibitors of cancer cell proliferation (Girardet et al., 2000).

The C5 position of pyrimidine nucleus is an attractive target for modification as it is located at the major groove surface in the duplex form and will not directly inhibit the hydrogen bonding in an A-T base pair (Cohen, 1989; Beanace and Iyer, 1993).

The most general and widely used route to synthesize pyrimidines involves the combination of a reagent containing the N-C-N skeleton with C-C-C unit. Urea, thiourea and guanidine are the most commonly used N-C-N agents and 1,3-diketones and diesters are the common agents to provide the C-C-C unit.

In the present method, ethylacetocetate is employed as the C-C-C unit and is condensed with urea to complete the pyrimidine ring.

The presence of carboxethoxy group at C-5 of the pyrimidine ring was crucial for linking of the triazolo and thiazololo groups at this position.

The carboxethoxy group was first converted into its hydrazide derivative that helped in better modification of the compound to the desired derivatives, by condensing with variety of cyclising agents.

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MATERIALS AND METHODS

Melting points were recorded in open capillary tubes and are uncorrected. IR spectra were recorded in KBr discs in Jasco FT-IR 470 plus spectrophotometer. $^1$H NMR data has been taken on a Bruker WM (300 MHz) NMR spectrometer using TMS as a reference compound (chemical shifts in $\delta$, ppm) and mass spectra on a Joel-JMS-D300 spectrometer.

A series of pyrimidine derivatives is synthesized using Biginelli condensation of ethylacetoacetate, urea and benzaldehyde.

The steps adapted in the synthesis of the pyrimidine derivatives are depicted in the scheme below (Fig. 1) adapted from the schemes already reported (Padhy et al., 2003) and (Upadhyay and Ram, 1999).

- Synthesis of 4-phenyl-5-carboxethoxy-6-methyl-3,4-dihydropyrimidine-2-one.
- Synthesis of 4-phenyl-5-carboxyhydrazide-6-methyl-3,4-dihydropyrimidine-2-one.
  (a). Synthesis of 4-phenyl-5-(2'-substituted-1', 3', 4'-triazolo)-6-methyl-3,4-dihydropyrimidine-2-one.
- (b). Synthesis of 4-phenyl-5-(1', 3', 4'-thiadiazolo)-6-methyl-3,4-dihydropyrimidine-2-one.
- (a). Synthesis of N-substituted-4-phenyl-5-(2'-substituted-1', 3', 4'-triazolo)-6-methyl-3,4-dihydropyrimidine-2-one.

Fig. 1: Synthesis of pyrimidine derivatives
Synthesis of 4-Pheny1-5-Carboxyloxy-6-Methyl-3, 4-Dihydropyrimidine-2-One

0.5 moles of urea (1), 0.75 moles of ethylacetocetate (3) and 0.5 moles of benzaldehyde (2) were mixed in 25 mL of ethanol. Catalytic amount of concentrated hydrochloric acid (5 drops) was added to the mixture and the mixture was refluxed until the completion of the reaction (approximately 3 h). On cooling, a solid separated which was filtered and recrystallized using ethanol to give the product 4. Completion of the reaction was monitored by TLC, yield 83%; m.p. 185-189°C; IR (5): 1570 (C=N), 1650 (amide), 1730 (ester), 3350 (NH) cm⁻¹; 1H NMR (CDCl₃): δ 1.5 (s, CH₃), 1.8 (t, COOCH₂CH₃), 2.5 (q, COOCH₂CH₃), 3.4 (s, C-NH-CHO), 5.4 (s, Ar-NH-CO), 6.4 (s, Ar-CH), 7-8 (m, 5H, ArH); MS: m/z 260 (M⁺).

Synthesis of 4-Phenyl-5-Carboxyhydrazide-6-Methyl-3, 4-Dihydropyrimidine-2-One

To 0.1 mole of the product 4 in 20 mL ethanol, 0.1 mole of hydrazine hydrate was added. To the mixture, catalytic amount of concentrated sulfuric acid was added. The mixture was refluxed until the completion of the reaction (approximately 2 h). On cooling, a solid separated, which was recrystallized from ethanol to give the product 5, yield 50%; m.p. 184-186°C; IR(KBr) cm⁻¹: 1570 (C=N), 1650 (amide), 3350 (N-H) cm⁻¹; 1H NMR (CDCl₃): δ 2.5 (d, 2H, NHNH₂), 3.4 (s, C-NH-CO), 5.1 (s, CO-NH-CO), 4.1 (t, CONH), 7-8 (m, 5H, ArH); MS: m/z 246 (M⁺).

Synthesis of 4-Phenyl-5-(2’-Substituted-1’, 3’, 4’-Triazolo)-6-Methyl-3, 4-Dihydropyrimidine-2-One

To 0.1 mole of product 5 in 20 mL acetic acid, a pinch of ammonium acetate was added, followed by the addition of 0.1 mole of Benzaldehyde/formaldehyde solution. The mixture was stirred for 24 h at room temperature. After 24 h, the reaction mixture was neutralized with ammonia solution, to give a solid, which was recrystallized from ethanol to give the product 6, yield 48%; m.p. 138-142°C; IR: 1650 (amide), 3350 (NH) cm⁻¹; 1H NMR (CDCl₃): δ 1.4 (s, -CH₃), 5.2 (s, C-NH-CO), 7-8 (m, 9H, ArH).

Synthesis of 4-Phenyl-5-(1’, 3’, 4’-Thiadiazolo)-6-Methyl-3, 4-Dihydropyrimidine-2-One

To a solution of 0.15 moles potassium hydroxide in ethanol and 0.15 mole of the compound 5 was added 0.15 mole of carbon disulfide. The mixture was diluted with ethanol and stirred at room temperature for 12-16 h. The mixture was then neutralized with concentrated hydrochloric acid and the precipitated solid was filtered, washed with water and recrystallized from ethanol to give the product 7, yield: 52%; m.p. 140-146°C; IR(KBr) cm⁻¹: 1570 (C=N), 1650 (amide), 2650 (SH), 3350 (NH) cm⁻¹; 1H NMR (CDCl₃): δ 1.4 (s, 3H, CH₃), 3.2 (s, 6H, OCH₃), 6.4 (s, ArCH), 7-8 (m, 3H, ArH).

General Method of Synthesis of the N-Substituted Compound

A mixture of 2.17 mmoles of 6 or 7, 4.35 mmoles of K₂CO₃, and 4.48 mmoles of the alkyl or aryl halide in 6 mL of DMF was stirred for 4 h at room temperature. The mixture was then diluted with water and the solid was filtered off and was recrystallized from ethanol to give the final product 8 or 9.

Table 1 summarizes the various synthesized compounds and the percent yield of each relative to the compound 6. The physical properties of synthesized compounds (Table 2).

Synthesis of 4-Phenyl-5-2’-(1’,3’, 4’-Triazolo-5’-Phenyl)-6-Methyl-3-N-Phenyl-Pyrimidine-2-One

To 2.17 mmole of 6, 4.35 mmole of K₂CO₃ and 4.48 mmole of chlorobenzene in 6 mL DMF was stirred for 4 h at room temperature. The mixture was then diluted with water and the solid was
Table 1: List of the synthesized compounds

<table>
<thead>
<tr>
<th>Compound code</th>
<th>R</th>
<th>X</th>
<th>Yield (%)</th>
</tr>
</thead>
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<tr>
<td>PYMTB-1</td>
<td></td>
<td></td>
<td>68</td>
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<td></td>
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<td></td>
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<tr>
<td>PYMTB-2</td>
<td>-CH₃</td>
<td></td>
<td>67</td>
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<td>PYMD-1</td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>PYMD-2</td>
<td>-CH₃</td>
<td></td>
<td>71</td>
</tr>
</tbody>
</table>

Table 2: Physical properties of the synthesized compounds

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Melting point (°C)</th>
<th>Absorption maximum (λmax) (nm)</th>
<th>Nitrogen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYMTB 1</td>
<td>C₁₂H₁₁N₂O</td>
<td>207.44</td>
<td>198-202</td>
<td>280.5</td>
<td>17.19</td>
</tr>
<tr>
<td>PYMTB 2</td>
<td>C₁₂H₁₁N₂O</td>
<td>207.44</td>
<td>192-196</td>
<td>280.0</td>
<td>19.80</td>
</tr>
<tr>
<td>PYMD 1</td>
<td>C₁₂H₁₁N₂S₂O</td>
<td>278.40</td>
<td>192-196</td>
<td>280.0</td>
<td>14.72</td>
</tr>
<tr>
<td>PYMD 2</td>
<td>C₁₂H₁₁N₂S₂O</td>
<td>278.40</td>
<td>184-186</td>
<td>280.0</td>
<td>14.01</td>
</tr>
</tbody>
</table>

filtered off and was recrystallized from ethanol to give the final product PYMTB 1, IR(KBr) cm⁻¹: 1570 (C-N), 1650 (amide), 2650 (SH), 3350 (NH) cm⁻¹; 1H NMR (CDCl₃): δ 1.4 (s, 3H, CH₃), 3.2 (s, 6H, OCH₃), 6.4 (s, ArCH), 7-8 (m, 3H, ArH); MS: m/z 407 (M⁺), Found: N, 16.76%.

**Synthesis of 4-Phenyl-5-2′-(1′,3′,4′-Triazolo-5′-Phenyl)-6-Methyl-3-N-Methyl-Pyrimidine-2-One**

To 2.17 mmoles of 6, 4.35 mmoles of K₂CO₃ and 4.48 mmoles of methyl chloride in 6 mL DMF was stirred for 4 h at room temperature. The mixture was then diluted with water and the solid was
Table 3: Antibacterial activity of the synthesized compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Proteus mirabilis (µg mL⁻¹)</th>
<th>Pseudomonas aeruginosa</th>
<th>Staphylococcus aureus</th>
<th>Bacillus subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYMTB-1</td>
<td>80</td>
<td>98</td>
<td>49</td>
<td>53</td>
</tr>
<tr>
<td>PYMTB-2</td>
<td>98</td>
<td>112</td>
<td>53</td>
<td>62</td>
</tr>
<tr>
<td>PYMD-1</td>
<td>71</td>
<td>107</td>
<td>89</td>
<td>85</td>
</tr>
<tr>
<td>PYMD-2</td>
<td>76</td>
<td>112</td>
<td>93</td>
<td>94</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>06</td>
<td>05</td>
<td>04</td>
<td>08</td>
</tr>
</tbody>
</table>

filtered off and was recrystallized from ethanol to give the final product PYMTB 2, IR (KBr) cm⁻¹: 1570 (C-N), 1650 (amide), 2650 (SH), 3350 (NH) cm⁻¹; 1H NMR (CDCl₃): δ 1.4 (s, 3H, CH₃), 3.2 (s, 6H, OCH₃), 6.4 (s, ArCH), 7-8 (m, 3H, ArH); MS: m/z 346 (M⁺); Found: N, 19.80%.

Synthesis of 4-Phenyl-5-2'-(1',3',4'-Thiadiazolo)-6-Methyl-3-N-Phenyl-Pyrimidine-2-One

To 2.17 mmoles of 6, 4.35 mmoles of K₂CO₃, and 4.48 mmoles of chlorobenzene in 6 mL DMF was stirred for 4 h at room temperature. The mixture was then diluted with water and the solid was filtered off and was recrystallized from ethanol to give the final product PYMTD-1, IR(KBr) cm⁻¹: 1570 (C-N), 1650 (amide), 2650 (SH), 3350 (NH) cm⁻¹; 1H NMR (CDCl₃): δ 1.4 (s, 3H, CH₃), 3.2 (s, 6H, OCH₃), 6.4 (s, ArCH), 7-8 (m, 3H, ArH); MS: m/z 380 (M⁺); Found: N, 14.01%.

Synthesis of 4-Phenyl-5-2'-(1',3',4'-Thiadiazolo)-6-Methyl-3-N-Methyl-Pyrimidine-2-One

To 2.17 mmoles of 6, 4.35 mmoles of K₂CO₃, and 4.48 mmoles of methyl chloride in 6 mL DMF was stirred for 4 h at room temperature. The mixture was then diluted with water and the solid was filtered off and was recrystallized from ethanol to give the final product PYMTD 2, IR (KBr) cm⁻¹: 1570 (C-N), 1650 (amide), 2650 (SH), 3350 (NH) cm⁻¹; 1H NMR (CDCl₃): δ 1.4 (s, 3H, CH₃), 3.2 (s, 6H, OCH₃), 6.4 (s, ArCH), 7-8 (m, 3H, ArH); MS: m/z 318 (M⁺); Found: N, 16.75%.

Antimicrobial Evaluation of the Synthesized Compounds

The antimicrobial activity of the synthesized compounds was tested against *Proteus mirabilis*, *Pseudomonas aeruginosa* (Gram negative), *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive) bacteria (Table 3).

RESULTS AND DISCUSSION

Characterization of the synthesized compounds was carried out by determining their melting points, UV absorption maxima, IR spectra, 1H NMR and nitrogen content studies by Kjeldahl method. All the compounds were found to exhibit the amide, amine, aromatic hydrogen and methoxy group shifts in the 1H NMR spectra.

The yield of all the synthesized compounds is found to be significant. The structural confirmation of the compounds is done by IR spectra and the percentage nitrogen content found in the synthesized compounds. All the synthesized compounds show peaks in the IR spectrum at wave number (cm⁻¹) 3500, 3120, 2980, 1690, 1570 and 1456. These peaks are characteristics of N—H, C—H (Aromatic), C—H, C = O stretching and C—H bending. Compounds PYMD-1 and PYMD-2 show peaks at 2525, which is the characteristic stretching of S—H group.

The compounds were evaluated for antibacterial activity by serial dilution method. All the synthesized compounds possessed anti bacterial activity. MIC value of the compounds was deduced from the antibacterial assay method employed.
The results obtained led to the conclusion that the activity of the pyrimidine derivatives as anti-microbial agents is affected by the type of the substituent at the 5-position of the pyrimidine nucleus. As envisaged from the literature review and SAR studies, the substitution of the nitrogen in pyrimidine ring led to significant anti-microbial activity. It could also be concluded from the results that the substitution of the 5-position with a substitution on the substituent yielded better activity than a non-substituted one. The substitution of the pyrimidine ring nitrogen with aryl groups yielded better activity than that obtained with alkyl substitution.

REFERENCES


