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Conventional and microwave assisted synthesis of novel pyrimidine derivatives as antimicrobial and antitubercular agent

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ABSTRACT

A simple and efficient approach towards single step synthesis of 6-amino-5-cyano-2-(hydroxy/mercapto)-4-substitutedpyrimidine derivatives has been developed by three component condensation of aromatic aldehydes, malononitrile and urea or thiourea using conventional heating and microwave irradiation technique. Some of these novel derivatives showed moderate to potent in-vitro antibacterial, antifungal and antitubercular activity.

Keywords: Pyrimidine derivatives, Conventional heating, MW irradiation, Antimicrobial activity, Antitubercular activity.

INTRODUCTION

Pyrimidine and its derivatives displays a wide spectrum of pharmacological activity such as antimicrobial [1-3], antitubercular[3-5], antimalarial[5,6], analgesic and anti-inflammatory [7,8], antiviral[9], antifilarial[10], antioxidant[11], anthelmintic[12], anti-HIV[13] and anticancer [3,14].

Biginelli in year 1893 reported one-step synthesis of 3,4-dihydropyrimidin-2(1*H*)-one by three-component condensation of aldehydes, ethyl acetoacetate and urea in alcohol using strong mineral acid[15].The method had certain limitations like low yield, longer reaction time especially with aliphatic and substituted aromatic aldehydes etc. The scope of the original Biginelli reaction was gradually extended by variation of all three building blocks, allowing access to a large number of multi functionalized dihydropyrimidinones. Many researchers reported the synthesis of dihydropyrimidinones including classical conditions with microwave irradiation. The different catalysts like conc. HCl, H_2SO_4, LiBr, $MgBr_2$, $ZrCl_4$, $BiCl_3$, $LiClO_4$, $LaCl_3$, $FeCl_3$, $KHSO_4$, PPE, $ZnCl_2$, $Bi(OTf)_3$, NH_4Cl, $Mn(OAc)_3$, $(MoCl_5)_2$, PPA, Iodine, NBS, $SiO_2/NaHSO_4$, $AlCl_3$, $Cu(OTf)_2$, H_3BO_3, $InCl_3$, CAN, $InBr_3$, $BF_3.OEt_2$, KSF clay, phosphorus pentoxide, triflates of lanthanide compounds, Cu/silica xerogel composite, ion-exchange resin etc. have been tried[16,17].However, out of several catalyst used, some of them are expensive, incompatible with other functional groups, difficult to prepare, require prolonged reaction time, gives unsatisfactory yields and require tedious workup procedures for the isolation of pure product. This requires the development of a new protocol for high yield and the use of inexpensive reagent, which requires shorter reaction time and with easier workup procedure. Even though high yields could be achieved by multi-step procedures, these methods lack the simplicity of original one-pot Biginelli protocol and are no longer used. Therefore, Biginelli reaction continues to attract the attention of synthetic chemists for the discovery of milder and efficient procedure.

Nowadays, development of solvent-free reactions and use of microwave irradiation has proved to be a powerful tool for both speeding up chemical optimizations and for efficient preparation of new target compounds[18-20]. They not only reduce the use of organic solvent, but also enhance the rate of many organic reactions.

So, keeping all above observations in mind, we planned to develop a single step synthesis of 6-amino-5-cyano-2-(hydroxy/mercapto)-4-substitutedpyrimidine derivatives by three-component condensation reaction of aromatic aldehydes, malononitrile and urea or thiourea under conventional heating and microwave irradiation in order to investigate its antimicrobial and antitubercular activity.

MATERIALS AND METHODS

Commercially available LR grade reagents and solvents were used as received without additional purification. The progress of the reaction and purity of the synthesized compounds were checked by TLC (Merck, silica gel F_{254}) and detection with iodine vapours and UV light at 254 nm. Melting points were recorded on Chemline CL726 melting point apparatus in an open capillary tube and are uncorrected. IR spectra in KBr (ν_{max}, cm^{-1}) were recorded at room temperature using Shimadzu FT-IR 157 spectrophotometer. The ^1H NMR spectra (400 MHz) were recorded in $CDCl_3$ or DMSO-d_6 on Bruker advance III NMR spectrophotometer. Chemical shifts are reported in ppm downfield from TMS as reference standard. The Mass spectra were scanned on Shimadzu GC-MS QP 2010 mass spectrometer.

General procedure for synthesis of pyrimidine derivatives (1a-1l and 2a-2l):

Conventional method

A mixture of different aromatic aldehyde (20 mM), malononitrile (20 mM), urea or thiourea (22 mM) and con.HCl (0.5 mL) was dissolved in 25 mL absolute ethanol in a 100 mL round bottom flask. The resulting mixture was heated at reflux on a water bath. There action mixture was poured on the crushed ice (about 200g) after the completion of the reaction. The solid was filtered, washed with little ice cold water, dried and recrystallized from suitable solvent.

Microwave assisted method

A mixture of substituted aldehyde (5 mM), malononitrile (5 mM), urea or thiourea (5 mM) and con.H_2SO_4 (1-2 drops) in absolute ethanol (5mL) were taken in 10 mL beaker and irradiated in the domestic type microwave oven 900 W with a frequency 2450 MHz (Kenstar OM-25 DCE, India) for 2-4 min (one pulse each of 30 sec). After the completion of reaction, mixture is allowed to stand at room temperature and the product formed was filtered off, washed with ethanol, dried and recrystallized from suitable solvent.

Antimicrobial activity

The MICs of synthesized compounds were carried out by broth micro dilution method according to reported procedure[21]. Preparation of nutrient broth, subculture, agar medium and peptone water was done as per the standard procedure. Serial dilutions of antimicrobial agents were made in Muller Hinton broth, after which a standardized bacterial/fungal suspension was added. Quantities of compound serially diluted in test tubes. One control test tube was prepared without compound which serves as growth control. All test tubes were inoculated with a calibrated suspension of the microorganism to be tested and incubated at 37°C for 24 h.

In primary screening 1000, 500, 200 and 100 µg/mL concentrations of the synthesized drugs were taken. The active drugs found in this primary screening were further diluted to obtain 75, 50and 25 µg/mL concentrations and tested for antimicrobial activity. MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. The highest dilution showing at least 99% inhibition was taken as MIC. The result of this is much affected by the size of the inoculums. All the test tubes contained 10^8 colony forming unit (CFU) microorganism and 50 µL sample.

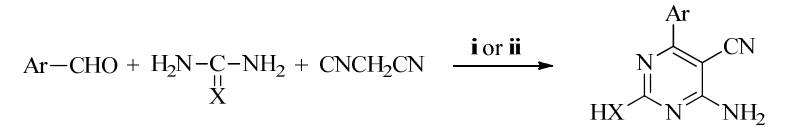
Antitubercular activity

MICs were determined and interpreted for *Mycobacterium tuberculosis* $H_{37}Rv$ according to the reported procedure by macro dilution reference method[22] using L. J. media. A culture of *M.tuberculosis* $H_{37}Rv$ growing on L.J. medium was harvested in 0.85% saline in bijou bottles. Compounds were taken at concentrations of 100, 50 and 25 µg/mL in DMSO. The bottles then inoculated with test compounds and incubated at 37°C for 24 h followed by streaking of *M. tuberculosis* $H_{37}Rv$ ($5*10^4$ bacilli per tube). These bottles were then incubated at 37°C and inspected for growth twice a week for a period of three weeks. Readings were taken at the end of 3 weeks. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* $H_{37}Rv$. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The appearance of turbidity was considered as growth and indicates resistance to the compound.

RESULTS AND DISCUSSION

Synthetic approach

Compounds 6-amino-5-cyano-2-hydroxy-4-substitutedpyrimidines (1a-1l) and 6-amino-5-cyano-2-mercapto-4-substitutedpyrimidines(2a-2l) were prepared in good yields under two different reaction conditions like conventional heating and microwave assisted synthesis. These reactions are summarized in Scheme 1.During the progress of reaction, the activated arylmethylenemalononitrile is likely to be formed via a Knoevenagel condensation reaction of aromatic aldehydes and malononitrile, which subsequently reacted with urea or thiourea to form desired product.



X = O, **1a-1l**; X = S, **2a-2l**

Reaction conditions: **i** = EtOH, con. HCl, reflux, 1.5-3 h (56-81%)
ii = EtOH, con. H_2SO_4, MWI, 2-3.5 min, (64-87%)

Scheme 1 Synthetic route of compounds 1a-1l and 2a-2l

In the presence of conc. HCl, reaction proceeds smoothly giving desired products in short time and in a quantitative yield. It was observed that the electron donating groups as well as electron withdrawing groups present in aryl aldehydes does not affect the yield of the reaction. The physicochemical data of all the newly synthesized compounds are summarized in Table 1.

Microwave assisted synthesis, besides being advantageous in simple reaction conditions and easy work-up procedures, less time consuming and eco-friendly, has resulted in better yields over the conventional method. Both synthetic methods are compared in terms of % yield and reaction time(Table 1).

Table 1Physicochemical data of synthesized compounds (1a-1l and 2a-2l)

Compds.	X	Ar	Melting Point (°C)*	R_f[$]	Conventional Method		Microwave Assisted Method	
					Time (h)	% Yield[#]	Time (min)	% Yield[#]
1a	O	C_6H_5-	179-180 [a]	0.72	3.0	78	3.5	84
1b	O	C_6H_5CH=CH-	151-153 [a]	0.67	2.5	60	3.0	67
1c	O	4-OHC_6H_4-	173-175 [b]	0.51	2.5	74	3.0	80
1d	O	2-ClC_6H_4-	180-182 [a]	0.65	2.0	81	2.5	86
1e	O	4-ClC_6H_4-	162-164 [a]	0.68	1.5	79	2.0	85
1f	O	4-$N(CH_3)_2C_6H_4$-	191-192 [a]	0.58	1.5	74	2.0	81
1g	O	4-$OMeC_6H_4$-	156-157 [a]	0.60	1.5	77	2.0	83
1h	O	4-OH-3-OMe-C_6H_3-	194-196 [a]	0.52	2.5	62	3.0	69
1i	O	2-$NO_2C_6H_4$-	199-200 [a]	0.59	1.5	81	2.0	87
1j	O	4-$NO_2C_6H_4$-	221-223 [a]	0.64	1.5	77	2.0	87
1k	O	3-$OMeC_6H_4$-	167-169 [a]	0.73	2.0	71	2.5	79
1l	O	3-$NO_2C_6H_4$-	160-162 [a]	0.69	2.0	72	2.5	80
2a	S	C_6H_5-	152-153 [a]	0.76	3.0	75	3.5	83
2b	S	C_6H_5CH=CH-	118-120 [a]	0.73	2.5	56	3.0	64
2c	S	4-OHC_6H_4-	141-142 [b]	0.48	2.5	72	3.0	79
2d	S	2-ClC_6H_4-	148-149 [a]	0.71	2.0	78	2.5	84
2e	S	4-ClC_6H_4-	123-124 [a]	0.75	1.5	73	2.0	80
2f	S	4-$N(CH_3)_2C_6H_4$-	172-173 [a]	0.63	1.5	76	2.0	83
2g	S	4-$OMeC_6H_4$-	120-121 [a]	0.55	1.5	71	2.0	78
2h	S	4-OH-3-OMe-C_6H_3-	167-168 [b]	0.67	2.5	64	3.0	70
2i	S	2-$NO_2C_6H_4$-	172-173 [a]	0.68	1.5	79	2.0	85
2j	S	4-$NO_2C_6H_4$-	190-191 [a]	0.73	1.5	75	2.0	82
2k	S	3-$OMeC_6H_4$-	129-130 [a]	0.71	2.0	71	2.5	77
2l	S	3-$NO_2C_6H_4$-	131-132 [a]	0.69	2.0	74	2.5	80

** Recrystallization Solvent:[a] = Ethanol, [b] = EtOH:H_2O (1:1);[$]Mobile Phase = Toluene : Ethyl acetate (7:3);*
[#] Yield refers to pure isolated product

Characterization

Structures of all the synthesized compounds 1a-1l and 2a-2lwere recognized on the basis of spectral study. The IR, ^{1}H NMR and mass spectral data of synthesized compounds are presented in Table 2. All the spectral data showed that the synthesized compounds are in full agreement with the proposed structures. For example, all IR spectra shows the presence of CN at region 2232-2211 cm^{-1} and two sharp bands at 3492-3420 and 3310-3220 cm^{-1} due to asymmetric and symmetric stretching of primary NH_2 group. In the ^{1}H NMR spectrum, the signals of the different protons were confirmed on the basis of their δ value and multiplicities. In ^{1}H NMR spectrum of 1e, two broad singlets at δ 5.30 and 7.76 ppm arises due to the presence of primary NH_2 group and phenolic proton respectively.

Table 2 Spectral data of synthesized compounds (1a-1l and 2a-2l)

Compds.	IR (KBr, *v*, cm^{-1})	^1H NMR ($CDCl_3$, δ, ppm)	MF (MW) m/z
1a	3473, 3233, 2221, 1635, 1286, 1170	5.44 (s, 2H, NH_2), 7.55-7.59 (t, 2H, aro. CH), 7.65-7.68 (t, 1H, aro. CH), 7.81 (s, 1H, OH), 7.93-7.95 (d, 2H, aro. CH)	$C_{11}H_8N_4O$ (212.21) 212 (M^+)
1b	3410, 3238, 3086, 2225, 1687, 1307	5.34 (s, 2H, NH_2), 7.26-7.27 (d, 1H, Ar-CH=CH-), 7.28-7.29 (d, 1H, Ar-CH=CH-), 7.42-7.48 (m, 3H, aro. CH), 7.61-7.62 (d, 2H, aro. CH), 7.82 (s, 1H, OH)	$C_{13}H_{10}N_4O$ (238.24) 238 (M^+)
1c	3493, 3307, 3233, 2231, 1627, 1288	DMSO-d_6: 5.40 (s, 2H, NH_2), 6.86-6.87 (d, 2H, aro. CH), 7.05 (s, 1H, OH), 7.48-7.50 (d, 2H, aro. CH), 7.65 (s, 1H, OH)	$C_{11}H_8N_4O_2$ (228.21) 228 (M^+)
1d	3463, 3225, 2226, 1578, 1290, 749	5.18 (s, 2H, NH_2), 7.46-7.49 (m, 1H, aro. CH), 7.57-7.58 (d, 2H, aro. CH), 7.82 (s, 1H, OH), 8.19-8.21 (d, 1H, aro. CH)	$C_{11}H_7ClN_4O$ (246.65) 246 (M^+), 248 (M^{+2})
1e	3445, 3214, 2220, 1571, 1240, 759	5.30 (s, 2H, NH_2), 7.53-7.56 (d, 2H, aro. CH), 7.76 (s, 1H, OH), 7.87-7.89 (d, 2H, aro. CH)	$C_{11}H_7ClN_4O$ (246.65) 246 (M^+), 248 (M^{+2})
1f	3434, 3225, 2215, 1565, 1293, 1169	3.17 (s, 6H, $N(CH_3)_2$), 5.33 (s, 2H, NH_2), 6.70-6.72 (d, 2H, aro. CH), 7.48 (s, 1H, OH), 7.83-7.84 (d, 2H, aro. CH)	$C_{13}H_{13}N_5O$ (255.28) 255 (M^+)
1g	3450, 3237, 2224, 1570, 1263, 1177	3.94 (s, 3H, OCH_3), 5.50 (s, 2H, NH_2), 7.03-7.05 (d, 2H, aro. CH), 7.68 (s, 1H, OH), 7.93-7.95 (d, 2H, aro. CH)	$C_{12}H_{10}N_4O_2$ (242.23) 242 (M^+)
1h	3486, 3322, 3227, 2221, 1630, 1165	3.98 (s, 3H, OCH_3), 5.14 (s, 2H, NH_2), 7.01-7.03 (d, 1H, aro. CH), 7.26 (s, 1H, OH), 7.30-7.32 (dd, 1H, aro. CH), 7.63 (s, 1H, OH), 7.73 (d, 1H, aro. CH)	$C_{12}H_{10}N_4O_3$ (258.23) 258 (M^+)
1i	3445, 3230, 2223, 1601, 1529, 1347	5.18 (s, 2H, NH_2), 7.82-7.85 (m, 2H, aro. CH), 7.87 (s, 1H, OH), 7.89-7.92 (m, 1H, aro. CH), 8.37-8.39 (dd, 1H, aro. CH)	$C_{11}H_7N_5O_3$ (257.20) 257 (M^+)
1j	3432, 3226, 2211, 1629, 1533, 1354	5.13 (s, 2H, NH_2), 7.91 (s, 1H, OH), 8.09-8.11 (d, 2H, aro. CH), 8.40-8.43 (d, 2H, aro. CH)	$C_{11}H_7N_5O_3$ (257.20) 257 (M^+)
1k	3445, 3243, 2215, 1570, 1256, 1170	3.93 (s, 3H, OCH_3), 5.33 (s, 2H, NH_2), 7.00-7.02 (d, 1H, aro. CH), 7.45-7.50 (m, 3H, aro. CH), 7.65 (s, 1H, OH)	$C_{12}H_{10}N_4O_2$ (242.23) 242 (M^+)
1l	3420, 3221, 2223, 1632, 1529, 1351	5.24 (s, 2H, NH_2), 7.77-7.79 (dd, 1H, aro. CH), 7.84 (s, 1H, OH), 8.11-8.13 (dd, 1H, aro. CH), 8.52 (m, 1H, aro. CH), 8.62 (s, 1H, aro. CH)	$C_{11}H_7N_5O_3$ (257.20) 257 (M^+)
2a	3464, 3219, 2227, 1623, 1347, 1017	4.81 (s, 1H, SH), 5.48 (s, 2H, NH_2), 7.56-7.59 (t, 2H, aro. CH), 7.64-7.68 (t, 1H, aro. CH), 7.93-7.95 (d, 2H, aro. CH)	$C_{11}H_8N_4S$ (228.27) 228 (M^+)
2b	3450, 3239, 3079, 2228, 1681, 1315	4.82 (s, 1H, SH), 5.23 (s, 2H, NH_2), 7.25-7.26 (d, 1H, Ar-CH=CH-), 7.28-7.29 (d, 1H, Ar-CH=CH-), 7.43-7.48 (m, 3H, aro. CH), 7.61-7.62 (d, 2H, aro. CH)	$C_{13}H_{10}N_4S$ (254.31) 254 (M^+)
2c	3499, 3323, 3219, 2220, 1634, 1294	DMSO-d_6:4.65 (s, 1H, SH), 5.42 (s, 2H, NH_2), 6.85-6.86 (d, 2H, aro. CH), 7.12 (s, 1H, OH), 7.49-7.50 (d, 2H, aro. CH)	$C_{11}H_8N_4OS$ (244.27) 244 (M^+)
2d	3421, 3234, 2221, 1596, 1260, 760	4.62 (s, 1H, SH), 5.26 (s, 2H, NH_2), 7.46-7.48 (m, 1H, aro. CH), 7.57-7.58 (d, 2H, aro. CH), 8.19-8.20 (d, 1H, aro. CH)	$C_{11}H_7ClN_4S$ (262.72) 262 (M^+), 264 (M^{+2})
2e	3421, 3228, 2232, 1585, 1215, 617	4.74 (s, 1H, SH), 5.46 (s, 2H, NH_2), 7.52-7.54 (d, 2H, aro. CH), 7.85-7.87 (d, 2H, aro. CH)	$C_{11}H_7ClN_4S$ (262.72) 262 (M^+), 264 (M^{+2})
2f	3419, 3242,2223, 1570, 1299, 1173	3.16 (s, 6H, $N(CH_3)_2$), 4.67 (s, 1H, SH), 5.26 (s, 2H, NH_2), 6.71-6.72 (d, 2H, aro. CH), 7.82-7.84 (d, 2H, aro. CH)	$C_{13}H_{13}N_5S$ (271.34) 271 (M^+)
2g	3446, 3249, 2229, 1563, 1278, 1183	3.92 (s, 3H, OCH_3), 4.68 (s, 1H, SH), 5.09 (s, 2H, NH_2), 7.03-7.05 (d, 2H, aro. CH), 7.93-7.94 (d, 2H, aro. CH)	$C_{12}H_{10}N_4OS$ (258.30) 258 (M^+)
2h	3491, 3330, 3211, 2222, 1636, 1157	3.97 (s, 3H, OCH_3), 4.63 (s, 1H, SH), 5.23 (s, 2H, NH_2), 7.02-7.03 (d, 1H, aro. CH), 7.26 (s, 1H, OH), 7.30-7.32 (dd, 1H, aro. CH), 7.73 (d, 1H, aro. CH)	$C_{12}H_{10}N_4O_2S$ (274.30) 274 (M^+)
2i	3441, 3223, 2221, 1596, 1523,1340	4.67 (s, 1H, SH), 5.19 (s, 2H, NH_2), 7.82-7.85 (m, 2H, aro. CH), 7.89-7.93 (m, 1H, aro. CH), 8.37-8.39 (dd, 1H, aro. CH)	$C_{11}H_7N_5O_2S$ (273.27) 273 (M^+)
2j	3412, 3220, 2213, 1629, 1533, 1354	4.80 (s, 1H, SH), 5.28 (s, 2H, NH_2), 8.08-8.11 (d, 2H, aro. CH), 8.40-8.42 (d, 2H, aro. CH)	$C_{11}H_7N_5O_2S$ (273.27) 273 (M^+)
2k	3434, 3244, 2219,	3.94 (s, 3H, OCH_3), 4.66 (s, 1H, SH), 5.27 (s, 2H, NH_2), 7.01-7.03 (d, 1H, aro. CH),	$C_{12}H_{10}N_4OS$

	1556, 1278, 1191	7.44-7.50 (m, 3H, aro. CH)	(258.30) 258 (M^+)
2l	3411, 3234, 2208, 1630, 1529, 1350	4.84 (s, 1H, SH), 5.30 (s, 2H, NH_2), 7.76-7.78 (dd, 1H, aro. CH), 8.11-8.13 (dd, 1H, aro. CH), 8.53 (m, 1H, aro. CH), 8.61 (s, 1H, aro. CH)	$C_{11}H_7N_5O_2S$ (273.27) 273 (M^+)

Two doublets at 7.53-7.56 and 7.87-7.89 ppm represent four aromatic protons. All the synthesized compounds gave M^+ peak in reasonable intensities. In mass spectrum of compound 1e, at the molecular ion region, two peaks were found at m/z 246 [M^+] and 248 [M^{+2}], separated by 2 m/z units with a ratio of 3:1 in the peak heights confirms the presence of chlorine atom.

Biological Screening

The *in-vitro* antimicrobial activities of the compounds 1a-1l and 2a-2l were tested against two gram (+)ve bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two gram (-)ve bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and one fungal species (*Candida albicans*).They also tested *in-vitro* for their antitubercular activity against *Mycobacterium tuberculosis* H_{37}Rv. The results of preliminary *in-vitro* antimicrobial and antitubercular screening of compounds 1a-1l and 2a-2l are shown in Table 3.

Table 3 Antimicrobial and antitubercular activity of synthesized compounds (1a-1l and 2a-2l)

Compds.	MIC (µg/mL)					
	Gram (+)ve bacteria		Gram (-)ve bacteria		Fungi	Acid fast
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*M. tuberculosis*
1a	200	200	200	100	200	>100
1b	200	1000	200	100	100	>100
1c	100	200	200	100	200	< 25
1d	100	100	50	100	200	50
1e	1000	200	1000	1000	50	>100
1f	100	200	200	200	>1000	>100
1g	100	200	1000	100	100	>100
1h	100	200	200	100	200	>100
1i	100	100	200	100	100	>100
1j	50	50	25	25	50	< 25
1k	1000	200	1000	100	100	>100
1l	100	100	200	100	1000	>100
2a	200	200	200	100	200	>100
2b	200	200	200	100	100	>100
2c	100	200	1000	100	200	>100
2d	100	100	100	100	1000	>100
2e	200	100	100	200	200	>100
2f	100	200	200	200	200	>100
2g	100	200	200	100	100	>100
2h	100	200	100	100	100	>100
2i	50	75	50	100	50	>100
2j	100	100	50	50	100	50
2k	1000	200	1000	100	100	>100
2l	100	100	200	200	1000	>100

Antimicrobial screening

The antimicrobial screening data shows that all the compounds possess weak to good inhibitory activity against all the tested strains of microorganisms. Amongst all the synthesized derivatives in series, compound 1d, 1i, 1j, 2d, 2i and 2j exhibited good antibacterial activity. Compound 1j was found to be the most potent against all the tested strains of microorganism. However, it shows lower MIC values against gram(-)ve bacteria compared to gram(+)ve bacteria. Moreover, compounds 1e, 1j and 2i exhibited potent activity against *C. albicans*. All the results also revealed that the substitution on benzene ring plays an important role on the potency of synthesized compounds. From data, it can be concluded that 2-chloro and 2-nitro substituent on benzene ring increases potency of the synthesized compounds, while 4-nitro substitution shows best potency as antimicrobial agent.

Antitubercular screening

From the results, it was found that compounds 1cand 1jwere most active (MIC < 25 µg/mL) while compounds 1d and 2j shows moderate activity (MIC =50 µg/mL). The other compounds were less active. The antitubercular activity results correlated well with those of antimicrobial activity. The results of antitubercular activity revealed that compounds 1d, 1j and 2jwhich have 2-chloro and 4-nitro group as substitution (electron withdrawing group) and 1cwhich has 4-hydroxyl group (electron releasing group) on phenyl ring enhanced the activity of pyrimidine derivatives while the other groups, such as 4-chloro, 4-dimethylamino, 4-methoxy, 3-methoxy, 3-nitro and 2-nitro groups substituted on phenyl ring did not influence the activity. Among the 4-nitro substituted compounds (1j and 2j**)** compound 1j**,** which has a hydroxyl group at 2nd position of pyrimidine ring system, exhibited the highest

activity. This suggests that electron withdrawing groups and hydroxyl groups substituted at 4th position on phenyl ring are responsible for the good antitubercular activity.

CONCLUSION

A conventional and microwave assisted method for the synthesis of 6-amino-5-cyano-2-(hydroxy/mercapto)-4-substitutedpyrimidinederivatives has been developed. Microwave assisted method is eco-friendly and efficient method which give excellent yields in short reaction times. All the synthesized compounds have been investigated for their *in-vitro* antimicrobial and antitubercular activity. Among the newly synthesized compounds, compound 1j, compounds 1e, 1j, 2i and compounds 1c, 1j shows highest antibacterial, antifungal and antitubercular activity respectively. To conclude, these novel classes of pyrimidine derivatives can be developed as a valuable lead that might be useful as antibacterial, antifungal and antitubercular agents.

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