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### 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 carboethoxy group at the C<sub>5</sub> position of the pyrimidine ring is converted to corresponding hydrazide which in turn is condensed with cyclizing agents such as aromatic aldehyde, CS<sub>2</sub> to give fused heterocycles. The fused heterocycles are then subjected to substitution to give N<sub>3</sub>-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, thiadiazolo, 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 (Joule and Smith, 1979). They are found to possess diverse biological properties like bactericide, fungicide, viricide, insecticide and meticide (Cheng, 1969). Many derivatives of pyrimidines have been used as other therapeutic agents (Garg and Prakash, 1971). Several triazolo and pyrazolopyrimidine derivatives are found to possess antifungal and antileishmanial activity (Ram, 1989). Certain pyrimidine derivatives are known to display antimalarial (Howells *et al.*, 1981) and antifilarial activities (Falco *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 C<sub>5</sub> 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; Beaucage 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, ethylacetoacetate is employed as the C-C-C unit and is condensed with urea to complete the pyrimidine ring.

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

The carboethoxy 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 cyclizing 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.  $^1\text{H}$  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-carboethoxy-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.

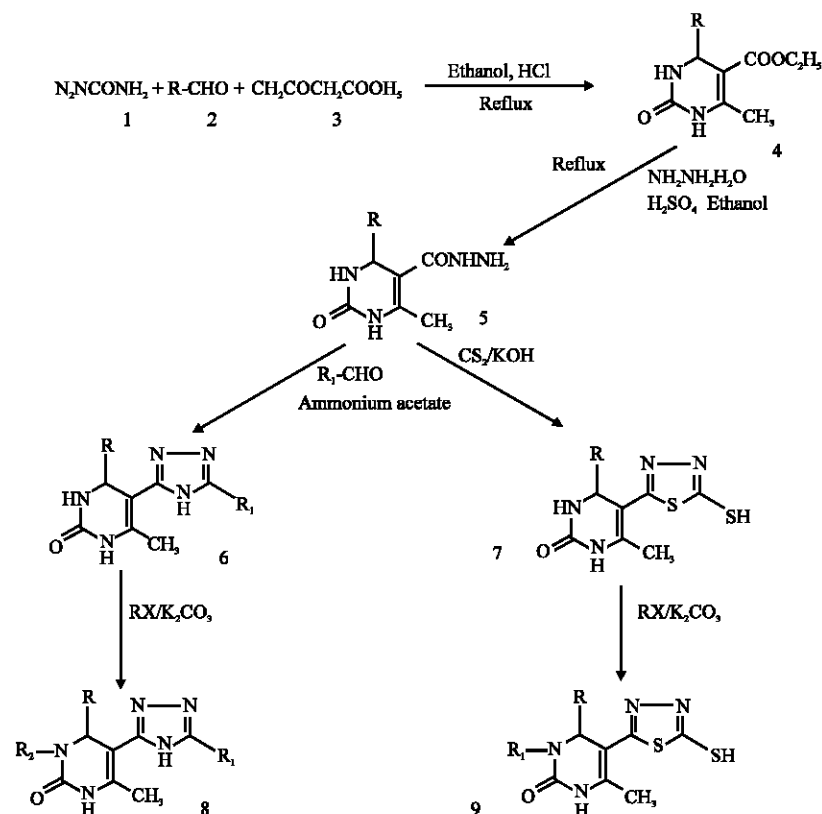


Fig. 1: Synthesis of pyrimidine derivatives

#### **Synthesis of 4-Phenyl-5-Carboethoxy-6-Methyl-3, 4-Dihydropyrimidine-2- One**

0.5 moles of urea (1), 0.75 moles of ethylacetoacetate (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)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.5 (s,  $\text{CH}_3$ ), 1.8 (t,  $\text{COOCH}_2\text{CH}_3$ ), 2.5 (q,  $\text{COOCH}_2\text{CH}_3$ ), 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 ( $\text{M}^+$ ).

#### **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 V, yield 50%; m.p. 184-186°C; IR(KBr)  $\text{cm}^{-1}$ : 1570 (C-N), 1650 (amide), 3350 (N-H)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.5 (d, 2H,  $\text{NHNH}_2$ ), 3.4 (s, C-NH-CO), 5.1 (s, CO-NH-CO), 4.1 (t, CONHN), 7-8 (m, 5H, ArH); MS: m/z 246 ( $\text{M}^+$ ).

#### **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)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.4 (s,  $-\text{CH}_3$ ), 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. 146-148°C; IR(KBr)  $\text{cm}^{-1}$ : 1570 (C-N), 1650 (amide), 2650 (SH), 3350 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.4 (s, 3H,  $\text{CH}_3$ ), 3.2 (s, 6H,  $\text{OCH}_3$ ), 6.4 (s, ArCH), 7-8 (m, 3H, ArH).

#### **General Method of Synthesis of the N-Substituted Compound**

A mixture of 2.17 nmoles of 6 or 7, 4.35 nmoles of  $\text{K}_2\text{CO}_3$  and 4.48 nmoles 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 nmoles of 6, 4.35 nmoles of  $\text{K}_2\text{CO}_3$  and 4.48 nmoles 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

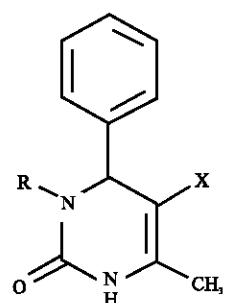
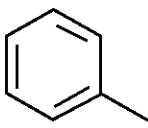
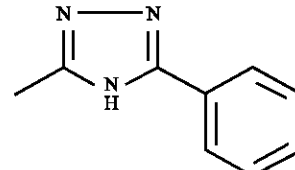
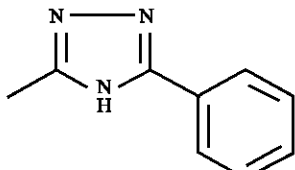
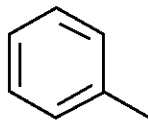
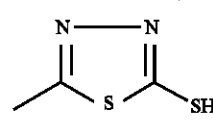
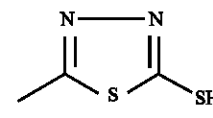
			
Compound code	R	X	Yield (%)
PYMTB-1			68
PYMTB-2	-CH <sub>3</sub>		67
PYMD-1			80
PYMD-2	-CH <sub>3</sub>		71

Table 2: Physical properties of the synthesized compounds

Compound name	Molecular formula	Molecular weight	Melting point (°C)	Absorption maximum ( $\lambda_{max}$ ) (nm)	Nitrogen (%)	
					Calculated	Found
PYMTB 1	C <sub>25</sub> H <sub>21</sub> N <sub>3</sub> O	407.48	198-202	280.5	17.19	16.76
PYMTB 2	C <sub>20</sub> H <sub>19</sub> N <sub>3</sub> O	345.41	192-196	280.0	20.28	19.80
PYMD 1	C <sub>19</sub> H <sub>16</sub> N <sub>4</sub> S <sub>2</sub> O	380.49	192-196	280.0	14.72	14.01
PYMD 2	C <sub>14</sub> H <sub>14</sub> N <sub>4</sub> S <sub>2</sub> O	318.42	184-186	280.0	17.60	16.75

filtered off and was recrystallized from ethanol to give the final product PYMTB 1, IR(KBr) cm<sup>-1</sup>: 1570 (C-N), 1650 (amide), 2650 (SH), 3350 (NH) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.4 (s, 3H, CH<sub>3</sub>), 3.2 (s, 6H, OCH<sub>3</sub>), 6.4 (s, ArCH), 7-8 (m, 3H, ArH); MS: m/z 407 (M<sup>+</sup>); 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 nmoles of 6, 4.35 nmoles of K<sub>2</sub>CO<sub>3</sub> and 4.48 nmoles 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

Compounds	MIC ( $\mu\text{g mL}^{-1}$ )			
	<i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
PYMTB-1	80	98	49	53
PYMTB-2	98	112	53	62
PYMD-1	71	107	80	85
PYMD-2	76	112	93	94
Norfloxacin	06	05	04	08

filtered off and was recrystallized from ethanol to give the final product PYMTB 2, IR (KBr)  $\text{cm}^{-1}$ : 1570 (C-N), 1650 (amide), 2650 (SH), 3350 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.4 (s, 3H,  $\text{CH}_3$ ), 3.2 (s, 6H,  $\text{OCH}_3$ ), 6.4 (s, ArCH), 7-8 (m, 3H, ArH); MS:  $m/z$  346 ( $\text{M}^+$ ); 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 nmoles of 6, 4.35 nmoles of  $\text{K}_2\text{CO}_3$  and 4.48 nmoles 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)  $\text{cm}^{-1}$ : 1570 (C-N), 1650 (amide), 2650 (SH), 3350 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.4 (s, 3H,  $\text{CH}_3$ ), 3.2 (s, 6H,  $\text{OCH}_3$ ), 6.4 (s, ArCH), 7-8 (m, 3H, ArH); MS:  $m/z$  380 ( $\text{M}^+$ ); 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 nmoles of 6, 4.35 nmoles of  $\text{K}_2\text{CO}_3$  and 4.48 nmoles 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)  $\text{cm}^{-1}$ : 1570 (C-N), 1650 (amide), 2650 (SH), 3350 (NH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.4 (s, 3H,  $\text{CH}_3$ ), 3.2 (s, 6H,  $\text{OCH}_3$ ), 6.4 (s, ArCH), 7-8 (m, 3H, ArH); MS:  $m/z$  318 ( $\text{M}^+$ ); 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,  $^1\text{H}$  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  $^1\text{H}$  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 ( $\text{cm}^{-1}$ ) 3500, 3120, 2980, 1690, 756, 1370 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 anti bacterial 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.

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