Design, Synthesis and Evaluation of 2-amino-5-cyano-4-hydroxy-6-(alkyl/aryl amino)pyrimidine as Potential Antibacterial Agents.

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Abstract: Increasing strain of microorganism with resistance is an alarming danger in current therapeutic era. WHO is giving red signal towards half hazard use of anti-microbials. Emergence of Mycobacterium producing MDR/RR-TB, XDR-TB are rising day by day. Discovery of newer molecule have been a fascinating and challenging task for researcher. Based on pharmacophore requirement for initial binding sites for antibacterial agent and basic structural requirement for various heterocycles a series of substituted pyrimidine 2-amino-5-cyano-4-hydroxy-6-(alkyl/aryl amino)pyrimidine have been synthesized. 2 component method for synthesis of pyrimidine was employed in which Condensation of Different cyanoketene S, N acetal with guanidine nitrate in presence of Sodium ethoxide yielded the designed series of substituted pyrimidines. All synthesized compounds were characterised by physical and spectral analysis. All were screened for antibacterial activity by cup-plate method against gram positive and gram-negative microorganism and zone of inhibition was compared to standard drug ciprofloxacin.

Key words: Pyrimidine, Antibacterial,

I. INTRODUCTION

Microbes are unique creatures that adapt to varying lifestyles and environment resistance in extreme or adverse conditions. The genetic architecture of microbe may bear a significant signature not only in the sequences position, but also in the lifestyle to which it is adapted. It becomes a challenge for the society to find new chemical entities which can treat microbial infections. The increase clinical importance of drug-resistance bacterial pathogens has lent additional urgency to microbiological and antibacterial research. Thus, there is a continuing need for new antibacterial agents against pathogenic bacteria, which have proven to be incredibly adaptable in their fight for survival. 70% of cases now involve strains that are resistant to at least one drug. in communities and hospitals around the world, the number of patients with antibiotic-resistant infections continues to climb [1-2]

Research have been focused on

1. New analogues of existing classes of antibacterial agents.
2. Novel classes of antibacterial agents lacking cross resistance
3. Novel antibacterial screening targets.

It has been well documented that resistance is mainly caused by continued overreliance on and imprudent use of these antibacterial agents [3] and increasing evidence is being obtained suggesting that the same may be true for the emergence of biocide resistance [4,5]. Of particular concern is the possible cross-resistance of antibiotics and biocide due to common resistance mechanism [6,7].

Pyrimidine is a diazine i.e., six-membered heterocyclic with two nitrogen atoms in the ring. Pyrimidine is planer Six-member heterocyclic ring two N present in 1st and 3rd positions – *m* configuration. [8]. Substituted Pyrimidine constitute an important class of natural and non-natural products, any of which exhibit useful biological activities and clinical applications.[9]. pyrimidine skeleton present in many natural products like DNA, RNA, vitamin B1 (thiamine) etc. Literature survey shows wide range of biological and pharmacological activities exhibited by pyrimidine derivatives like Antimicrobial Activity [10], Antifungal activity [11], antileishmanial [12], anti-inflammatory [13] etc. Several pyrimide derivative and fused pyrimidine have been synthesized and reported to have comparable antibacterial action [14-17]. Various approaches are used for synthesis of substituted and fused pyrimidine compounds [18-22].

II. AIM OF PRESENT WORK

Boehringer M. and co-workers have reported some of the basic pharmacophore requirement for the antibacterial agent, particularly DNA gyrase inhibitors [23]. They have proposed initial binding sites and basic structural requirement for various heterocycles.

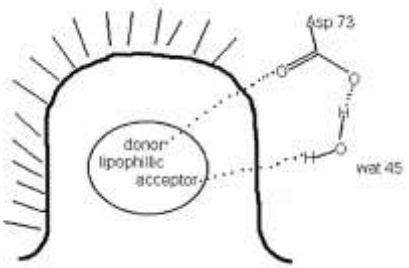


Fig 1: Pharmacophoric requirement

For pyrimidine ring, the basic structural requirements are:

1. Hydrogen atom donor at C-4 and
2. Hydrogen atom acceptor at 3rd position (it should be unsubstituted);

All other positions should be suitably substituted:

So it was thought of interest to synthesize a series of 2-amino-5-cyano-4-hydroxy-6-(alkyl/aryl amino)pyrimidine as a potential antibacterial agent. Where at C-6 different aryl and alkyl amines were substituted. We selected aryl and long chain alkylamine with higher lipophilicity which may help compounds to Penetrate through bacterial cell wall.

III. MATERIALS AND METHODS

3.1 Chemistry

Melting points of all the compounds were determined in open capillary and are uncorrected. Infrared spectra were recorded in potassium bromide disc on Perkin-Elmer Model-841 spectrophotometer. spectra were recorded on Shimadzu 640-A UV-Visible spectrophotometer. Nuclear magnetic spectra were taken on Varian A-60 Spectrophotometer at 60 MHz and the chemical shifts are given in parts per million (δ), down field from Tetramethyl silane (TMS) as internal standard. Splitting patterns are designated as follow. s = singlet, d = doublet, t = triplet, q = quartet and m = muptiplet. Mass spectra were obtained on Perkin Elmer LC-MS PE SCIEX API 165 spectrophotometer. The thin layer chromatography was performed on microscopic slides (2 x 7.5 cms.) coated with silica gel G and spots were visualized by UV radiation and exposure to iodine.

Synthetic grade chemicals were used.

Synthesis of 2-amino-5-cyano-4-hydroxy-6-(alkyl/aryl amino)pyrimidine

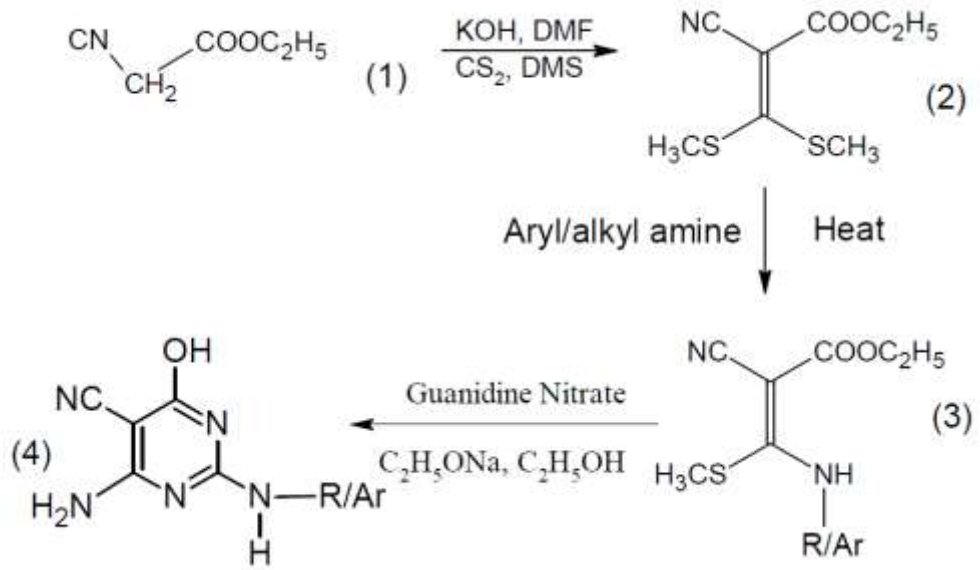


Figure 2: Scheme for synthesis of designed series

Sarting materials (2) and (3) were prepared as reported method.

3.1.1 Procedure for Synthesis of ethyl 2,2-di(methylmercapto)methylenecyanoacetate [S,S acetal] (2)

To an ice-cold solution of 13.2 gm (0.2 mole) of potassium hydroxide (85%) in 10 ml of water and 30 ml of dimethylformamide was added with cooling and stirring, 11.3 ml (0.1 mole) of ethylcyanoacetate followed by 7.6 gm (0.1 mole) of carbon disulfide. The mixture was stirred for one hour at room temp., cooled and treated dropwise with 25.2 ml (0.2 mole) of dimethylsulphate, maintaining the temperature at 20 °C. The reaction mixture was allowed to stand at room temp. for 12 hours and poured in to ice-water. The solid obtained was filtered, washed with water and dried and recrystallised from methanol to get yellow crystalline compound.

3.1.2 General Procedure for Synthesis of ethyl (1-methylmercapto-l-amino)methylenecyanoacetate [S,N acetal] (3)

For Alkylamine: Ethyl di(methylthio)methylenecyanoacetate (2) 4.34g (0.02 moles) was reacted with 9.02 moles alkyl amine in 25ml of ethanol by refluxing for 1 hours. After allowing to stand at room temperature for 24 hours the reaction mixture was added in ice cooled water and the solid obtained was filtered, washed with cold ethanol and dried. The crude product obtained was recrystalized from chloroform-n-hexane to obtain the product.

For arylamine: Ethyl di(methylthio)methylenecyanoacetate 4.34g (0.02 moles) was reacted with 0.02 moles of aryl amine in 25ml of ethanol by refluxing for 3-4 hours. After allow to stand at room temperature for 24 hours the reaction mixture was added in ice cooled water and the solid obtained was filtered, washed with cold ethanol and dried. The crude product obtained was recrystalized from chloroform-n-hexane to obtain the product.

3.1.3 Procedure for Synthesis of designated series: 2-amino-5-cyano-4-hydroxy-6-(alkyl/aryl amino)pyrimidine (4)

Solution of guanidine nitrate (0.01moles) and sodium ethoxide [prepared by dissolving sodium (0.02 moles) in refluxing ethanol (35 ml)] in ethanol (35ml) is added S,N-acetal (0.01 moles) and the reaction mixture is refluxed for 12 hours. the ethanol is removed under reduced pressure, the residue is diluted with ice cold water (40ml) and acidified with 4N hydrochloric acid (15ml). The crude, white oxopyrimidines are filtered and purified by crystallization from acetic acid.

3.2 Microbiological screening

The microbiological assay is based upon a comparison of inhibition of growth of organism by measured concentration of antibacterial to be examined with that mM produced by the known concentration of a standard preparation of antibiotics having a known activity.

Two methods are generally employed.

1) The cylinder plate (cup-plate) method.
2) The turbidimetric (tube assay) method.

3.2.1 The cylinder plate method

Depends upon diffusion of antibiotics from a vertical cylinder through a solidified agar layer in a Petridis or plate to an extent such that growth of added microorganism is prevented entirely in a zone around the cylinder containing solution of the antibiotics.

3.2.2 The turbidimetric method

Depends upon inhibition of growth of a microbial culture in a uniform solution of antibiotics in a fluid medium that is favourable to its rapid growth in absence of antibiotics.

3.2.3 The Dilution Method for Determination of Susceptibility to antimicrobial agent

In the dilution method for determining the susceptibility of an organism to anti-microbial agents, specific amount of antibiotics, prepared in decreasing Concentration in broth or agar by serial dilution technique are inoculated with a Standard suspension of bacterium to be tested. The susceptibility of an organism is determined by, after suitable period of incubation, by microscopic observation of presence or absence of growth, in varying concentration of drug demonstrating NO observable growth is a measure of the bacteriostatic effect of agent on the bacterium and is commonly referred to the minimum inhibitory concentration (MIC). When using a broth medium, technique can be further adapted to determine of bactericidal effect of an antibiotic of the minimum bactericidal Concentration (MBC).

Following factor must be considered in establishing dilution procedure and in the evaluation of test results.

1) The medium in which the effect are performed.
2) The Drug's stability.
3) The quantity of inoculum.
4) The organism's rate of growth.
5) The period of incubation of the tests.

3.2.4 The CUP-PLATE method

This method is similar to the broth dilution method except that a solid medium is used.

Preparation of medium:

Nutrient agar ……2%
Peptone ..……...1%
Beef extract ……1%
Sodium Chloride ……0.5%
Distilled water ……..…….up to 100ml

The ingredients were added to water and heated on water bath for about one and half hour till the solution becomes clear. Then the media is sterilized.

Inoculum of the plates:

Inoculum (1.0ml) is added to the sterilized plate (9" x 9") and then melted agar is added, mixed gently and allowed to set. Wells are bored in the agar plate and solution are filled in the bore at a constant volume and allowed to diffuse for a Period of 90 minutes. The plates are then incubated at 37°C for 24 hours after which the zone of inhibition is measured.

3.2.5 Filter paper method:

Disks are impregnated with various antimicrobial agents of specific concentration and are carefully placed on an agar plate that has been inoculated with a culture of bacterium to be tested. The plate is inoculated overnight and observed the following morning for zone of inhibition around disks containing the antimicrobial agents. Organisms that grow up to the edge of disks are resistant. This method has the advantage of Simplicity, 2) Speed of performance, Economy

3.3 Antibacterial Screening:

The series 4 (a-g) was screened for antibacterial activity against

E. Coli. (Gram negative bacteria)

S. aureus (gram positive bacteria) |

Four wells of diameter 20mm were bored in each agar plate containing the media Sterile suspension (1.0ml) of the microorganism in water and were set.

All the compounds were dissolved in DMF at concentration of 1 x 10^{-4} M and each well was filled with 0.2ml of the solution. The diffusion period was 90 minutes. After incubation at 37°C for 24 hours, zone of inhibition of all the compounds was observed.

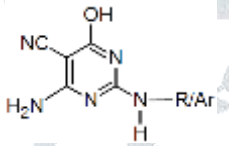
IV RESULTS AND DISCUSSION:

4.1 Chemistry:

Synthesis of designed derivatives was accomplished as per scheme in Figure 2.

4.1.1 Physical data

All the compounds are insoluble in water and organic solvents like methanol, ethanol, acetone, chloroform, benzene. Slightly soluble in sodium hydroxide and highly soluble in solvents like dimethylformaide, dimethylsulphoxide. Melting points of all compounds are above 300°C except 61g.



4(a-g)

Table 1: Physical data of 2-amino-5-cyano-4-hydroxy-6-(alkyl/aryl amino)pyrimidine

No.	R/Ar	Mol. for.	Mol. Wt.	Rf*	MP(°C)	%
4a	p-$OCH_3C_6H_4$	$C_{12}H_{11}N_50_2$	257.3	0.38	320-325	61
4b	p-F C_6H_4	$C_{11}H_8FN_50$	245.0	0.40	Above 350	60
4c	p-Cl C_6H_4	$C_{11}H_8CIN_50$	261.5	0.41	Above 350	58
4d	p-CF_3 C_6H_4	$C_{12}H_8F_3N_50$	295.1	0.40	Above 350	60
4e	-CH_2-CH2-N(C_2H_5)$_2$	$C_{11}H_{18}N_60$	250.4	0.38	Above 350	60
4f	-CH_2-CH_2-CH_2-N(C_2H_5)$_2$	$C_{12}H_{20}N_60$	264.5	0.37	Above 350	55
4g	-CH-(CH_2)$_3$-N(C_2H_5)$_2$ | CH_2	$C_{14}H_{24}N_60$	292.4	0.37	276-280	50

* Solvent system: Benzene: Methanol (8:2)

4.1.2 Spectral analysis

4.1.2.1 U.V. Spectra:

The U.V. Spectra of all the compounds were studied in 0.1N sodium hydroxide.

All the compounds exhibited two peaks, in the range 225 to 232 and 265 to 275 nm, which indicates that all the compounds belong to the same series.

4.2.2.1 I.R. Spectra:

The I.R. Spectra of all synthesized compounds exhibited characteristic bands in region of 3480-3100 cm^{-1} due to (NH) stretching. Bands are obtained in the region 1680-1720 cm^{-1} due to carbonyl stretching. They also exhibited characteristic band of CN around 2200^{-1}.

4.2.2.3 Mass Spectra:

The mass spectra of compounds were studied and all compounds found to show M^+, M^{+1} peaks corresponding to their molecular weight except 4c, which was found to show M^{+2} peak cue to presence of chloride.

Table 2: Spectral data of 2-amino-5-cyano-4-hydroxy-6-(alkyl/aryl amino)pyrimidine

No.	R/Ar	U.V. λmax (nm)	I.R. Spectra (cm^{-1})	Mass Peak (m/e)
4a	p-$OCH_3C_6H_4$	230, 271	3480, 3420, 3300 (NH) 2210 (C≡N), 1680 (C=O)	258 (M^{+1}) 257 (M^{+})
4b	p-F C_6H_4	225, 275	3440, 3320, 3200 (NH) 2200 (C≡N), 1680 (C=O)	246 (M^{+1}) 245 (M^{+})
4c	p-Cl C_6H_4	230, 272	3480, 3420, 3260 (NH) 2210 (C≡N), 1680 (C=O)	264 (M^{+2}) 262 (M^{+})
4d	p-CF_3 C_6H_4	227, 270	3440, 3400, 3360 (NH) 2220 (C≡N), 1670 (C=O)	295 (M^{+1}) 296 (M^{+})
4e	-CH_2-CH2-N(C_2H_5)$_2$	225, 265	3420, 3340 3280 (NH) 2220 (C≡N), 1680 (C=O)	251 (M^{+1}) 250 (M^{+})
4f	-CH_2-CH_2-CH_2-N(C_2H_5)$_2$	227, 270	3440, 3420, 3340 (NH) 2210 (C≡N), 1680 (C=O)	266 (M^{+1}) 265 (M^{+})
4g	-CH-(CH_2)$_3$-N(C_2H_5)$_2$ | CH_2	230, 268	3480, 3420, 3300 (NH) 2210 (C≡N), 1680 (C=O)	293 (M^{+1}) 292 (M^{+})

4.2 Microbial screening

4.2.1 Antibacterial activity:

It was observed from the data that all the compounds 4(a-g) exhibited very little activity against E. Coli and S. aureus. The most active derivative in the series against gram-negative and gram-positive bacteria found was.

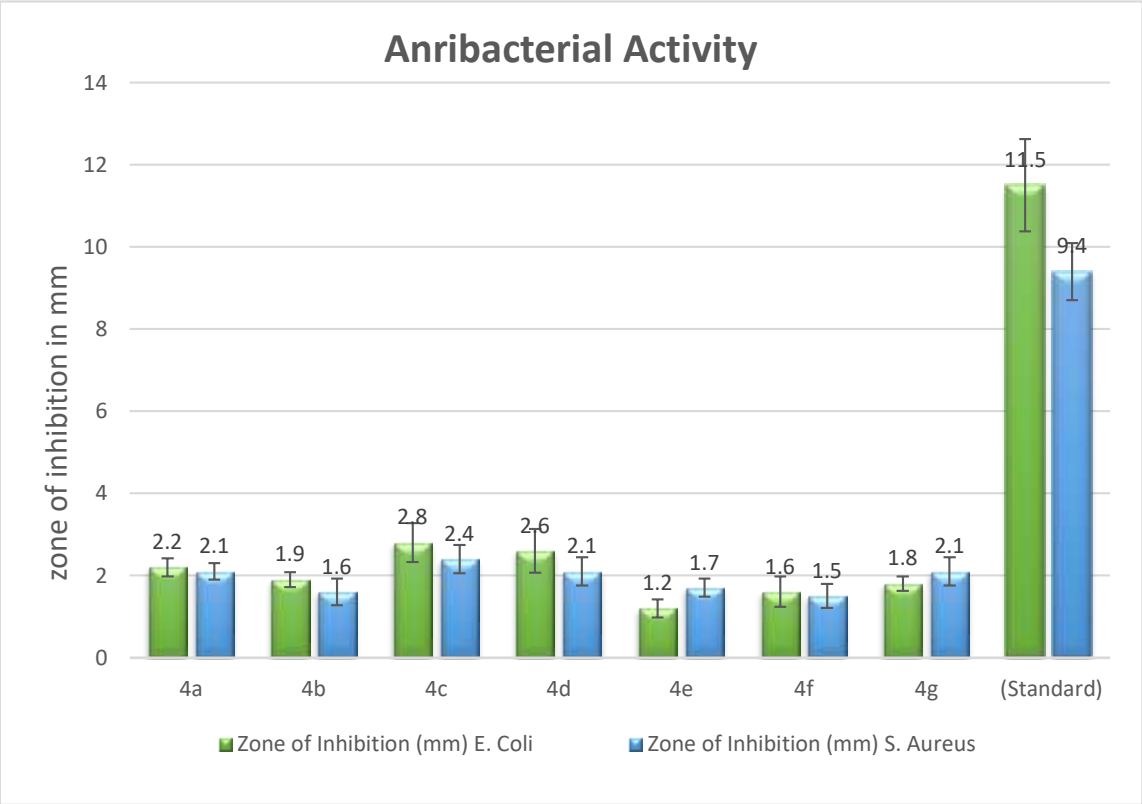
Gram positive ------- p-Cl (4c)

Gram negative ------ p-Cl (4c)

All the compounds had comparatively lower activity than the standard ciprofloxacin at a concentration of 1 x 10^{-4}M.

Table 3: Antibacterial activity of target compounds (4a-4g) in comparison to Ciprofloxacin

		Zone of Inhibition (mm)	
No.	**R/Ar**	E. Coli	S. Aureus
4a	p-$OCH_3C_6H_4$	02.2 ± 0.22	2.1 ± 0.21
4b	p-F C_6H_4	01.9 ± 0.18	1.6 ± 0.32
4c	p-Cl C_6H_4	02.8 ± 0.47	2.4 ± 0.34
4d	p-CF_3 C_6H_4	02.6 ± 0.53	2.1 ± 0.34
4e	-CH_2-CH2-N(C_2H_5)$_2$	01.2 ± 0.22	1.7 ± 0.22
4f	-CH_2-CH_2-CH_2-N(C_2H_5)$_2$	01.6 ± 0.37	1.5 ± 0.29
4g	-CH-(CH_2)$_3$-N(C_2H_5)$_2$ •| CH_2	01.8 ± 0.18	2.1 ± 0.34
Ciprofloxacin (Standard)		11.5 ± 1.12	9.4 ± 0.69



V. CONCLUSION:

A series of S, N-acetal 3 (a-g) were synthesized by reaction of alkyl/aryl amine with S, S-acetal and their condensation with guanidine gave the target compounds 2-amino-5-cyano-4-hydroxy-6-(alkyl/aryl amino) pyrimidine 4(a-g). All the synthesized compounds were characterized by T.L.C., U.V and I.R spectral analysis. Compounds were characterized by mass spectra also. The title compounds 4(a-g) were evaluated for invitro antibacterial activity against E. Coli and S. Aureus by the agar cup-plate method. All the compounds were found to be less active than standard drug (ciprofloxacin 3 x 10^{-6}M) at concentration 1 x 10^{-4} M.

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