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Research Article

SYNTHESIS AND ANTITUBERCULAR EVALUATION OF SOME NOVEL PYRIMIDINE DERIVATIVES

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ABSTRACT

A series of some new pyrimidine derivatives were synthesized by condensation of different 2-acetyl-5-chloropyrazine derived chalcones with guanidine hydrochloride. The synthesized pyrimidines were characterized by IR, ^{1}H NMR and elemental analysis. When these compounds were evaluated for antitubercular activity, some of them found to possess significant biological activity when compared to standard drug.

Keywords: Chalcone, Pyrimidine, Antitubercular activity.

INTRODUCTION

Pyrimidine **(Fig 1)** is a six-membered heterocycle with two nitrogen atoms situated in a 1,3- arrangement. It is also known as *m*-diazine or 1,3-diazine. Both nitrogen atoms are like the pyridine nitrogen. Each has its lone pair of electrons in the *sp2* hybrid orbital in the plane of the aromatic ring. These lone pairs are not needed for the aromatic sextet, and they are basic, like the lone pair of pyridine. The most common pyrimidine synthesis[1] belong to the (3 + 3) or NCN = CCC route in which one component synthon is an amine and the other is a 1, 3-dipolar component. The amine component mostly used is urea, thiourea, guanidine, amidines, imidines and substituted urea and amines. A large variety of compounds like 1,3-diketones, *β*-keto aldehydes, carboxylic acids, esters, α, *β*-unsaturated carbonyl compounds are used in the condensation reactions. Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms. Purines, uric acid, alloxan, barbituric acid and a group of antimalarial and antibacterials also contain the pyrimidine ring. Pyrimidine derivatives are associated with diverse spectrum of biological activities like antimicrobial[2], antifungal[3], anticancer[4], anti-inflammatory[5], herbicidal[6], antiviral[7], antitubercular[8], antimalarial[9-13], antileishmanial[14], neuroprotective[15] and antihyperlipidemic[16] activities.

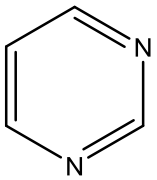


Fig. 1: General structure of chalcone

In the current work, we synthesized of different pyrimidine derivatives by condensation of 2-acetyl-5-chloropyrazine derived chalcones with guanidine hydrochloride in the presence of pyridine as catalyst and ethanol as solvent to form different pyrimidine derivatives (**D1** to **D20**). The structures of various synthesized pyrimidine derivatives were characterized on the basis of elemental analyses, IR and 1H NMR spectral data. The compounds were evaluated for their antitubercular activity by using MABA assay method.

MATERIALS AND METHODS

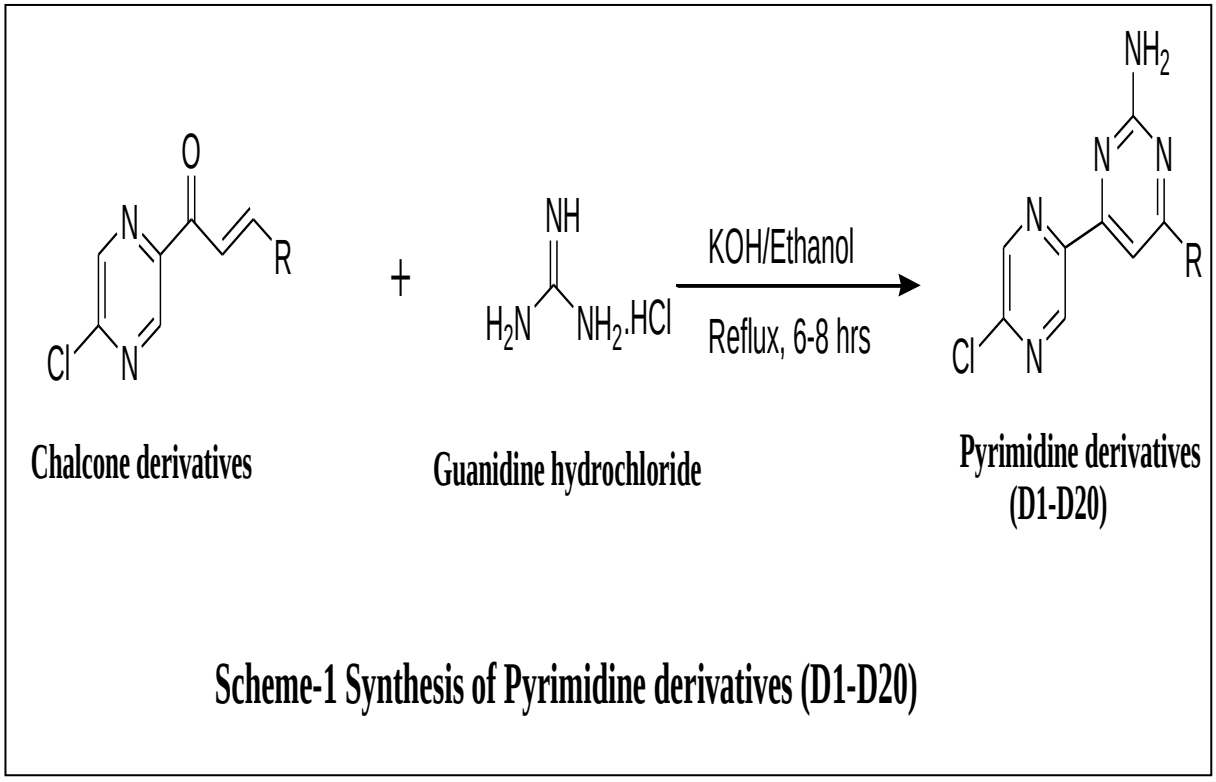
Chemistry

Melting points were determined on an open capillary melting point apparatus and are uncorrected. ^{1}H NMR was recorded in CDCl3 on Bruker WM 400 MHz spectrometer with

TMS as internal standard. Infrared spectra were recorded (KBr) on a Perkin-Elmer AC-1 spectrophotometer. Microanalyses were performed on Carloerba EA-1108 element analyzer and were within the ± 0.4 % of the theoretical values. Reaction completion was identified by TLC using Silica gel-G for TLC (Merck). All the chalcones have been purified by column chromatography performed on Silica gel (100-200 mesh, Merck).

General procedure for the preparation of pyrimidines

Chalcones of 5-chloro-2-acetylpyrazine (0.005 mol) and guanidine hydrochloride (0.005 mol) were dissolved in absolute ethanol (10 ml). To this mixture ethanolic potassium hydroxide (0.3 ml) was added drop wise at room temperature. After that the mixture was refluxed for 6-8 hrs and the solvent was evaporated completely. The reaction mixture was poured into ice-cold water and the solid that separated out was filtered, dried and purified by column chromatography with ethyl acetate/ hexane and crystallized from chloroform **(Scheme-1)**.



Scheme-1 Synthesis of Pyrimidine derivatives (D1-D20)

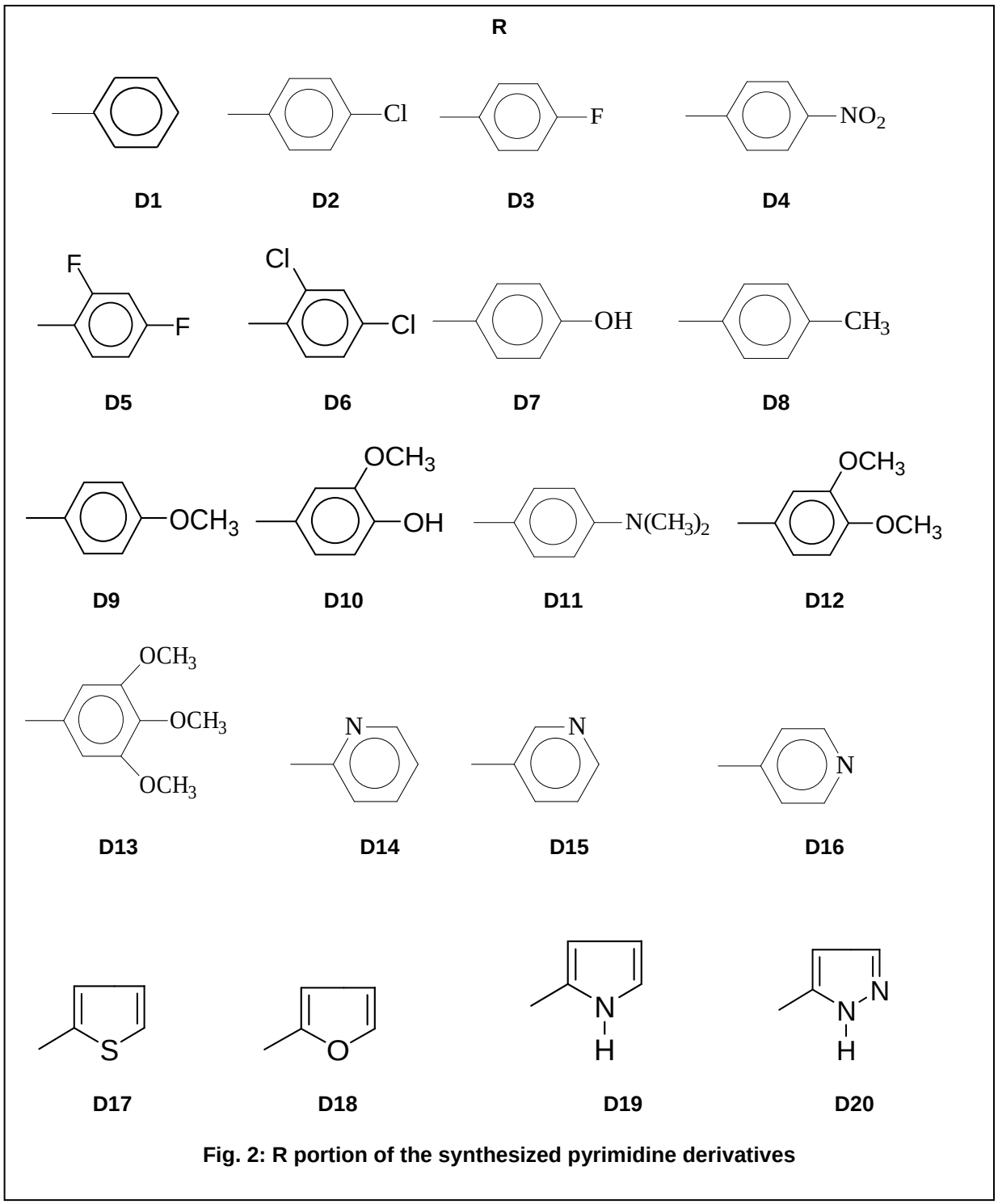


Fig. 2: R portion of the synthesized pyrimidine derivatives

Table 1: Physical characterization and elemental analysis data of chalcones (C1-C20)

Compound	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (°C)	Yield (%)	Elemental analysis					
					%Calculated			%Found		
					C	H	C	H	C	H
D1	$C_{14}H_{10}ClN_5$	283.72	164-166	85	49.72	2.68	12.42	49.36	2.49	12.12
D2	$C_{14}H_9Cl_2N_5$	318.16	153-155	90	47.15	2.26	11.78	47.01	2.02	11.46
D3	$C_{14}H_9ClFN_5$	301.71	169-171	87	51.15	3.15	11.93	50.95	3.00	11.82
D4	$C_{14}H_9ClN6O_2$	328.71	183-185	85	52.61	3.86	15.34	52.25	3.52	15.11
D5	$C_{14}H_8ClF_2N_5$	319.70	119-121	81	42.99	1.80	10.74	42.65	1.62	10.33
D6	$C_{14}H_8ClN_5$	352.61	131-133	91	62.57	3.10	9.95	62.12	2.91	9.71
D7	$C_{14}H_{10}ClN_5O$	299.72	194-196	76	53.58	3.30	12.50	53.28	3.15	12.22
D8	C15H12ClN5	297.74	146-148	82	52.19	2.82	13.04	51.99	2.41	12.94
D9	C15H12ClN50	313.7	203-205	77	49.43	2.37	12.35	49.11	2.05	12.02
D10	$C_{15}H_{12}ClN_2O_2$	329.74	250-252	83	50.27	3.43	10.99	50.01	3.12	10.44
D11	$C_{16}H_{15}ClN6$	326.78	173-175	88	49.52	3.67	10.26	49.12	3.48	10.01
D12	$C_{16}H_{14}ClN_5O_2$	343.77	164-166	87	45.79	2.20	15.26	45.33	2.04	15.12
D13	$C_{17}H_{16}ClN_50_3$	373.79	208-210	84	45.79	2.20	15.26	45.33	2.04	15.12
D14	$C_{13}H_9ClN_6$	284.71	156	76	48.31	2.49	17.34	48.01	2.05	17.11
D15	$C_{13}H_9ClN_6$	284.71	176	92	48.31	2.49	17.34	48.01	2.05	17.11
D16	$C_{13}H_9ClN_6$	284.71	146	72	48.31	2.49	17.34	48.01	2.05	17.11
D17	$C_{12}H_8ClN_5S$	289.74	206	74	46.32	2.59	18.00	46.06	2.23	17.88
D18	$C_{12}H_8ClN_5O$	273.68	174	65	43.91	2.15	12.80	43.42	1.98	12.35
D19	$C_{12}H_9ClN_6$	272.70	199	56	46.17	2.26	13.46	45.95	2.11	13.12
D20	$C_{11}H_8ClN_7$	273.68	194	72	49.19	2.48	11.47	49.01	2.25	11.21

Table 2: IR (KBr disc) and ^{1}H NMR spectral data of chalcones (C1-C20)

Compound	Position of absorption band (cm^{-1})	Chemical shift (δ) in ppm
D1	3340 (NH2), 1630 (C=N), 1575 (C=C), 866 (C-Cl)	7.34 (1H, s, C-5-H), 5.35 (2H, s, C-2-NH2), 7.13-8.04 (7H, m, Ar-H)
D2	3342 (NH2), 1628 (C=N), 1580 (C=C), 856 (C-Cl)	7.30 (1H, s, C-5-H), 5.34 (2H, s, C-2- NH2), 7.15-7.98 (6H, m, Ar-H)
D3	3335 (NH2), 1630 (C=N), 1575 (C=C), 1120 (C-F), 861 (C-Cl)	7.30 (1H, s, C-5-H), 5.17 (2H, brs, C-2-NH2), 7.14-7.74 (6H, m, Ar-H)
D4	3335 (NH2), 1635 (C=N), 1575 (C=O), 1510 (N=O, asymmetric), 1330 (N=O, symmetric), 862 (C-Cl)	7.39 (1H, s, C-5-H), 5.18 (2H, brs, C-2- NH2), 7.16-8.90 (6H, m, Ar-H)
D5	3413 (NH2), 1605 (C=N), 1572 (C=C), 762 (C-Cl)	7.07 (1H, s, C-5-H), 5.47 (2H, s, C-2- NH2), 7.11-8.55 (5H, m, Ar-H)
D6	3348 (NH2), 1635 (C=N), 1582 (C=C), 850 (C-Cl), 868 (C-Cl)	7.18 (1H, s, C-5-H), 5.42 (2H, s, C-2- NH2), 7.11-8.51 (5H, m, Ar-H)
D7	3348 (NH2), 1628 (C=N), 1580 (C=C), 856 (C-Cl), 1052 (C-S), 3200 (-OH)	7.26 (1H, s, C-5-H), 5.31 (2H, s, C-2- NH2), 4.75 (1H, s, Ar-OH), 7.11-7.88 (6H, m, Ar-H)
D8	3350 (NH2), 1630 (C=N), 1580 (C=C), 760 (C-Cl)	7.31 (1H, s, C-5-H), 5.34 (2H, s, C-2-NH2), 2.14 (3H, s, C-4''-CH3), 7.14-7.98 (6H, m, Ar- H)
D9	3345 (NH2), 1625 (C=N), 1590 (C=C), 1165 (OCH3), 864 (C-Cl)	7.68 (1H, s, C-5-H) , 4.90 (2H, s, C-2-NH2) , 3.88 (3H, s, C-4''-OCH3), 7.18-8.00 (6H, m, Ar-H)
D10	3414 (NH2), 1641 (C=N), 1519 (C=C), 1145 (OCH3), 862 (C-Cl), 3205 (-OH)	7.30 (1H, s, C-5-H), 5.22 (2H, brs, C-2-NH2), 5.49 (1H, s, -OH), 3.98 (3H, s, -OCH3), 6.94- 7.59 (5H, m, Ar-H)
D11	3338 (NH2), 1633 (C=N), 1588 (C=C), 1185 (N(CH3)2), 867 (C-Cl)	7.27 (1H, s, C-5-H), 5.39 (2H, s, C-2- NH2), 3.09 (6H, s, C-4''-N(CH3)2), 7.14-8.00 (6H, m, Ar-H)
D12	3414 (NH2), 1641 (C=N), 1519 (C=C), 1145 (-O-CH3), 862 (C-Cl)	7.30 (1H, s, C-5-H), 5.22 (2H, brs, C-2-NH2), 3.93 (3H, s, -OCH3), 3.98 (3H, s, -OCH3), 7.04-7.59 (5H, m, Ar-H)
D13	3361 (NH2), 1602(C=N), 1572 (C=C), 1120 (-O-CH3), 864 (C-Cl)	7.29 (1H, s, C-5-H), 5.21 (2H, s, -NH2), 3.90 (3H, s, -OCH3), 3.98 (6H, s, =2X-OCH3), 7.16-7.28 (4H, m, Ar-H)
D14	3332 (NH2), 1638 (C=N), 1566 (C=C), 864 (C-Cl)	7.37 (1H, s, C-5-H), 5.09 (2H, brs, C-2- NH2), 7.13-7.75 (6H, m, Ar-H)
D15	3335 (NH2), 1633 (C=N), 1572 (C=C), 868 (C-Cl)	7.35 (1H, s, C-5-H), 5.21 (2H, brs, C-2- NH2), 7.15-9.24 (6H, m, Ar-H)
D16	3338 (NH2), 1635 (C=N), 1570 (C=C), 864 (C-Cl)	7.37 (1H, s, C-5-H), 5.21 (2H, brs, C-2- NH2), 7.15-8.75 (6H, m, Ar-H)
D17	3335 (NH2), 1048 (C-S), 1570 (C=C), 871 (C-Cl)	7.37 (1H, s, C-5-H), 5.09 (2H, brs, C-2- NH2), 7.13-7.75 (5H, m, Ar-H)
D18	3329 (NH2), 1125 (C-O), 1573 (C=C), 878 (C-Cl)	7.34 (1H, s, C-5-H), 5.12 (2H, brs, C-2- NH2), 7.17-7.78 (5H, m, Ar-H)
D19	3328 (NH2), 1638 (C=N), 1572 (C=C), 874 (C-Cl)	7.35 (1H, s, C-5-H), 5.18 (2H, brs, C-2- NH2), 4.78 (1H, s, -NH), 7.18-7.86 (5H, m, Ar-H)
D20	3338 (NH2), 1133 (C-O), 1576 (C=C), 866 (C-Cl)	7.41 (1H, s, C-5-H), 5.14 (2H, brs, C-2-NH2), 4.22 (1H, s, -NH), 7.13-7.75 (5H, m, Ar-H)

Antitubercular activity

The preliminary antitubercular screening for test compounds was obtained for *M. tuberculosis* $H_{37}Rv$, the MIC of each drug was determined by broth dilution assay [17-19] and is defined as the lowest concentration of drug, which inhibits ≤ 99% of bacterial population present at the beginning of the assay. A frozen culture in Middle brook 7H9 broth supplemented with 10% albumin-dextrose-catalase and 0.2% glycerol was thawed and diluted in broth to 105 cfu mL-1 (colony forming unit/mL) dilutions. Each test compound was dissolved in DMSO and then diluted in broth twice at the desired concentration. The final concentration of DMSO in the assay medium was 1.3%. Each U-tube was then inoculated with 0.05 mL of standardized culture and then incubated at 37 °C for 21 days. The growth in the U-tubes was compared with visibility against positive control (without drug), negative control (without drug and inoculum) and with standard pyrazinamide. Results of the antitubercular activity are illustrated in table 3.

RESULTS AND DISCUSSIONS

Chemistry

All the above pyrimidines exhibited characteristic absorption bands in the IR spectra (cm^{-1}) in between 3300-3400 (NH_2), 1600-1700 (C=N), 1510-199 (C=C), 800-900 (C-Cl) and at other regions of the spectrum depending upon the specific substituents present in each compound. The ^1H NMR spectra of the compounds shown peaks in the region 7.00-7.50 (1H, s, C-5-H), and 5-6 (2H, s, C-2-NH_2) characteristic of amino pyrimidines. The spectra also showed the peaks accounting for the aromatic protons, in between the corresponding regions of the spectrum. The elemental analyses carried out for all the compounds supported the given molecular formula.

Anti-tubercular activity

Results of the anti-tubercular activity clearly notify that the pyrimidines exhibited anti-tubercular activity with altered MIC values against the tested organisms, but not as much of the standard. Anti-tubercular potency of most of the pyrimidines was less compared to standard pyrazinamide. The compounds **D3**, **D5**, **D6** and **D12** with 4''-fluorophenyl, 2'',4''-difluorophenyl, 2'',4''-dichlorophenyl and 3'',4''-dimethoxyphenyl moieties were active with a MIC value of 32 µg/mL. Compounds **D2**, was potent with an MIC value of 64 µg/mL. All the other compounds were somewhat potent with MIC values ranging between 128-512 µg/mL. However, among the pyrimidines, again compounds with both electron releasing like methoxy and electron withdrawing groups like halogens enhanced the activity, a similar observation as seen in the case of chalcones.

Table 3: Antitubercular activity data of the chalcones (C1-C20)

Compound	R	MIC values (µg/mL) of M. tuberculosis H37Rv
D1	phenyl	256
D2	4''-chlorophenyl	8
D3	4''-fluorophenyl	8
D4	4''-nitrophenyl	8
D5	2'',4''-difluorophenyl	4
D6	2'',4''-dichlorophenyl	4
D7	4''-hydroxyphenyl	256
D8	4''-methylphenyl	64
D9	4''-methoxyphenyl	256
D10	3''-methoxy-4''-hydroxyphenyl	256
D11	4''-dimethylaminophenyl	64
D12	3'',4''-dimethoxyphenyl	256
D13	3'',4'',5''-trimethoxyphenyl	32
D14	2''-pyridinyl	128
D15	3''-pyridinyl	64
D16	4''-pyridinyl	32
D17	2''-thienyl	64
D18	2''-furfuryl	128
D19	2''-pyrrolyl	128
D20	5''-pyrazolyl	64
Standard (Pyrazinamide)		1

CONCLUSION

In conclusion, we synthesized a series of pyrimidine derivatives by condensing 2-acetyl-5-chloropyrazine derived chalcones with guanidine hydrochloride and evaluated for their antitubercular activity. The compounds **D3**, **D5**, **D6** and **D12** with 4''-fluorophenyl, 2'',4''-difluorophenyl, 2'',4''-dichlorophenyl and 3'',4''-dimethoxyphenyl found to be the most potent of the series. Further studies needs to be performed to determine the usefulness of these halogenated and methoxylated compounds as potential antitubercular agents.

CONFLICT OF INTERESTS

There is no conflict of interests.

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