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Synthesis and characterization of 2-aminopyrimidine-5-carbonitrile derivatives and their antibiotic screening

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ABSTRACT

The target compound2-(arylamino)-4-(4-fluorophenyl/4-bromophenyl/isobutyl)-1,6-dihydro-1-methyl-6-oxopyri -midine -5-carbonitrilehave been synthesized by the condensation of 4-(4-fluorophenyl/4-bromophenyl/ isobutyl)-1,6-dihydro-1-methyl-2-(methylthio)-6-oxopyrimidine-5-carbonitrile with different aromatic amines using ethanol as solvent. Here three different aldehyde used in synthesis of 4-(4-fluorophenyl/4-bromophenyl/isobutyl)-1,6-dihydro-1-methyl-2-(methylthio)-6-oxopyrimidine-5-carbonitrile.The obtained products were characterized by ^{1}H-NMR, Mass and IR Spectra and screened for their antibiotic activity against different gram positive and gram negative bacterial strain.

Keyword: 2-Aminopyrimidine, Pyrimidine-5-carbonitrile, Antibiotic.

INTRODUCTION

Heterocyclic systems are found in variety of naturally occurring and synthetic compounds and are essential to life. They are important components of alkaloids, antibiotics, hormones and large number of synthetic drugs and dyes[1].

The nitrogen heterocycls are of great importance as they are present in nucleic acids, vitamins, proteins and other biologically important molecular systems[2].

The 2-aminopyrimidine as a common structural moiety are useful intermediates for the synthesis of important pharmaceuticals[3-6]that show a broad spectrum of biological activities including inhibitory activity against specific kinases.

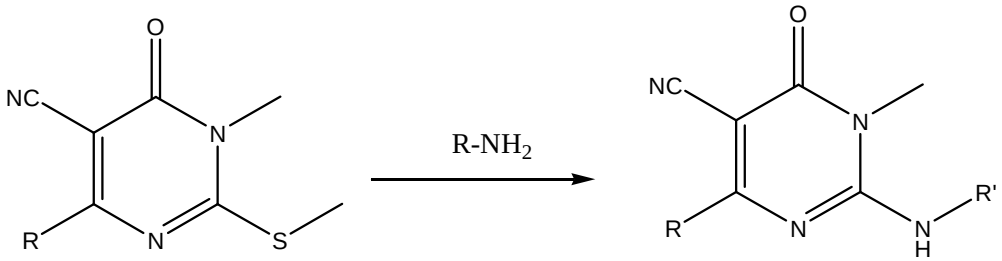
The 3,4-dihydropyrimidine derivatives are known to exhibit traditional antithyroid activity of the 5-fluoro-2-thiouracil[7]. Furthermore, dihydropyrimidine derivatives are also reported to have showed different pharmacological activities like antitumor[8], analgesic[9], antineoplastic[10],cardiovascular[11], antiallergic[12] etc. Substituted 2-aminopyrimidines are commonly obtained by the introduction of amino groups into a previously synthesized pyrimidine ring[13-15]by displacement of halides or other leaving groups at C-2 by nucleophilic aromatic substitution or metal-catalyzed C−N bond forming reactions.

Going through the references and in search of newer pharmacologically active pyrimidine-5-carbonitrile derivatives, we have synthesized some new 2-(aryl amino)-4-(4-fluorophenyl/4-bromophenyl/isobutyl)-1,6-dihydro-1-methyl-6-oxopyrimidine-5-carbonitrile by condensation 4-(4-fluorophenyl/4-bromophenyl/isobutyl)-1,6-dihydro-1-methyl-2-(methylthio)-6-oxopyrimidine-5-carbonitrile) with different aromatic amine using condensation method[16]. Compounds have been synthesized by reported method[17] and screened all the series of compounds for antibiotic activity.

EXPERIMENTAL SECTION

Melting points were taken in open capillary and are not corrected. Purity of synthesized compounds have been checked by TLC. Mass spectra were determined on Shimadzu-QP2010 spectrometer.IR spectra were recorded on Shimadzu-FTIR-8400 using KBr pallet. ^{1}H-NMR spectra were recorded in Bruker-Avance-II(400MHz) using DMSO-$d_6$as a solvent and TMS as an internal standard and the chemical shifts are reported as parts per million(ppm).

Reaction Scheme



(1) R = -C_6H_4-4-F,
= -C_6H_4-4-Br,
= -CH_2-CH-$(CH_3)_2$

(2a-o)
R' = Aryl

1.1Synthesis of 4-(4-fluorophenyl/4-bromophenyl/isobutyl)-1,6-dihydro-1-methyl-2-(methylthio)-6-oxopyri -midine-5-carbonitrile(1)[18]

A solution of 1,2,3,4-tetrahydro-6-(4-fluorophenyl/4-bromophenyl/isobutyl)-4-oxo-2-thioxopyrimidine-5-carbonitrile(0.05mol) in DMF(70ml) was stirred for 3hr with potassium carbonate(0.1mol) and methyl iodide(0.1mol). After completion of reaction, the reaction mixture was poured in to crushed ice and washed with water. Solid product was filtered, dried and crystallized from DMF.

2.1Synthesis of 2-(arylamino)-4-(4-fluorophenyl/4-bromophenyl/isobutyl)-1,6-dihydro-1-methyl-6-oxo pyrimidine-5-carbonitrile(2)

A mixture of4-(4-fluorophenyl/4-bromophenyl/isobutyl)-1,6-dihydro-1-methyl-2-(methylthio)-6-oxopyrimidine-5-carbonitrile(0.01mol) and different aromatic amine(0.01mol) in absolute alcohol(30ml) was refluxed for 10hr.Theprogressof reaction was monitored by thin layer chromatography. After completion of reaction, the reaction mixture was poured in tocrushed ice. The Productobtained was isolated and Crystalized from absolute alcohol.

2.2 Spectral analysis of novel arylamine derivatives

(2a)4-(4-fluorophenyl)-1,6-dihydro-1-methyl-6-oxo-2-(phenylamino)pyrimidine-5-carbonitrile:

Mass M^+= 320; IR(KBr) V(cm^{-1}), 3431(-NH,secondary),2930(-CH_3,Asym.), 2840(-CH_3,Sym.), 2209(-CN), 1638(-CO), 1090(N-C), 1240(C-F);^{1}HNMR(δ ppm)(400MHz, DMSO)δ 3.4(s,3H,N-CH_3), δ 7.8-7.9(m,9H,Ar-H).

(2b)2-(4-methoxyphenylamino)-4-(4-fluorophenyl)-1,6-dihydro-1-methyl-6-oxopyrimidine-5-carbonitrile:

Mass M^+=350; IR(KBr)V(cm^{-1}), 3430(-NH,secondary), 2922(-CH_3,Asym.), 2830(-CH_3,Sym.), 2200(-CN), 1635(-CO), 1090(N-C), 1270(C-F);^{1}HNMR(δ ppm) (400MHz, DMSO) δ 3.4(s,3H,N-CH_3), δ 2.3(s,3H,Ar-O-CH_3),δ 7.3-7.4(m,8H,Ar-H).

(2c)2-(2,4-dimethylphenylamino)-4-(4-fluorophenyl)-1,6-dihydro-1-methyl-6-oxopyrimidine-5-carbonitrile:

Mass M^+=348; IR(KBr)V(cm-1),3432(-NH,secondary), 2920(-CH_3,Asym.), 2830(-CH_3,Sym.),2210(-CN), 1095(N-C), 1255(C-F);^{1}H NMR(δ ppm)(400MHz, DMSO) δ 3.3(s,3H,N-CH_3), δ 2.4(s,3H,Ar-CH_3),δ 2.6(s,3H,Ar-CH_3), δ 7.2-7.3(m,7H,Ar-H).

(2d)2-(3-chlorophenylamino)-4-(4-fluorophenyl)-1,6-dihydro-1-methyl-6-oxopyrimidine-5-carbonitrile:

Mass M^+=354; IR(KBr)V(cm-1),3436(-NH,secondary), 2925(-CH_3,Asym.), 2836(-CH_3,Sym.), 2217(-CN), 1636(-CO), 1092(N-C), 1251(C-F), 763(C-Cl);^{1}H NMR(δ ppm)(400MHz, DMSO) δ 3.4(s,3H,N-CH_3), δ 7.0-7.2(m,8H,Ar-H).

(2e)2-(3,4-dimethylphenylamino)-4-(4-fluorophenyl)-1,6-dihydro-1-methyl-6-oxopyrimidine-5-carbonitrile:

Mass M^+=348; IR(KBr)V(cm-1),3431(-NH,secondary), 2922(-CH_3,Asym.), 2830(-CH_3,Sym.), 2221(-CN), 1646(-

CO), 1089(N-C), 1248(C-F); ^{1}H NMR(δ ppm)(400MHz, DMSO),δ 3.3(s, 3H,N-CH_3),δ 2.1(s,3H,Ar-CH_3), δ 2.3(s,3H,Ar-CH_3),δ7.5-7.6(m,7H,Ar-H).

(2f)4-(4-bromophenyl)-1,6-dihydro-1-methyl-6-oxo-2-(phenylamino)pyrimidine-5-carbonitrile:
Mass M^+=380; IR(KBr) V(cm^{-1}),3490(-NH, secondary), 2952(-CH_3, Asym.), 2872(-CH_3,Sym.), 2200(-CN), 1680(-CO), 1090(N-C), 680(C-Br); ^{1}H NMR(δ ppm)(400MHz, DMSO) δ 3.4(s,3H,N-CH_3), δ 7.2-7.9(m,9H,Ar-H).

(2g)2-(4-methoxyphenylamino)-4-(4-bromophenyl)-1,6-dihydro-1-methyl-6-oxopyrimidine-5-carbonitrile:
Mass M^+=410; IR(KBr) V(cm^{-1}),3480(-NH,secondary), 2962(-CH_3,Asym.), 2870(-CH_3,Sym.), 2220(-CN), 1675(-CO),1090(N-C), 700(C-Br);^{1}H NMR(δ ppm)(400MHz, DMSO) δ 3.4(s,3H,N-CH_3), δ 2.34(s,3H,Ar- O-CH_3), δ 7.38(m,8H,Ar-H).

(2h)2-(2,4-dimethylphenylamino)-4-(4-bromophenyl)-1,6-dihydro-1-methyl-6-oxopyrimidine-5-carbonitrile:
Mass M^+=408;IR(KBr) V(cm-1), 3482(-NH, secondary), 2950(-CH_3,Asym.), 2868(-CH_3,Sym.),1680(-CO),2210(-CN), 715(C-Br);^{1}H NMR(δ ppm)(400MHz, DMSO) δ 3.3(s,3H,N-CH_3),δ 2.6(s,3H,Ar-CH_3), δ 2.3(s,3H,Ar-CH_3), δ 7.2-7.8(m,7H,Ar-H).

(2i)2-(3-chlorophenylamino)-4-(4-bromophenyl)-1,6-dihydro-1-methyl-6-oxopyrimidine-5-carbonitrile:
Mass M^+=413;IR(KBr) V(cm-1), 3496(-NH, secondary), 2958(-CH_3,Asym.), 2863(-CH_3,Sym.), 2225(-CN), 1667(-CO), 1092(N-C), 680(C-Br),770(C-Cl);^{1}H NMR(δ ppm)(400MHz, DMSO) δ 3.4(s,3H,N-CH_3), δ 7.1-7.5(m ,8H,Ar-H).

(2j)2-(3,4-dimethylphenylamino)-4-(4-bromophenyl)-1,6-dihydro-1-methyl-6-oxopyrimidine-5-carbonitrile:
Mass M^+=408;IR(KBr) V(cm-1), 3439(-NH, secondary), 2967(-CH_3,Asym.), 2860(-CH_3,Sym.), 2229(-CN), 1665(-CO), 1089(N-C), 685(C-Br);^{1}H NMR(δ ppm)(400MHz, DMSO),δ 3.6(s,3H,N-CH_3), δ 2.1(s,3H,Ar-CH_3), δ 2.3(s,3H,Ar-CH_3), δ 7.4-7.8(m ,7H,Ar-H).

(2k) 1,6-dihydro-4-isobutyl-1-methyl-6-oxo-2-(phenylamino)pyrimidine-5-carbonitrile:
Mass M^+=282; IR(KBr) V(cm-1), 3429(-NH, secondary), 2965(-CH3,Asym.), 2870(-CH3, Sym.), 2221(-CN), 1667(-CO), 1084(N-C); ^{1}H NMR(δ ppm) (400MHz, DMSO), δ 1.0(d,6H,CH3), δ 2.0(m,1H,CH), δ 2.7(d, 2H, CH2), δ 3.2(s,3H,N-CH3), δ 7.4-7.8(m,5H,Ar-H).

(2l) 2-(4-methoxyphenylamino)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile:
Mass M^+=312; IR(KBr) V(cm-1), 3438(-NH,secondary), 2963(-CH3,Asym.), 2873(-CH3,Sym.), 2223(-CN), 1672(-CO), 1074(N-C); ^{1}H NMR(δ ppm)(400MHz, DMSO), δ 0.9(d,6H,CH3), δ 2.1(m,1H,CH), δ 2.3(s,3H,Ar-OCH_3), δ 2.5(d,2H,CH2), δ 3.2(s,3H,N-CH3), δ 7.2-7.9(m,4H,Ar-H).

Table–1: Physical constant

No.	Comp.	R	R'	M.F.	M.W.	MP °C	Yield %	N% Cal. (Found)
1.	2a	-C_6H_4-4-F	-C_6H_6	$C_{18}H_{13}FN_4O$	320	160	65	17.19(16.90)
2.	2b	-C_6H_4-4-F	-4-OCH_3-C_6H_4	$C_{19}H_{15}FN_4O_2$	350	184	63	15.99(15.50)
3.	2c	-C_6H_4-4-F	-2,4-$(CH_3)_2$- C_6H_3	$C_{20}H_{17}FN_4O$	348	156	65	16.08(15.90)
4.	2d	-C_6H_4-4-F	-3-Cl-C_6H_4	$C_{18}H_{12}FN_4OCl$	354	200	59	15.79(15.60)
5.	2e	-C_6H_4-4-F	-3,4-$(CH_3)_2$- C_6H_3	$C_{20}H_{17}FN_4O$	348	192	62	16.08(17.97)
6.	2f	-C_6H_4-4-Br	-C_6H_6	$C_{18}H_{13}BrN_4O$	380	268	68	14.70(14.35)
7.	2g	-C_6H_4-4-Br	-4-OCH_3-C_6H_4	$C_{19}H_{15}BrN_4O_2$	410	256	64	13.62(13.20)
8.	2h	-C_6H_4-4-Br	-2,4-$(CH_3)_2$- C_6H_3	$C_{20}H_{17}BrN_4O$	408	240	69	13.69(13.56)
9.	2i	-C_6H_4-4-Br	-3-Cl-C_6H_4	$C_{18}H_{12}BrN_4OCl$	413	258	62	15.48(15.25)
10.	2j	-C_6H_4-4-Br	-3,4-$(CH_3)_2$- C_6H_3	$C_{20}H_{17}BrN_4O$	408	252	65	13.69(13.50)
11.	2k	-CH_2-CH-$(CH_3)_2$	-C_6H_5	$C_{16}H_{18}N_4O$	282	118	49	19.84 (19.81)
12.	2l	-CH_2-CH-$(CH_3)_2$	-4-OCH_3-C_6H_4	$C_{17}H_{20}N_4O_2$	312	110	52	17.94 (17.94)
13.	2m	-CH_2-CH-$(CH_3)_2$	-2,4-$(CH_3)_2$- C_6H_3	$C_{18}H_{22}N_4O$	310	92	55	18.05 (18.06)
14.	2n	-CH_2-CH-$(CH_3)_2$	-3-Cl-C_6H_4	$C_{16}H_{17}N_4OCl$	316	98	54	17.69 (17.71)
15.	2o	-CH_2-CH-$(CH_3)_2$	-3,4-$(CH_3)_2$- C_6H_3	$C_{18}H_{22}N_4O$	310	102	57	18.05 (18.02)

(2m)2-(2,4-dimethylphenylamino)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile:
MassM^+=310; IR(KBr) V(cm-1), 3428(-NH,secondary), 2959(-CH3,Asym.), 2867(-CH3,Sym.), 2220(-CN), 1680(-CO), 1071(N-C);^{1}H NMR(δ ppm)(400MHz, DMSO), δ 0.9(d,6H,CH3), δ 2.0(m,1H,CH), δ 2.3(s,3H,Ar-CH_3), δ 2.5(s,3H,Ar-CH_3),δ 2.7(d,2H,CH2), δ 3.2(s,3H,N-CH3), δ 7.2(m,3H,Ar-H).

(2n)2-(3-chlorophenylamino)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile:
MassM^+=316; IR(KBr) V(cm-1),3417(-NH, secondary), 2968(-CH3,Asym.), 2858(-CH3,Sym.), 2222(-CN), 1670(-CO), 1069(N-C);^{1}H NMR(δ ppm)(400MHz, DMSO), δ 1.0(d,6H,CH3), δ 2.0(m,1H,CH), δ 2.6(d,2H,CH2), δ 3.4(s,3H,N-CH3), δ 7.6(m,4H,Ar-H).

(2o)2-(3,4-dimethylphenylamino)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile:
Mass M^+=310; IR(KBr)V(cm-1), 3437(-NH, secondary), 2961(-CH3,Asym.), 2872(-CH3,Sym.),2219(-CN), 1673(-CO),1077(N-C);^{1}H NMR(δ ppm)(400MHz, DMSO), δ 0.9(d,6H,CH3), δ 2.1(m,1H,CH),δ 2.2(s,3H,Ar-CH_3), δ 2.4(s,3H,Ar-CH_3), δ 2.6(d,2H,CH2), δ 3.3(s,3H,N-CH3), δ 7.7(m,3H, Ar-H).

3.1 Antibiotic Evolution

The compounds were tested for bacterial growth inhibition activity against a primary panel including *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Stock solutions ware prepared at 10mg/mL in DMSO, according to the weight for each compound.

Table–2:Single Point Bacterial Inhibition

100%inhibitionat 32µg/mL	50% inhibitionat 32µg/mL	> 32 µg/mL

No.	Compound	E. coli (ATCC 25922)	K. pneumoniae (ATCC 700603)	A. buamanii (ATCC 19606)	P. aeruginosa (ATCC 27853)	S. aureus (ATCC 43300)
1.	2a	>32	>32	>32	>32	>32
2.	2b	>32	32	>32	>32	>32
3.	2c	>32	>32	>32	>32	>32
4.	2d	>32	>32	>32	>32	>32
5.	2e	>32	>32	>32	32	>32
6.	2f	32	>32	>32	>32	>32
7.	2g	>32	>32	>32	>32	>32
8.	2h	>32	>32	>32	>32	>32
9.	2i	>32	>32	32	>32	>32
10.	2j	>32	>32	>32	>32	>32
11.	2k	>32	>32	>32	>32	>32
12.	2l	>32	32	>32	>32	>32
13.	2m	>32	>32	>32	>32	32
14.	2n	>32	>32	>32	>32	>32
15.	2o	>32	>32	>32	>32	>32

- **Compound preparation:**

Stock solutions ware prepared at 10mg/mL in DMSO, according to the weight for each compound. Gentle heating and sonication was required to solubilize the compounds. The highest concentration tested for each compound was 32µg/mL.

- **Single point bacterial inhibition assay:**

The primary bacteria panel, including *Escherichia coli* ATCC25922(GN_001), *Klebsiella pneumonia* ATCC700603(GN_003), *Acinetobacter baumannii* ATCC19606(GN_034), *Pseudomonas aeruginosa* ATCC27853 (GN_042) and *Staphylococcus aureus* ATCC43300(MRSA)(GP_020) were cultured in Muller Hinton broth(MHB) at 37°C overnight. A sample of each culture was then diluted 40-fold in fresh MHB broth and incubated at 37°C for 1.5-3hr. The compounds were plated at a single test concentration of 64µg/mL.Colistin, Polymyxin B, Vancomycin and Daptomycin were serially diluted two-fold across the wells, with compound concentrations ranging from 0.5 to 64 µg/mL, as controls of bacterial inhibitors. The resultant mid-log phase cultures were diluted to the final concentration of 5×105CFU/mL, then 50µL was added to each well of the compound containing 96-well plates(Corning;Cat.No-3641,NBS), giving a final compound concentration range of 0.25µg/mL to 32µg/mL for control inhibitors and 32µg/mL for test compounds. All the plates were covered and incubated at 37°C for 24hr. Inhibition of bacterial growth was determined visually(details Table – 2).

RESULTS AND DISCUSSION

Total 15 compounds have been synthesized and all compounds screen for antibiotic activity among them four compounds (2b, 2f, 2i, 2l)found to be active at 50%bacterial inhibition and two compounds (2e, 2m) found to be

active at 100% bacterial inhibition at32µg/mL in single point bacterial inhibition against different bacterial strain. Elemental analysis of all synthesized compound was carried out.

CONCLUSION

Rarely reported 2-Arylamino derivatives of 2-Arylaminepyrimidine-5-carbonitrile targeted to be prepared by condensing 4-(4-fluorophenyl/4-bromophenyl/4-isobutyl)-1,6-dihydro-1-methyl-2-(methylthio)-6-oxopyrimidine-5-carbonitrile with different arylamine**(2a-o)(Scheme-1)** in good yield**(Table-1)**.Result of biological evaluation given in **Table no.2**. Result of constitutional characterization of the obtained products by IR, ^{1}H-NMR and Mass Spectroscopy showed good agreement with the constitution of the targeted molecules.

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