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SYNTHESIS, ANTIVIRAL, ANTITUBERCULOSTIC AND ANTIBACTERIAL ACTIVITIES OF SOME NOVEL, 4-(4'-SUBSTITUTED PHENYL)-6-(4''-HYDROXYPHENYL)-2-(SUBSTITUTED IMINO) PYRIMIDINES

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Abstract: A variety of novel 4-[(4'-substituted phenyl)-6-(4''-hydroxyphenyl)-2-substituted imino]pyrimidines were synthesized by reacting 4-(4'-substituted phenyl)-6-(4''-hydroxyphenyl)-2-aminopyrimidines with different substituted aromatic aldehydes, with coumarin and with chloroisatin. The 4-(4'-chlorophenyl)-6-(4''-hydroxyphenyl)-2-aminopyrimidines were synthesized by reacting 3-(4'-substituted phenyl)-1-(4''-hydroxyphenyl)-2-propen-1-ones with guanine hydrochloride. The 3-(4'-substituted phenyl)-1-(4''-hydroxyphenyl)-2-propen-1-ones were synthesized by reacting 4-hydroxyacetophenone with different *para* substituted aromatic aldehydes. Spectral data (IR, NMR, and mass spectra) confirmed the structures of the synthesized compounds. The synthesized compounds were investigated for their antiviral, antituberculostic and antibacterial activities. The results indicated that the synthesized compounds have mild to potent activities with reference to their appropriate reference standards. However, mechanism related studies could be carried out to predict the structure activity relationship for all the compounds.

Keywords: pyrimidines, *in vitro* cytotoxic, antiviral, antituberculostic, antibacterial.

Many human illnesses are especially caused by infections with microbes like virus or bacteria or fungi. Amongst various illnesses to the human beings, certain viral, tubercular, bacterial and fungal infections are more common and because of their tendency to develop new strains under any circumstances for developing resistance with the available drugs, they paved the way for scientists to make the efforts to work on several molecules for coming out with novel entities to combat the illnesses caused by them. Infectious microbial diseases remain an important worldwide problem, because microbes have resisted prophylaxis or therapy longer than any other life form. For viral diseases, till today no antiviral candidate tested is able to inhibit completely the replication of any virus. Since the efficiency of currently used antiviral compounds are doubtful, there is a need to envisage new class of antiviral agents from both synthetic and natural sources. Pyrimidine and its derivatives are noteworthy for their physiological and biological importance. They paved the attention of medicinal chemists due to their wide range of biological activities like anticancer (1-4), antiviral (5-11), antituberculostic (12, 13), and antibacterial (14-17) activities. In view of above facts and inspired by the research envisaged on pyrimidine and its derivatives, particularly in relation to microbial infections, in the present study, we aimed to synthesize some novel,4-[4'-(substituted phenyl)-6-(4''-hydroxyphenyl)-2-substituted imino]pyrimidines. The title compounds were synthesized by reacting 4-(4'-substituted phenyl)-6-(4''-hydroxyphenyl)-2-aminopyrimidines with different substituted aromatic aldehydes, with coumarin and chloroisatin. The 4-(4'-chlorophenyl)-6-(4''-hydroxyphenyl)-2-aminopyrimidines were synthesized by reacting 3-(4'-substituted phenyl)-1-(4''-hydroxyphenyl)-2-propen-1-ones with guanine hydrochloride. The 3-(4'-substituted phenyl)-1-(4''-hydroxyphenyl)-2-propen-1-ones were synthesized by reacting 4-hydroxyacetophenone with different *para* substituted aromatic aldehydes (Scheme 1). Spectral

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data (IR, NMR, and mass spectra) confirmed the structures of the synthesized compounds, whereas the purity of these compounds was ascertained by microanalysis (Table 1). The synthesized compounds were evaluated for their antiviral, antituberculostic and antibacterial activities.

CHEMISTRY

Melting points were determined in open capillaries on a Veego VMP-1 melting point apparatus and are uncorrected. The IR spectra were recorded in KBr on a Perkin-Elmer infra-red spectrophotometer, the NMR spectra (in DMSO) on a Jeol Fx-100 FT-NMR spectrophotometer at 100 MHz using TMS as an internal standard and mass spectra were obtained on a Jeol Bx 102/DA-6000 spectrometer. The purity of the compounds was ascertained by microanalysis. Elemental analysis (C, H and N) indicated that the calculated and observed values were within the acceptable limits (± 0.4%). Elemental analyses were performed on Carlo Erba 1108 instrument. The progress of the reaction was monitored on silica gel plates (Merck) using chloroform-methanol (9:1, v/v) as a solvent system. Iodine was used as a reagent for visualization.

Synthesis of 4-(4'-substituted phenyl)-6-(4"-hydroxyphenyl)-2-(substituted imino)pyrimidines

The overall reaction involves three steps. For instance, synthesis and characterization of compound **6** is as follows:

Synthesis of 3-(4'-chlorophenyl)-1-(4"-hydroxyphenyl)-2-propen-1-one (1)

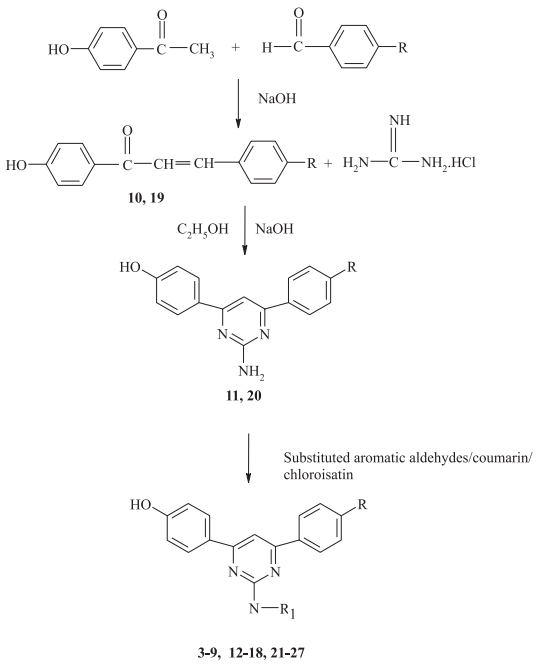
An aqueous solution of sodium hydroxide (10% w/v, 10 mL) was added to a solution of 4-chlorobenzaldehyde (0.02 mol) and 4-hydroxyacetophenone (0.02 mol) in ethanol (6 mL). The reaction mixture was stirred at room temperature overnight and poured into water (100 mL). After neutralization with HCl (10% w/v), a pale yellow solid which separated, was recrystallized from ethanol to obtain pale yellow crystalline compound, yield 85 %, m.p. 128-130°C; IR (KBr, cm^{-1}): 3437 (Ar-OH), 2920 (CH), 1661 (CO), 1601 (CH=CH), 725 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.82 (d, 1H, J = 8.7 H_Z, =CH-Ar), 7.47-7.94 (m, 8H, Ar-H), 8.05 (d, 1H, J=8.7 H_Z, CO=CH), 10.42 (s, 1H, -OH); MS (m/z): 258 (M^+), 260 (M^++2); Analysis: calcd. for $C_{15}H_{11}O_2Cl$: C, 69.64; H, 4.28; found: C, 69.66; H, 4.31. Adopting this procedure, starting materials, **10** and **19** were prepared.

Synthesis of 4-(4'-chlorophenyl)-6-(4"-hydroxyphenyl)-2-aminopyrimidine (2)

A mixture of 3-(4'-chlorophenyl)-1-(4"-hydroxyphenyl)-2-propen-1-one (**1**) (0.01 mol) and guanidine hydrochloride (0.015 mol) was added to NaOH (0.045 mol, water, 2 mL) and ethanol (50 mL). The reaction mixture was refluxed for 8 h and poured onto crushed ice. The resultant solid was washed with diluted ethanol, dried and recrystallized from ethanol-chloroform mixture to yield yellow crystalline solid, yield = 78.5 %, m.p. 125-127°C; IR (KBr, cm^{-1}): 3484 (OH), 3437 (NH_2), 3046 (CH), 1637 (CN), 776 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.70 (s, 2H, NH_2), 6.43-8.22 (m, 9H, Ar-H); MS (m/z): 297 (M^+), 299 (M^++2); Analysis: calcd. for $C_{16}H_{12}N_3OCl$: C, 64.54; H, 4.06; N, 14.11; found: C, 64.58; H, 4.10; N, 14.15. Adopting this procedure, intermediates **11** and **20** were prepared.

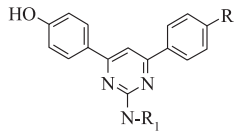
Synthesis of 4-(4'-chlorophenyl)-6-(4"-hydroxyphenyl)-2-(3,4,5-trimethoxy phenylmethylimino)pyrimidine (6)

Equimolar quantities of 4-(4'-chlorophenyl)-6-(4"-hydroxyphenyl)-2-amino pyrimidine **(2)** (0.002 mol) and 3,4,5-trimethoxybenzaldehyde (0.002 mol)



Scheme 1. Synthesis of 4-(4'-substituted phenyl)-6-(4"-hydroxyphenyl)-2-(substituted imino)pyrimidines.

Table 1. Physical data for of 4-(4'-R-phenyl)-6-(4"-hydroxyphenyl)-2-(R_1-imino) pyrimidines.



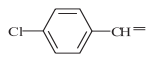
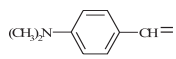
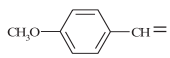
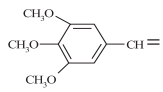
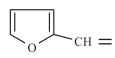
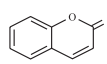
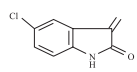
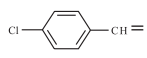
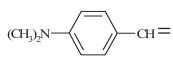
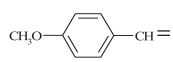
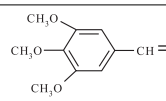
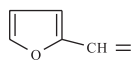
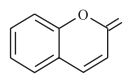
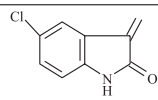
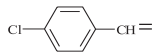
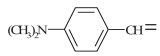
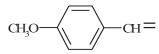
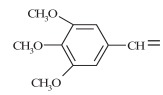
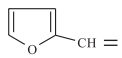
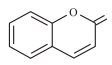
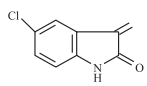
Comp. No.	R	R_1	Molecular formula (Mol. Wt.)	Elemental analysis calcd. (found) (%)			Melting point (°C)	Yield %
				C	H	N		
3	-Cl		$C_{23}H_{15}N_3OCl_2$ (421)	65.72 (65.74)	3.59 (3.57)	9.99 (9.98)	160–162	68
4	-Cl		$C_{25}H_{21}N_4OCl$ (429)	70 (69.6)	4.93 (4.94)	13.06 (13.08)	81-83	70
5	-Cl		$C_{24}H_{18}N_3O_2Cl$ (416)	69.31 (69.33)	4.36 (4.38)	10.10 (10.09)	164-166	72
6	-Cl		$C_{26}H_{22}N_3O_4Cl$ (476)	65.61 (65.63)	4.65 (4.63)	8.82 (8.83)	137-139	65
7	-Cl		$C_{21}H_{14}N_3O_2Cl$ (376)	67.11 (67.09)	3.75 (3.72)	11.18 (11.20)	154-155	70
8	-Cl		$C_{25}H_{16}N_3O_2Cl$ (426)	70.50 (70.51)	3.78 (3.80)	9.86 (9.90)	115-117	68
9	-Cl		$C_{24}H_{14}N_4O_2Cl_2$ (462)	62.48 (62.50)	3.05 (3.01)	12.14 (12.16)	115-117	71
12	-$N(CH_3)_2$		$C_{25}H_{21}N_4OCl$ (469)	70.00 (70.01)	4.93 (4.95)	13.06 (13.04)	84-86	69
13	-$N(CH_3)_2$		$C_{27}H_{27}N_5O$ (438)	74.11 (74.09)	6.21 (6.20)	16.00 (16.03)	61-63	73
14	-$N(CH_3)_2$		$C_{26}H_{24}N_4O_2$ (425)	73.56 (73.58)	5.69 (5.71)	13.19 (13.18)	118-120	69
15	-$N(CH_3)_2$		$C_{28}H_{28}N_4O_4$ (485)	69.40 (69.42)	5.82 (5.81)	11.56 (11.54)	175-177	75
16	-$N(CH_3)_2$		$C_{23}H_{20}N_4O_2$ (385)	71.85 (71.83)	5.24 (5.22)	14.57 (14.58)	96-98	72
17	-$N(CH_3)_2$		$C_{27}H_{22}N_4O_2$ (435)	74.63 (74.65)	5.10 (5.12)	12.89 (12.91)	120-122	75
18	-$N(CH_3)_2$		$C_{26}H_{20}N_5O_2Cl$ (470)	66.45 (66.44)	4.28 (4.26)	14.90 (14.89)	95-97	68

Table 1. cont.

Comp. No.	R	R_1	Molecular formula (Mol. Wt)	Elemental analysis calcd. (found) (%)			Melting point (°C)	Yield %
				C	H	N		
21	$-OCH_3$		$C_{24}H_{18}N_3O_2Cl$ (416)	69.31 (69.32)	4.36 (4.34)	10.10 (10.12)	96-98	62
22	$-OCH_3$		$C_{26}H_{24}N_4O_2$ (425)	73.56 (73.54)	5.69 (5.67)	13.19 (13.20)	88-90	70
23	$-OCH_3$		$C_{25}H_{21}N_3O_3$ (412)	72.97 (72.99)	5.14 (5.15)	10.21 10.19)	76-78	78
24	$-OCH_3$		$C_{27}H_{25}N_3O_5$ (472)	68.77 (68.79)	5.34 (5.35)	8.91 (8.94)	103-105	65
25	$-OCH_3$		$C_{22}H_{17}N_3O_3$ (372)	71.14 (71.15)	4.61 (4.63)	11.31 (11.29)	175-176	75
26	$-OCH_3$		$C_{26}H_{19}N_3O_3$ (422)	74.09 74.06	4.54 (4.55)	9.97 (9.99)	54-56	70
27	$-OCH_3$		$C_{25}H_{17}N_4O_3Cl$ (457)	65.72 65.74	3.75 (3.74)	12.26 (12.27)	121-123	78

were dissolved in warm ethanol (75 mL) containing glacial acetic acid (1 mL). The reaction mixture was refluxed for 11 h and poured onto the crushed ice. The resultant yellow solid was dried and recrystallized from ethanol-water yielding yellow crystalline solid, yield = 65%, m.p. 137-139°C; IR (KBr, cm^{-1}): 3386 (Ar-OH), 2941 (CH), 1586 (C=N), 845 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.80 (s, 9H, 3 × OCH_3), 6.52 (s, 1H, =CH), 6.82-8.18 (m, 11H, Ar-H), 9.88 (s, 1H, OH); MS (m/z) 476 (M^+), 478 (M^++2); Analysis: calcd. for $C_{26}H_{22}N_3O_4Cl$: C, 65.61; H, 4.65; N, 8.82; found: C, 65.63; H, 4.63; N, 8.83. Adopting this procedure, compounds **3**-**9**, **12**-**18** and **21**-**27** were prepared.

4-(4'-Chlorophenyl)-6-(4"-hydroxyphenyl)-2-(furfuryl-2-yl-methylimino)pyrimidine (7)

IR (KBr, cm^{-1}): 3406 (Ar-OH), 2945 (CH), 1575 (C=N), 815 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.53 (s, 1H, =CH), 6.82-8.15 (m, 11H, Ar-H), 9.98 (brs, 1H, OH); MS (m/z) 376 (M^+), 378 (M^++2); Analysis: calcd. for $C_{21}H_{14}N_3O_2Cl$: C, 67.11; H, 3.75; N, 11.18; found: C, 67.09; H, 3.72; N, 11.20.

4-(4'-Chlorophenyl)-6-(4"-hydroxyphenyl)-2-(5-chloroisatin-3-yl-imino)pyrimidine (9)

IR (KBr, cm^{-1}): 3406 (Ar-OH), 2945 (CH), 1575 (C=N), 815 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.80-8.18 (m, 12H, Ar-H), 9.98 (brs, 1H, OH); MS (m/z) 462 (M^++1), 464 (M^++2); Analysis: calcd. for $C_{24}H_{14}N_4O_2Cl_2$: C, 62.48; H, 3.05; N, 12.14; found: C, 62.50; H, 3.01; N, 12.16.

4-(4'-N-dimethylaminophenyl)-6-(4"-hydroxyphenyl)-2-(4-N-dimethylphenylmethylimino)pyrimidine (13)

IR (KBr) cm^{-1}: 3439 (Ar-OH), 2918 (CH), 1602 (C=N); ^{1}H-NMR (DMSO-d_6) d: 3.06 (s, 12H, 2 × $N(CH_3)_2$), 6.63 (s, 1 H, =CH), 7.67-8.21 (m,

Table 2. Cytotoxicity and antiviral activity of 4-(4'-substituted phenyl)-6-(4"-hydroxyphenyl)-2-(substituted imino)pyrimidines in E_6SM cell cultures.

Compound No.	Minimum Cytotoxic Concentration[a] (μg/mL)	Minimum Inhibitory Concentration[b] (mg/mL)				
		Herpes Simplex Virus-1 (KOS)	Herpes Simplex Virus-2 (G)	Vaccinia Virus	Vesicular Stomatitis Virus	Herpes Simplex Virus-1 TK KOS ACV
3	> 80	> 80	> 80	> 80	> 80	> 80
4	≥ 400	> 400	> 400	> 400	> 400	> 400
5	400	> 80	> 80	> 80	> 80	> 80
6	400	> 80	> 80	> 80	> 80	> 80
7	400	> 80	> 80	> 80	> 80	> 80
8	400	> 80	> 80	> 80	> 80 (240)	> 80
9	400	> 80	> 80	> 80	> 80	> 80
12	> 400	> 400	240	240	> 400	240
13	> 400	240	240	240	240	240
14	80	> 16	> 16	> 16	> 16	> 16
15	>400	240	240	240	240	240
16	80	> 16	> 16	> 16 (48)	> 16 (240)	> 16
17	80	> 16	> 16	> 16	> 16	> 16
18	400	> 80	> 80	> 80	> 80	> 80
21	80	> 16	> 16	> 16	> 16	> 16
22	> 400	240	> 400	240	240	240
23	80	> 16	> 16	> 16	> 16	> 16
24	> 400	240	400	240	240	240
25	80	> 16	> 16	> 16	> 16	> 16
26	400	9.6	48	9.6	240	9.6
27	16	> 3.2	> 3.2	> 3.2	> 3.2	> 3.2
Brivudin	> 400	0.0768	240	1.92	> 400	80
Ribavirin	> 400	240	240	48	240	240

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus- induced cytopathogenicity by 50%.

12H, Ar-H); MS (m/z) 438 (M^+), 440 (M^+ +2); Analysis: calcd. for $C_{27}H_{27}N_5O$: C, 74.11; H, 6.21; N, 16.00; found: C, 74.09; H, 6.20; N, 16.03.

4-(4'-N-dimethylaminophenyl)-6-(4"-hydroxyphenyl)-2-(5-chloroisatin-3-yl-imino)pyrimidine (18)

IR (KBr) cm^{-1}: 3567 (OH), 3446 (NH), 2911 (CH), 1599 (CO), 1578 (C=N), 734 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.03 (s, 6H, $N(CH_3)_2$), 6.55-7.78 (m, 13H, Ar-H), 8.45 (s, 1H, CONH), 9.66 (s, 1H, OH); MS (m/z) 470 (M^+), 472 (M^+ +2); Analysis: calcd. for $C_{26}H_{20}N_5O_2Cl$: C, 66.44; H, 4.28; N, 14.90; found: C, 66.44; H, 4.26; N, 14.89.

4-(4'-Methoxyphenyl)-6-(4"-hydroxyphenyl)-2-(4-methoxyphenylmethylimino)pyrimidine (23)

IR (KBr) cm^{-1}: 3350 (OH), 1560 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.80 (s, 6H, 2 × OCH_3), 6.48 (d, 1H, =CH), 6.89-8.18 (m, 13H, Ar-H), 9.98 (brs, 1H, OH); MS *(*m/z*)* 412 (M^+), 414(M^+ +2); Analysis: calcd. for $C_{25}H_{21}N_3O_3$: C, 72.97; H, 5.14; N, 10.21; found: C, 72.99; H, 5.15; N, 10.19.

4-(4'-Methoxyphenyl)-6-(4"-hydroxyphenyl)-2-(coumarinylimino)pyrimidine (26)

IR (KBr) cm^{-1}: 3580 (OH), 3055 (CH), 1704 (cyclic C-O-C), 1604 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.85 (s, 3H, OCH_3), 6.48 (d, 1H, =CH), 6.90 (d, 1H, =CH), 7.35-8.08 (m, 13H, Ar-H); MS (m/z) 422 (M^+), 424 (M^+ +2); Analysis: calcd. for $C_{26}H_{19}N_3O_3$: C, 74.09; H, 4.54; N, 9.97; found: C, 74.06; H, 4.55; N, 9.99.

Table 3. *In vitro* antitubercular activity of 4-(4'-substituted phenyl)-6-(4"-hydroxyphenyl)-2-(substituted imino)pyrimidines.

Compound No.	Concentration (µg/mL)	Percentage of inhibition
3	6.25	62
4	6.25	59
5	6.25	57
6	6.25	61
7	6.25	32
8	6.25	70
9	6.25	65
12	6.25	47
13	6.25	36
14	6.25	29
15	6.25	42
16	6.25	12
17	6.25	35
21	6.25	50
22	6.25	46
26	6.25	37
27	6.25	47
Rifampicin	0.25	98
Isoniazid	0.031	95
Tobramycin[1]	10.0	99
Ethionamide[1]	1.17	99
PAS[1]	2.31	99

[1]The concentration represents their MIC's.

Table 4. Antibacterial activity of 4-(4'-substituted phenyl)-6-(4"-hydroxyphenyl)-2-(substituted imino)pyrimidines.

Compound	Concentration (µg/mL)	Zone of inhibition (in mm)			
		Gram positive		Gram negative	
		Staph. aureus	***M. luteus***	*E. coli*	*K. pneumoniae*
3	100	-	-	-	-
	200	-	-	-	-
	500	-	-	-	-
4	100	-	-	-	-
	200	15	-	-	13
	500	19	-	-	18
5	100	-	-	17	20
	200	-	-	21	22
	500	20	-	24	25
6	100	-	-	-	-
	200	-	21	-	-
	500	-	23	-	22
7	100	-	-	-	16
	200	-	-	-	19
	500	-	-	-	22
8	100	-	-	-	-
	200	-	17	-	17
	500	-	20	-	21

Table 4. cont.

Compound	Concentration (μg/mL)	Zone of inhibition (in mm)			
		Gram positive		Gram negative	
		Staph. aureus	*M. luteus*	*E. coli*	*K. pneumoniae*
9	100	-	-	-	15
	200	-	-	-	18
	500	-	-	-	20
12	100	-	-	-	-
	200	-	-	-	-
	500	17	-	-	18
13	100	-	-	-	-
	200	16	-	18	-
	500	21	-	21	-
14	100	-	-	-	-
	200	-	-	-	15
	500	17	19	16	17
15	100	-	-	-	-
	200	-	15	-	-
	500	-	18	-	-
16	100	-	17	-	-
	200	-	20	-	-
	500	-	22	-	-
17	100	-	20	-	-
	200	-	22	-	-
	500	-	24	-	-
18	100	-	-	16	-
	200	-	-	18	-
	500	-	-	21	-
21	100	-	20	-	-
	200	-	22	-	-
	500	-	24	-	-
22	100	-	-	16	-
	200	-	-	18	-
	500	-	-	22	-
23	100	-	-	-	-
	200	-	-	-	-
	500	-	-	-	-
24	100	-	-	-	17
	200	-	-	-	20
	500	-	-	-	22
25	100	-	-	-	15
	200	-	-	-	17
	500	-	-	-	20
26	100	14	-	-	-
	200	17	-	-	-
	500	19	-	-	-
27	100	-	15	-	-
	200	18	19	-	-
	500	21	21	16	-
Ampicillin	50	23	21	25	21
Chloramphenicol	50	22	25	23	20

– shows no antibacterial activity

BIOLOGICAL EVALUATION

The synthesized compounds were evaluated for their antiviral, antituberculostic and antibacterial activities.

Cytotoxicity assay (18)

Each synthesized compound was separately dissolved in 1 mL of distilled dimethyl sulfoxide (DMSO) and volume was made up to 10 mL with maintenance medium to obtain a stock solution of 1 mg/mL concentration, sterilized by filtration and further dilutions were made from the stock. The cytotoxicity assay was carried out using 0.1 mL of the cell suspension, containing 10,000 cells seeded in each well of a 96 well microtitre plate (Tarsons India Pvt. Ltd., Kolkata). Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Control cells were incubated without test sample and with DMSO. The little percentage of DMSO present in the wells (maximal 0.2%) was found not to affect the experiment. The microtitre plates were incubated at 37°C for a period of 72 h. Sixteen wells were used for each concentration of the test sample. The morphology of the cells was inspected daily and observed for microscopically detectable alterations i.e., loss of monolayer, granulation and vacuolization in the cytoplasm. The CTC_{50} values (the minimum concentration of test drug required to kill 50% of exposed cell population) of each test drug were determined by the standard MTT assay.

Antiviral assay (MIC or EC_{50}) (19, 20)

Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50 % of the cell cultures. After 1-2 h of virus adsorption period, residual virus was removed, and the cell cultures were incubated at 37°C in the presence of varying concentrations of the test compounds (dilutions were made based upon CTC_{50}). Viral cytopathogenicity was recorded as soon as it reached completion in the control virus infected cell cultures that were not treated with the test compounds after 7-8 days of post infection, microscopically. The antiviral activity of the compounds was expressed as the effective concentration required for inhibiting the viral cytopathic effect by 50% (MIC or EC_{50}). The CTC_{50} and MIC of the test compounds were compared with the standard drugs Brivudin (BVDU) and Ribavirin under similar conditions. By adopting the above procedure, the MIC or EC_{50} for all the synthesized compounds were determined.

Antitubercular activity [Alamar blue susceptibility test (MABA)] (21, 22)

Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either dimethyl sulfoxide or distilled deionized water, and subsequent twofold dilutions were performed in 0.1 mL of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1:2 in 7H9GC, and 0.1 mL was added to wells. Subsequent determination of bacterial titers yielded 1×10^6 CFU/mL in plate wells for *M. tuberculosis* H37Rv. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium followed by a 1:50 dilution in 7H9GC. Addition of 1/10 mL to wells resulted in final bacterial titers of 2.0×10^5 CFU/mL for *M. tuberculosis* H_{37}Rv. Wells containing drug only were used to detect autofluorescence of compounds. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37°C. Starting at day 4 of incubation, 20 µL of 10× alamar Blue solution (Alamar Biosciences/Accumed, Westlake, Ohio) and 12.5 mL of 20% Tween 80 were added to one B well and one M well, and plates were reincubated at 37°C. Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of 50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 h, reagent was added to the entire plate. If the well remained blue or 50,000 FU was measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were added to all remaining wells. Plates were then incubated at 37°C, and results were recorded at 24 h post-reagent addition. Visual minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that prevented a color change. For fluorometric MICs, a background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined as 1 – (test well FU/mean FU of triplicate B wells)×100. The lowest drug concentration effecting an inhibition of > 90% was considered the MIC. The percentage inhibition of bacterial growth at 6.25 µg/mL of all the selected synthesized compounds is presented in Table 3 along with the MIC's of standard drugs.

Antibacterial activity (23)

The synthesized compounds were tested for their antibacterial activity by cup plate method. The selected standard microbial strains viz., *Staphylococcus aureus, Micrococcus luteus*, *Escherichia coli* and *Klebsiella pneumoniae* were procured from NCL, Pune, India. The zone of inhibition of the test compounds were compared with the reference standard antibiotics namely ampicilin (50 µg/mL and chloramphenicol (50 µg/mL).

RESULTS AND DISCUSSION

The results of cytotoxicity assay indicates that all the compounds shown varying degree of cytotoxicity (i.e. from 80 to 400 µg/mL). Based on the CTC_{50} nontoxic concentrations, all the synthesized compounds were subjected for antiviral activity determination against different viral strains. The results of antiviral activity (Table 2) indicate that pyrimidines with $–OCH_3$ and coumarinyl (**26**) and isatinyl (**27**), substitution were excelled in their action. Next in the order, pyrimidines with $-N(CH_3)_2$ and p-chlorophenyl, p-N-dimethylaminophenyl and 3,4,6-trimethoxyphenyl substitutions exhibited comparable activity with the reference drugs brivudin and ribavirin. The rest of the synthesized compounds exhibited mild activity. The results of antituberculostic activity (Table 3) indicate that the pyrimidines with chloro and p-chlorophenyl (**3**), trimethoxyphenyl (**6**) and coumarinyl (**8**) substituents showed comparable activity with the reference drugs. Pyrimidines with $-OCH_3$ and isatinyl (**27**) substitution had moderate activity. The rest of the compounds exhibited mild activity. In antibacterial screening, almost all the compounds presented comparable activity against *K. pneumonia*. Against other bacterial strains, only few compounds demonstrated comparable activity with reference to the standard drugs, especially against *S. aureus* only few compounds presented mild to moderate activity. However, a mechanism related studies should be carried out to predict the structure activity relationship for all the compounds.

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