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Synthesis, characterization and pharmacological studies of some novel 2-N-(aryl substituted)-4-methyl-6-phenylpyrimidine-5-carboxylic acids

Ashoka[a], Balakrishna Kalluraya[a*], Anish kumar and B. Sunil Kumar[b]

[a]*Department of Studies in Chemistry, Mangalore University, Mangalagangothri-574199, Karnataka, India*
[b]*Department of Pharmaceutical Chemistry, N.E.T. Pharmacy College, Raichur-584103, Karnataka, India*

ABSTRACT

*A novel series of 2-N-(aryl substituted)-4-methyl-6-phenylpyrimidine-5-carboxylic acid**(5a-m)** were synthesized from ethyl2-N-(aryl substituted)-4-methyl-6-phenylpyrimidine-5-carboxylate**(4a-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. Among them compounds* **5a**, **5b**, **5g**, **5h**, **5k**, **5l** and **5m** *have shown significant activity.*

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

INTRODUCTION

Heterocyclic compounds are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, harmones and antibiotics [1-2], hence they have attracted considerable attention in the design of biologically active molecules [3-4]and in drug design[5-6].

Pyrimidine derivatives possess several interesting biological activities [7] such as antimicrobial [8], antitumor [9], and antifungal [10]. Many of the natural and synthetic polymers also contain pyrimidine nucleus.

In specific, 2,4,5- trisubstituted pyrimidine derivatives have shown potent anticancer activity as CDK inhibitors – a CDK(cyclin-depedent kinase) inhibitor is a chemical that inhibits the function of CDKs. It is used to treat cancers by preventing overproliferation of cancer cells. Piromidic acid and pipemidic acid are commercially available 2, 4, 5-trisubstituted 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[11] we planned to synthesize a hitherto unreported novel series of 2-N-(aryl substituted)-4-methyl-6-phenylpyrimidine-5-carboxylic acids in order to study their antibacterial activity.

EXPERIMENTAL SECTION

Chemistry

Thin layer chromatography was used to analyze the reaction progress and purity of the compounds synthesized. ^{1}H NMR spectra were recorded on Brucker spectrometer(400 MHz) in DMSO-d_6/$CDCl_3$ using TMS as an internal standard, ^{13}C NMR spectra were recorded on Brucker spectrometer(100 MHz) in $DMSOd_6$/$CDCl_3$. Mass spectra were recorded on Agilent6320 Ion Trap method.

General procedure for synthesis of ethyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate(1)[12]
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 6 h. Progress of the reaction was monitored by TLC(ethylacetate/petroleum ether,1:1, v/v). After completion of the reaction, the contents were 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**.

^{1}H NMR (DMSO-d_6) δ: 1.09(t, 3H, *J* = 7.2 Hz, -*CH2*CH3), 2.25 (s, 3H, Py-CH3), 3.98(q, 2H, *J* = 7.2Hz, -CH2*CH3*), 5.14(d, 1H, NH), 7.25(m, 3H, Ar-H), 7.32(m, 2H, Ar-H), 7.73(bs, 1H, NH), 9.19(bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 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)[13]
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^o C and stirred at room temperature for overnight. Progress of the reaction was monitored by LCMS. After completion of the reaction, the reaction mass was concentrated to remove acetone and the aqueous phase was extracted, dried over anhydrous sodium sulfate, filtered and concentrated to afford compound **2**.

^{1}H NMR (DMSO-d_6) δ: 0.82(t, 3H, *J* = 7.2 Hz, -*CH2*CH3), 2.39(s, 3H, Py-CH3), 3.94(q, 2H, *J* = 7.2Hz, -CH2*CH3*), 7.45(m, 5H, Ar-H), 12.39(bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 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)[14]
Compound **2**(3.87 mmol) and $POCl_3$(38.71 mmol) was heated at 120^o C for 2 h. Progress of the reaction was monitored by TLC(ethylacetate/petroleum ether,1:1, v/v). After completion of the reaction, the reaction mass was concentrated, the residue was dissolved in chloroform, 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 60-120 mesh silica gel column chromatography using ethyl acetate and petroleum ether as eluents. The product was eluted with 20% ethyl acetate and concentrated to afford compound **3**.

^{1}H NMR (DMSO-d_6) δ: 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); ^{13}C NMR (DMSO-d_6) δ: 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)[11]
To a solution of **3**(3.61 mmol) and substituted aniline(4.33 mmol) in 1,4-dioxane(25 mL), 4.0M HCl in 1,4-dioxane(2 mL) was added and was heated at 100^o C for 12 h. Progress of the reaction was monitored by TLC (ethylacetate/petroleum ether,1:1, v/v). After completion of the reaction, the reaction mass was concentrated, the residue was dissolved in ethylacetate and was washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to afford crude compounds(**4a-m**). The crude compounds were purified by 60-120 mesh silica gel column chromatography using ethyl acetate and petroleum ether as eluents. The products were eluted with 25% ethyl acetate and concentrated to afford pure compounds(**4a-m**).

Ethyl-2-[(2,4-difluorophenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylate(**4a**)
^{1}H NMR (DMSO-d_6) δ: 0.93(t, 3H, *J* = 7.2 Hz, -*CH2*CH3), 2.40(s, 3H, Py-CH3), 4.05(q, 2H, *J* = 7.2Hz, -CH2*CH3*), 7.07(t, 1H, *J* = 8.4 Hz, Ar-H), 7.3(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); ^{13}C NMR (DMSO-d_6) δ: 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; Off white solid; M.P : 100-102^o C; yield: 45 %.

Ethyl-2-[(4-fluorophenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylate(**4c**)
^{1}H NMR (DMSO-d_6) δ: 0.94(t, 3H, *J* = 7.08 Hz, -*CH2*CH3), 2.47(s, 3H, Py-CH3), 4.05(q, 2H, *J* = 7.12Hz, -CH2*CH3*), 7.15(t, 1H, *J* = 8.8Hz,Ar-H), 7.54(m, 5H, Ar-H), 7.81(m, 2H, Ar-H), 10.05(s, 1H, N-H); ^{13}C NMR (DMSO-d_6) δ: 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; Off white solid; M.P : 85-87^o C; yield: 57 %.

Ethyl-2-[(2-bromo-4-chloro-6-methyl phenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylate (**4h**)
^{1}H NMR (DMSO-d_6) δ: 0.92(t, 3H, *J* = 7.2 Hz, -*CH2*CH3), 2.23(s, 3H, Py-CH3), 4.04(q, 2H, *J* = 7.2 Hz, -CH2*CH3*), 7.46(m, 6H, Ar-H), 7.67(m, 2H, Ar-H), 9.43(s, 1H, N-H); ^{13}C NMR (DMSO-d_6) δ: 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; Off white solid; M.P : 146-148^o C; yield: 63 %.

Ethyl-4-methyl-2-[(2-methyl phenyl)amino]-6-phenylpyrimidine-5-carboxylate (**4l**)
^{1}H NMR (DMSO-d_6) δ: 0.92(t, 3H, *J* = 7.12 Hz, -*CH2*CH3), 2.24(s, 3H, Py-CH3), 2.39(s, 3H, Ar-CH3), 4.04(q, 2H, *J* = 7.12 Hz, -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); ^{13}C NMR (DMSO-d_6) δ: 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; Brown solid; M.P : 78-80^o C; yield: 82 %.

General procedure for synthesis of 2-N-(arylsubstituted)-4-methyl-6-phenylpyrimidine-5-carboxylic acid(5a-m)
To a solution of **4(a-m)**(3.61 mmol) in methanol(25 mL) and water(15 mL), NaOH(18.05 mmol) was added and was heated at 100^o C for 12 h. Progress of the reaction was monitored by TLC(ethylacetate/petroleum ether,1:1, v/v). After completion of the reaction, the reaction mass was concentrated, the residue was diluted with water, acidified with 1.5N HCl and solid precipitated was filtered and vacuum dried to afford pure compounds(**5a-m**)

2-[(2,4-difluorophenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylic acid(**5a**)
^{1}H NMR (DMSO-d_6) δ: 2.40(s, 3H, Ar-CH3), 7.07(t, 1H, *J* = 8.4 Hz,Ar-H), 7.31(t, 1H, *J*= 8.0 Hz, Ar-H), 7.45(m, 5H, Ar-H), 7.65(q, 1H, *J*=8.8, Ar-H), 9.55(s, 1H, N-H),13.17(s, 1H, COO-H); ^{13}C NMR (DMSO-d_6) δ: 22.8,103.4, 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: 342.32.

2-[(-4-chlorophenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylic acid (**5b**)
^{1}H NMR (DMSO-d_6) δ: 2.23(s, 3H, Ar-CH3), 7.35(m, 2H, Ar-H), 7.51(m, 3H, Ar-H),7.67(m, 2H, Ar-H), 7.85(m, 2H, Ar-H), 10.08(s, 1H, N-H), 13.17(s, 1H, COO-H); ^{13}C NMR (DMSO-d_6) δ: 22.7, 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: 340.77.

2-[(-3-bromophenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylic acid(**5d**)
^{1}H NMR (DMSO-d_6) δ: 2.23(s, 3H, Ar-CH3), 7.17(d, 1H,*J* = 8.0 Hz,Ar-H), 7.25(t, 1H, *J*= 8.0 Hz, Ar-H), 7.51(m, 3H, Ar-H), 7.71(m, 2H, Ar-H), 8.21(m, 1H, Ar-H), 10.15(s, 1H, N-H); ^{13}C NMR (DMSO-d_6) δ: 13.0, 18.5, 23.03, 118.1, 118.8, 121.6, 121.9, 124.5, 128.2, 128.8, 130.4, 130.9, 138.4, 142.3, 158.7, 163.9, 166.1, 169.7; LCMS M+1: 385.22.

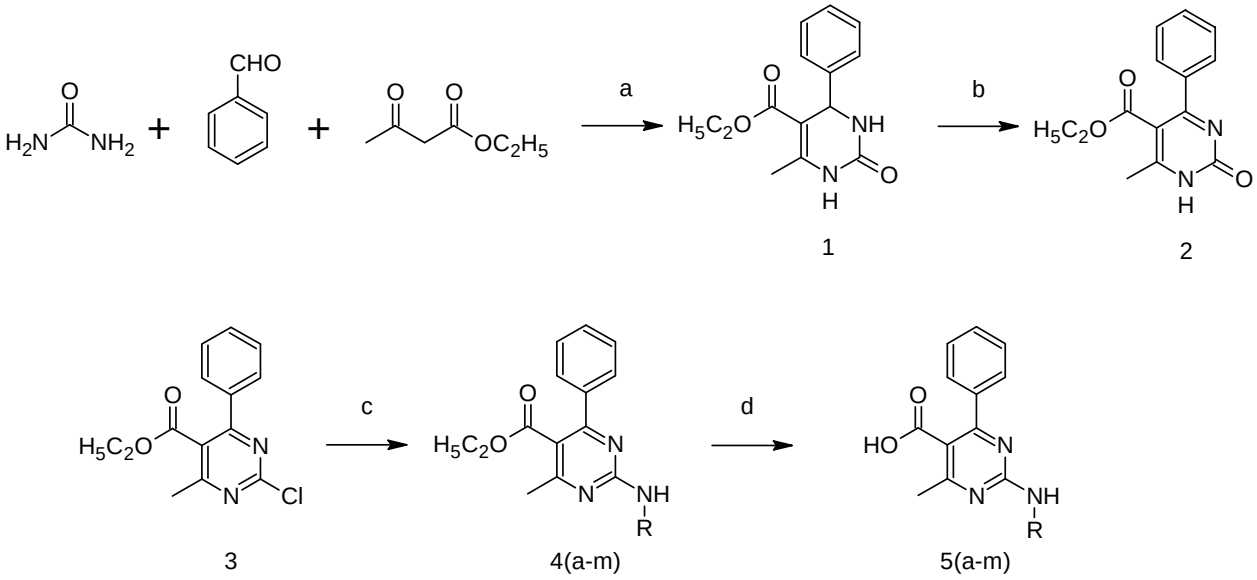
2-[(2-ethyl-6-methylphenyl)amino]-4-methyl-6-phenylpyrimidine-5-carboxylic acid(**5k**)
^{1}H NMR (DMSO-d_6) δ: 0.91(t, 3H, *J* = 8.0 Hz, -*CH2*CH3), 2.20(m, 5H, Ar-CH3 and CH2*CH3*), 2.23(s, 3H, Ar-CH3), 7.11(s, 3H, Ar-H), 7.48(m, 5H, Ar-H), 8.99(s, 1H, N-H); 12.98(bs, 1H, COO-H); ^{13}C NMR(DMSO-d_6) δ: 23.03, 118.1, 118.8, 121.6, 121.9, 124.5, 128.2, 128.8, 130.4, 130.9, 138.4, 142.3, 158.7, 163.9, 166.1, 169.7; LCMS M+1: 348.34.

Biological activity
Antibacterial activity
The newly synthesized compounds were screened for their antibacterial activity against four bacterial strains namely *Staphylococcus aureus, Enterococcus faecalis, Klebsiellapneumoniae* and *Eschericia.coli* byMinimum Inhibitory concentration (MIC) values were observed[15].

Procedure
1.9 Dilutions of each drug have to be done with BHI for MIC.
2.In the initial tube 20 microliter of drug was added into the 380 microliter of BHI broth.
3.For dilutions 200 microliter of BHI broth was added into the next 9 tubes separately.
4.Then from the initial tube 200 microliter was transferred to the first tube containing 200 microliter of BHI broth. This was considered as 10^{-1} dilution.
5.From 10^{-1} diluted tube 200 microliter 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, 5 microliter was taken and added into 2 mL of BHI (brain heart infusion) broth.
8.In each serially diluted tube 200 microliter of above culture suspension was added.
9.The tubes were incubated for 24 h and observed for turbidity.

Table 1: Characterization data of compounds 5a-m

Compound No.	Mol.formula	R	Colour and crystal nature	Yield (%) M.P. (^oC)	Analysis (%) found (calculated)		
					C	H	N
5a	$C_{18}H_{13}F_2N_3O_2$	2,4-Dichloro phenyl	Brown solid	55 240-250	63.31 (63.34)	3.87 (3.84)	12.35 (12.31)
5b	$C_{18}H_{14}ClN_3O_2$	4-Chloro phenyl	Off white solid	59 265-270	63.65 (63.63)	4.13 (4.15)	12.40 (12.37)
5c	$C_{18}H_{14}FN_3O_2$	4-Fluro phenyl	Yellow solid	70 205-210	66.88 (66.87)	4.38 (4.36)	13.02 (13.00)
5d	$C_{18}H_{14}BrN_3O_2$	3-Bromo phenyl	Off white solid	55 255-260	56.28 (56.27)	3.68 (3.67)	10.97 (10.94)
5e	$C_{18}H_{15}N_3O_2$	Phenyl	Off white solid	72 185-190	70.82 (70.81)	4.96 (4.95)	13.79 (13.76)
5f	$C_{18}H_{13}Cl_2N_3O_2$	2,6-Dichloro phenyl	Brown solid	76 265-270	57.80 (57.77)	3.53 (3.50)	11.21 (11.23)
5g	$C_{21}H_{21}N_3O_2$	2,4,6-Trimethyl phenyl	white solid	62 305-310	72.61 (72.60)	6.11 (6.09)	12.13 (12.10)
5h	$C_{18}H_{13}BrClN_3O_2$	2-Bromo-4-chloro-6-methyl phenyl	White solid	69 175-180	51.66 (51.64)	3.16 (3.13)	10.02 (10.04)
5i	$C_{19}H_{17}N_3O_3$	2-Methoxy phenyl	Yellow solid	49 180-185	68.02 (68.05)	5.13 (5.11)	12.55 (12.53)
5j	$C_{19}H_{17}N_3O_3$	3-Methoxy phenyl	Off white solid	53 190-195	68.02 (68.05)	5.13 (5.11)	12.55 (12.53)
5k	$C_{21}H_{21}N_3O_2$	2-Methyl-6-ethyl phenyl	Brown solid	89 255-260	72.57 (72.60)	6.10 (6.09)	12.11 (12.10)
5l	$C_{19}H_{17}N_3O_2$	2-Methyl phenyl	Brown solid	68 195-190	71.48 (71.46)	5.40 (5.37)	13.18 (13.16)
5m	$C_{19}H_{14}N_4O_2$	4-Cyano phenyl	White solid	50 190-195	69.04 (69.08)	4.09 (4.07)	16.98 (16.96)



***Conditions**:(a) conc.HCl(cat.), ethanol, 75^o C,12 h; (b) ceric ammonium nitrate, $NaHCO_3$, water and acetone, RT; (c) $POCl_3$, reflux, 2 h; (d) aryl substituted anilines, 4.0N HCl in 1,4-dioxane, 1,4- dioxane, reflux, 12 h;(e) NaOH, methanol, water, 100^o C, 12 h.*

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, ethyl acetoacetate, urea and a catalytic amount of conc. HCl. 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)**. 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 aryl substituted anilines gaveethyl 2-N-(aryl substituted)-4-methyl-6-phenylpyrimidine-5-

carboxylate**(4a-m)**. The ethyl-2-N-(aryl substituted)-4-methyl-6-phenylpyrimidine-5-carboxylate was treated with NaOH to get 2-N-(aryl substituted)-4-methyl-6-phenylpyrimidine-5-carboxylic acid (**5a-m**).

All the new compounds were characterized by elemental analysis, ^{1}H NMR, ^{13}C NMR and mass spectral studies. The yield and physical data of synthesized compounds are summarized in Table 1.

Table 2: Antibacterial activity data of the compounds(5a-m) MIC values in µg/mL

Compound No.	*E.Coli*	*Klebsiella*	*S.aureus*	*K.fecalis*
5a	100	100	0.2	0.8
5b	25	100	0.2	0.4
5c	100	100	25	1.6
5d	100	100	0.4	3.12
5e	-	-	3.12	6.25
5f	50	100	3.12	3.12
5g	50	100	1.6	1.6
5h	25	12.5	0.2	0.2
5i	100	-	3.12	1.6
5j	50	100	25	1.6
5k	50	100	0.4	0.8
5l	100	-	1.6	1.6
5m	50	100	1.6	3.2
Ciprofloxacin(std)	2.0	1.0	2.0	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 values lower than that of the standard against *E.fecalis*. Similarly compounds **5a**, **5b**, **5g**, **5h**, **5k**, **5l** and **5m** 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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