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Synthesis, characterization and pharmacological studies of some novel pyrimidine derivatives

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ABSTRACT

*A novel series of ethyl 2-N-(aryl amino)-4-methyl-6-phenylpyrimidine-5-carboxylate **(4a-m)** were synthesized from ethyl 2-chloro-4-methyl-6-phenylpyrimidine-5-carboxylate **(3)** by reaction with appropriate anilines **(a-m)**. The structures of these compounds were established on the basis of spectral and analytical data. These novel compounds were screened for their antibacterial activity. The promising compounds **4a** and **4h** have been identified.*

Key words: 2,4,5,6-tetrasubstituted pyrimidine derivatives; antibacterial activity.

INTRODUCTION

Among the variety of existing heterocycles, the nitrogen containing heterocylic derivatives are well known due to its broad spectrum of interesting biological activities. These compounds have been widely used in the fields of agriculture [1], medicine [2], and microbiology [3].

As pyrimidine is a basic nucleus in DNA and RNA, its derivative were found to be associated with diverse biological activies[4] such as antimicrobial[5], antitumor[6], and antifungal[7]. Many pyrimidine derivatives are used as drugs in the treatment of thyroid and leukemia. Many of the natural and synthetic polymers also contain pyrimidine nucleus.

In specific, 2,4- disubstitued and 2,4,5- tri-substituted pyrimidine derivatives have shown potent anticancer activity as CDK inhibitors . It is used to treat cancers by preventing over proliferation of cancer cells-[5]. Piromidic acid and pipemidic acid are commercially available 2,4,5- tri-substituted antibacterial drugs. Also 2,4,6-trisubstituted pyrimidine derivatives such as pyrimethanil, mepanipyrin and cyprodinil are commercial fungicides.

Prompted by these observations and in continuation of our work on biologically potent heterocycles[8-10] we planned to synthesize a hitherto unreported novel series of ethyl 2-N-(aryl substituted)-4-methyl-6-phenylpyrimidine-5-carboxylate and to study their antibacterial activity.

MATERIALS AND METHODS

Chemistry

Thin layer chromatography was used to analyze the reaction progress and purity of the compounds synthesized. 1H NMR spectra were recorded on Brucker spectrometer (400 MHz) in DMSO-d6/CDCl3 using TMS as an internal standard, 13C NMR spectra were recorded on Brucker spectrometer (100 MHz) in DMSOd6/CDCl3. Mass spectra were recorded by Agilent6320 Ion Trap method.

General procedure for synthesis of ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate(1)

To a solution of benzaldehyde (9.41 mmol) in ethyl alcohol (4 mL), ethylacetoacetate (10.35 mmol), urea (10.35 mmol) and a catalytic amount of conc. hydrochloric acid were added and resulting mixture was heated under reflux for 6h. Progress of the reaction was monitored by TLC (ethyl acetate/petroleum ether,1:1, v/v). After completion of the reaction, the reaction mixture was cooled to room temperature and poured into ice cold water. The precipitated solid was filtered under vacuum, washed with water, dried and recrystallized from ethyl alcohol to afford compound **1.**

1H NMR (DMSO-d6) d :1.09 (t, 3H, *J* = 7.2 Hz, -*CH2*CH3), 2.25 (s, 3H, Ar-CH3), 3.98 (q, 2H, *J* = 7.2Hz, -CH2*CH3*), 5.14 (d, 1H, CH),7.25 (m, 3H, Ar-H), 7.32 (m, 2H, Ar-H), 7.73 (bs, 1H, NH), 9.19 (bs, 1H, NH);13C NMR (DMSO-d6) d: 14.5, 18.2, 54.4, 59.6, 99.7, 126.7, 127.7,128.8,145.33, 148.7, 152.5, 165.7; LCMS M+1: 261.28.

General procedure for synthesis of ethyl- 6-methyl-2-oxo-4-phenyl-1,2-dihydropyrimidine-5-carboxylate(2)

To a solution of **1** (3.84 mmol) in acetone (40 mL), ceric ammonium nitrate (11.52 mmol) in water (40 ml) and sodiumbicarbonate(19.21 mmol) were added at 0 ^{0}C and stirred at room temperature for overnight. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mass was concentrated to remove acetone and the aqueous phase was extracted with ether, dried over anhydrous sodium sulfate, filtered and concentrated to afford compound **2**.

1H NMR (DMSO-d6) d: 0.82 (t, 3H, *J* = 7.2 Hz, -*CH2*CH3), 2.39 (s, 3H, Ar-CH3), 3.94 (q, 2H, *J* = 7.2Hz, -CH2*CH3*), 7.45 (m, 5H, Ar-H), 12.39 (bs, 1H, NH); 13C NMR (DMSO-d6) d: 13.6, 61.2, 126.6, 127.9,128.6, 128.8, 130.5, 166.3; LCMS M+1: 259.28.

General procedure for synthesis of ethyl 2-chloro-4-methyl-6-phenylpyrimidine-5-carboxylate (3)

A solution of **2** (3.87mmol) and $POCl_3$(38.71 mmol) was heated at 120 ^{0}C for 2h. Progress of the reaction was monitored by TLC (ethyl acetate/petroleum ether, 1:1, v/v). After completion of the reaction, the reaction mass was concentrated, the residue was dissolved in chloroform and was washed with water and saturated solution of $NaHCO_3$. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to afford crude compound **3**. The crude compound was purified by column chromatography using ethyl acetate and petroleum ether as eluents. The product was eluted with 20% ethyl acetate.

1H NMR(DMSO-d6) d: 1.03 (t, 3H, *J* = 7.2 Hz, -*CH2*CH3), 2.56 (s, 3H, Ar-CH3), 4.21 (q, 2H, *J* = 7.2Hz, -CH2*CH3*), 7.58 (m, 5H, Ar-H); 13C NMR (DMSO-d6) d: 13.8, 22.6, 62.6, 79.6, 124.7, 128.6, 129.3, 131.4, 136.1, 159.9, 166.0, 166.7, 169.2; LCMS M+1: 277.2.

General procedure for synthesis of ethyl 2-N-(aryl substituted)-4-methyl-6-phenylpyrimidine-5-carboxylate (4a-m)

To a solution of **3** (3.61mmol) and substituted aniline (4.33 mmol) in 1,4-dioxane(20ml), 4.0M HCl (2ml) was added and was heated at 100 ^{0}C for 12h. Progress of the reaction was monitored by TLC (ethyl acetate/petroleum ether, 1:1, v/v). After completion of the reaction, the reaction mass was concentrated, the residue was dissolved in ethyl acetate and was washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to afford crude compounds **4(a-m)**. The crude compounds were purified by column chromatography using ethyl acetate and petroleum ether as eluents. The products were further purified by recrystalliztion from ethanol to afford pure compounds **4(a-m)**.

Ethyl 2-[(2,4-difluorophenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylate (**4a**)
1H NMR (DMSO-d6) d: 0.93 (t, 3H, *J* = 7.2 Hz, -*CH2*CH3), 2.40 (s, 3H, Ar-CH3), 4.05 (q, 2H, *J* = 7.2Hz, -CH2*CH3*), 7.07 (t, 1H, *J* = 8.4 Hz, Ar-H), 7.31(t, 1H, *J* = 8.0, Ar-H), 7.45 (m, 5H, Ar-H), 7.65(q, 1H, *J* =8.8, Ar-H), 9.55(s, 1H, N-H); 13C NMR (DMSO-d6) d: 13.5, 22.8, 61.4, 103.4, 110.9, 117.9, 121.83, 123.9, 128.0, 128.5, 130.8, 138.3, 156.4, 158.8, 165.9, 167.3, 168.2; LCMS M+1: 370.2.

Ethyl 2-[(4-fluorophenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylate (**4c**)
1H NMR (DMSO-d6) d: 0.94 (t, 3H, *J* = 7.08 Hz, -*CH2*CH3), 2.47 (s, 3H, Ar-CH3), 4.05 (q, 2H, *J* = 7.12Hz, -CH2*CH3*), 7.15 (t, 2H, *J* = 8.8 Hz, Ar-H), 7.54(m, 5H, Ar-H), 7.81 (m, 2H, Ar-H), 10.05 (s, 1H, N-H); 13C NMR (DMSO-d6) d: 13.5, 22.9, 61.3, 115.3, 115.6, 117.3, 121.0, 128.0, 128.4, 129.8, 135.1, 138.5, 157.4, 158.6, 159.8, 165.9, 167.3, 168.4 ; LCMS M+1: 352.2.

Table 1: Characterization data of compounds 3 and 4a-m

Compound No.	Mol. Formula	R	Colour and crystal nature	Yield (%) M.P. (0C)	Analysis (%) Found (Calculated)		
					C	H	N
3	$C_{14}H_{13}ClN_2O_2$	-	Brown liquid	63 -	60.73 (60.77)	4.73 (4.74)	10.11 (10.12)
4a	$C_{20}H_{17}F_2N_3O_2$	2,4-Dichloro phenyl	Off white solid	45 100-102	65.07 (65.03)	4.62 (4.64)	11.39 (11.38)
4b	$C_{20}H_{18}ClN_3O_2$	4-Chloro phenyl	Off white solid	60 95-98	65.35 (65.31)	4.91 (4.93)	11.44 (11.42)
4c	$C_{20}H_{18}FN_3O_2$	4-Fluro phenyl	Off white solid	57 85-87	68.32 (68.36)	5.14 (5.16)	11.98 (11.96)
4d	$C_{20}H_{18}BrN_3O_2$	3-Bromo phenyl	White solid	72 100-102	58.29 (58.26)	4.38 (4.40)	10.21 (10.19)
4e	$C_{20}H_{19}N_3O_2$	Phenyl	Off white solid	45 130-132	72.08 (72.05)	5.76 (5.74)	12.61 (12.60)
4f	$C_{20}H_{17}Cl_2N_3O_2$	2,6-Dichloro Phenyl	White solid	68 125-128	59.68 (59.71)	4.27 (4.26)	10.46 (10.45)
4g	$C_{23}H_{25}N_3O_2$	2,4,6-Trimethyl phenyl	Off white solid	73 134-136	73.61 (73.57)	6.73 (6.71)	11.21 (11.19)
4h	$C_{21}H_{19}BrClN_3O_2$	2-Bromo-4-chloro-6-methyl phenyl	Off white solid	63 146-148	54.70 (54.74)	4.18 (4.16)	9.13 (9.12)
4i	$C_{21}H_{21}N_3O_3$	2-Methoxy phenyl	White solid	68 120-122	69.46 (69.41)	5.79 (5.82)	11.55 (11.56)
4j	$C_{21}H_{21}N_3O_3$	3-Methoxy phenyl	Off white solid	42 110-112	69.46 (69.41)	5.79 (5.82)	11.55 (11.56)
4k	$C_{23}H_{25}N_3O_2$	2-Methyl 6-ethyl phenyl	White solid	49 104-106	73.61 (73.57)	6.72 (6.71)	11.21 (11.19)
4l	$C_{21}H_{21}N_3O_2$	2-Methyl phenyl	Brown solid	82 78-80	72.57 (72.60)	6.10 (6.09)	12.11 (12.10)
4m	$C_{21}H_{18}N_4O_2$	4-Cyano phenyl	Off white solid	64 127-129	67.59 (67.55)	5.15 (5.13)	18.77 (18.76)

Ethyl 2-[(2-bromo-4-chloro-6-methylphenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylate (**4h**)
1H NMR (DMSO-d6) d: 0.92 (t, 3H, *J* = 7.2 Hz, -*CH2*CH3), 2.23 (s, 3H, Ar-CH3), 4.04 (q, 2H, *J* = 7.2Hz, -CH2*CH3*), 7.46 (m, 6H, Ar-H), 7.67 (m, 2H, Ar-H), 9.43 (s, 1H, N-H); 13C NMR (DMSO-d6) d: 13.5, 19.6, 22.7, 61.3, 117.3, 123.0, 123.3, 128.0, 128.4, 129.7, 130.1, 132.3, 134.3, 138.2, 140.0, 159.4, 166.0, 167.4, 168.5; LCMS M+1: 461.2.

Ethyl 4-methyl-2-[(2-methylphenyl) amino]-6-phenylpyrimidine-5-carboxylate (**4l**)
1H NMR (DMSO-d6) d: 0.92 (t, 3H, *J* = 7.12 Hz, -*CH2*CH3), 2.24 (s, 3H, Ar-CH3), 2.39 (s, 3H, Ar-CH3), 4.04 (q, 2H, *J* = 7.12Hz, -CH2*CH3*), 7.08 (t, 1H, Ar-H), 7.20 (m, 2H, Ar-H), 7.47 (m, 6H, Ar-H), 9.25 (s, 1H, N-H); 13C NMR (DMSO-d6) d: 13.5, 18.2, 22.8, 61.3, 117.1, 121.7, 123.8, 126.5, 128.1, 128.3, 128.4, 129.8, 130.5, 136.9, 138.5, 158.9, 166.0, 167.1, 168.4; LCMS M+1: 348.2.

Biological activity
Antibacterial activity
The newly synthesized compounds were screened for their antibacterial activity against four bacterial strains namely

Staphylococcus aureus, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Eschericia.coli* by dilution technique and the minimum inhibitory concentration (MIC) values were observed [11].

Procedure

1. 9 dilutions of each drug have been done with BHI for MIC.
2. In the initial tube 20microliter of drug was added into the 380microliter of BHI broth.
3. For dilutions 200microliter of BHI broth was added into the next 9 tubes separately.
4. Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10^{-1} dilution.
5. From 10^{-1} diluted tube 200microliter was transferred to second tube to make 10^{-2} dilution.
6. The serial dilution was repeated up to 10^{-9} dilution for each drug.
7. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI (brain heart infusion) broth.
8. In each serially diluted tube 200microliter of above culture suspension was added.
9. The tubes were incubated for 24 hours and observed for turbidity

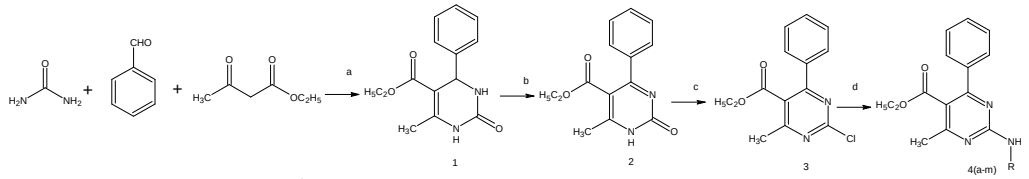
RESULTS AND DISCUSSION

Chemistry

The ethyl- 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(1)** was prepared by the reaction of benzaldehyde, ethylacetoacetate , urea and a catalytic amount of conc. hydrochloric acid **[9]**. The ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(1)** when treated with ceric ammonium nitrate and sodium bicarbonate gave ethyl- 6-methyl-2-oxo-4-phenyl-1,2-dihydropyrimidine-5-carboxylate **(2)(scheme 1)**.

The ethyl 2-chloro-4-methyl-6-phenylpyrimidine-5-carboxylate **(3)** was obtained by treating ethyl 6-methyl-2-oxo-4-phenyl-1, 2-dihydropyrimidine-5-carboxylate **(2)** with $POCl_3$. The ethyl- 2-chloro-4-methyl-6-phenylpyrimidine-5-carboxylate **(3)** treated with substituted anilines gave ethyl 2-(arylamino)-4-methyl-6-phenylpyrimidine-5-carboxylate **(4a-m) (scheme 1).**

All the new compounds were characterized by elemental analysis, 1H, 13C and mass spectral studies. The characterization data of newly synthesized compounds are summarized in Table 1.



Conditions*: (a) conc.HCl(cat.), ethanol, 75°C, 12h, (b) ceric ammonium nitrate, $NaHCO_3$,water and acetone, RT, (c) $POCl_3$, reflux, 2h, (d) substituted anilines, 4.0 N HCl , 1,4- dioxane, reflux, 12h.*

Table-2: Antibacterial activity data of compounds (4a-m) MIC Values in µg/ml

Compound No.	*E.Coli*	*Klebsiella*	*S.aureus*	*E.fecalis*
4a	25	100	0.2	0.2
4b	25	50	0.8	0.4
4c	3.12	-	-	0.8
4d	12.5	50	50	0.8
4e	50	-	12.5	0.4
4f	12.5	25	3.12	0.8
4g	50	100	0.2	0.2
4h	3.12	12.5	0.2	0.2
4i	50	-	1.6	0.8
4j	25	100	12.5	0.8
4k	12.5	100	1.6	1.6
4l	50	-	1.6	0.8
4m	25	100	6.25	6.25
Ciprofloxacin (Std)	20	2.0	1.0	2.0

BIOLOGICAL ACTIVITY

Antibacterial activity

The results of antibacterial activity were given in Table-2.Ciprofloxacin was used as the standard drug. Among the newly synthesized compounds most of them showed MIC value lower than that of the standard against E.fecalis. Similarly compounds 4a,4b,4g &4h were active against the microorganism S.aureus at concentration lower than that of the standard drug. However against E.coli and Klebsiella none of the newly synthesized compounds showed any significant activity comparable with the standard drug.

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