Full Paper

Synthesis, Antiviral, Antituberculostic, and Antibacterial Activities of Some Novel, 4-(4-substituted phenyl)-6-(4-nitrophenyl)-2-(substituted imino)pyrimidines

Anees A. Siddiqui[1], Ramadoss Rajesh[1], Mojahid-Ul-Islam[1], Veerachamy Alagarsamy[2], and Erick De Clercq[3]

[1] Department of Pharmaceutical Chemistry, Hamdard University, New Delhi, India
[2] Department of Pharmaceutical Chemistry, Arulmigu Kalasalingam College of Pharmacy, Krishnan Koil, India
[3] Rega Institute for Medical Research, Katholieke University, Leuven, Belgium

A variety of novel 4-(4-substituted phenyl)-6-(4-nitrophenyl)-2-substituted imino) pyrimidines were synthesized by reacting 4-(4-substituted phenyl)-6-(4-nitrophenyl)-2-amino pyrimidines with different substituted aromatic aldehydes, coumarin chloroisatin. The 4-(4-substituted phenyl)-6-(4-nitrophenyl)-2-amino pyrimidines were synthesized by reacting 3-(4′-substituted phenyl)-1-(4-nitrophenyl)-2-propen-1-ones with guanine hydrochloride. 3-(4-Substituted phenyl)-1-(4-nitrophenyl)-2-propen-1-ones were synthesized by reacting 4-nitroacetophenone 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 of antiviral, antituberculostic, and antibacterial activities indicated that the synthesized compounds exhibited mild to potent activities compared to the respective reference standards.

Keywords: Antibacterial / Antituberculostic / Antiviral / Pyrimidine

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Introduction

Many human illnesses are caused by infections with microbes like viruses or bacteria or fungi. Amongst those various illnesses to human beings certain viral, bacterial, tubercular, and fungal infections are more common because of their tendency to develop new strains under any circumstance and to develop resistance against the available drugs. This stimulated scientists to engage in the development of several compounds with novel entities to combat the illnesses caused by them. Infectious microbial diseases remain a pressing problem worldwide, because microbes have resisted prophylaxis or therapy longer than any other form of life. For viral diseases, till today, no antiviral candidate substance tested has been able to completely inhibit the replication of the virus. Since the efficiency of currently used antiviral compounds are doubtful, there is a need to envisage new classes of antiviral agents from both synthetic and natural sources. Pyrimidine and its derivatives are noteworthy for their physiological and biological importance. They attracted 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 the above mentioned facts and inspired by the research envisaged on pyrimidine and its derivatives, particularly in relation to microbial infections [1 – 17], in the present study, we aimed to synthesize some novel 4-(4-substituted phenyl)-6-(4-nitrophenyl)-2-(substituted imino) pyrimidines for their antiviral, antituberculostic, and antibacterial activities. The title compounds were synthesized by reacting 4-(4-substituted phenyl)-6-(4-nitrophenyl)-2-amino pyrimidines with different substituted aromatic aldehydes, chloroisatin and coumarin. The 4-(4-substituted phenyl)-

Correspondence: Ramadoss Rajesh, Huclin Research Ltd., 4th Floor, Ticel Bio Park, CSIR Road, Taramani, Chennai-600 113, Tamilnadu, India
E-mail: rrajeshmpharm@yahoo.com
Fax: +91 44 22540-211

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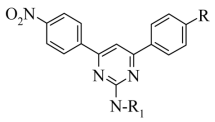
6-(4-nitrophenyl)-2-amino pyrimidines were synthesized by reacting 3-(4-substituted phenyl)-1-(4-nitrophenyl)-2-propen-1-ones with guanine hydrochloride. The starting material 3-(4-substituted phenyl)-1-(4-nitrophenyl)-2-propen-1-ones was synthesized by reacting 4-nitroacetophenone with different *para*-substituted aromatic aldehydes (Scheme 1). Spectral data (IR, NMR, and mass spectra) confirmed the structures of the synthesized compounds; the purity of these compounds was ascertained by microanalysis (Table 1).

Results and discussion

The results of the cytotoxicity assay indicate that all the compounds showed varying degrees of cytotoxicity, *i.e.* from 80 to 400 μg/mL. Based on the CTC_{50} (the minimum concentration of test drug required to kill 50% of exposed cell population), nontoxic concentrations of all the synthesized compounds were subjected for antiviral activity against different viral strains. The results of the antiviral activity testing (Table 2) indicate that pyrimidines with chlorophenyl, dimethylaminophenyl **4**, and pyrimidines with methoxyphenyl, furfuryl **25** substitutions were excellent in their action. Next in the order, pyrimidines with chlorophenyl, methoxyphenyl **5**, and trimethoxyphenyl **6** substitutions, pyrimidines with dimethylaminophenyl, methoxyphenyl, and trimethoxyphenyl **13–15** substitutions, pyrimidines with methoxyphenyl and *p*-chlorophenyl **21**, *p*-*N*-dimethylaminophenyl **22**, methoxyphenyl **23**, 3,4,6-trimethoxyphenyl **24** substitutions have exhibited comparable activity with the reference drugs brivudin and ribavirin. The rest of the synthesized compounds exhibited mild activity. The

Table 1. Physical data of 4-(4′-substituted phenyl)-6-(4′-nitrophenyl)-2-(substituted imino)pyrimidines.



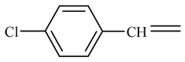
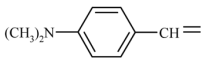
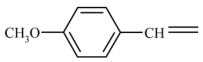
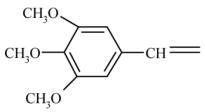
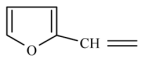
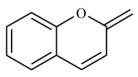
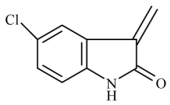
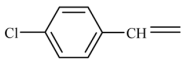
Comp.	R	R_1	Molecular formula[a)]	Molecular weight	Mp (°C)	Yield %
3	–Cl		$C_{23}H_{14}N_4O_2Cl_2$	449	82	68
4	–Cl		$C_{25}H_{20}N_5O_2Cl$	458	102	70
5	–Cl		$C_{24}H_{17}N_4O_3Cl$	445	127	72
6	–Cl		$C_{26}H_{21}N_4O_5Cl$	503	92–94	65
7	–Cl		$C_{21}H_{13}N_4O_3Cl$	405	172–-175	70
8	–Cl		$C_{25}H_{15}N_4O_3Cl$	453	90–92	68
9	–Cl		$C_{24}H_{13}N_5O_3Cl_2$	490	110	71
12	$-N(CH_3)_2$		$C_{25}H_{20}N_5O_2Cl$	457	138	69

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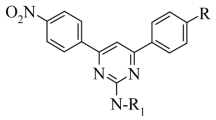
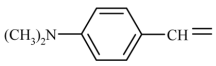
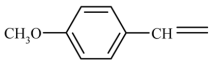
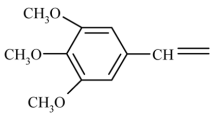
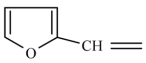
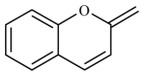
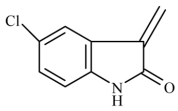
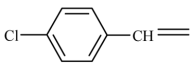
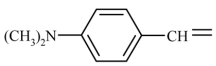
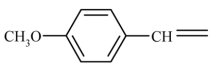
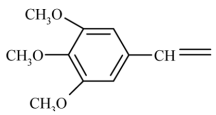
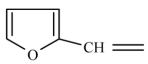
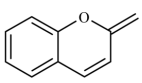
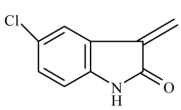
Table 1. Physical data of 4-(4′-substituted phenyl)-6-(4′-nitrophenyl)-2-(substituted imino)pyrimidines.



Comp.	R	R_1	Molecular formula[a)]	Molecular weight	Mp (°C)	Yield %
13	$-N(CH_3)_2$		$C_{27}H_{26}N_6O_2$	442	83	73
14	$-N(CH_3)_2$		$C_{26}H_{23}N_5O_3$	453	98	69
15	$-N(CH_3)_2$		$C_{27}H_{27}N_5O_5$	501	123	75
16	$-N(CH_3)_2$		$C_{23}H_{19}N_5O_3$	413	152	72
17	$-N(CH_3)_2$		$C_{27}H_{20}N_5O_3$	462	131	75
18	$-N(CH_3)_2$		$C_{26}H_{19}N_6O_3Cl$	498	107	68
21	$-OCH_3$		$C_{24}H_{17}N_4O_3Cl$	444	186	62
22	$-OCH_3$		$C_{26}H_{23}N_5O_3$	454	135	70
23	$-OCH_3$		$C_{25}H_{20}N_4O_4$	440	105	78
24	$-OCH_3$		$C_{27}H_{24}N_4O_6$	500	76	65
25	$-OCH_3$		$C_{22}H_{16}N_4O_4$	400	152	75
26	$-OCH_3$		$C_{26}H_{17}N_4O_4$	449	85 – 87	70
27	$-OCH_3$		$C_{25}H_{16}N_5O_4Cl$	485	110	78

[a)] All compounds gave satisfactory elemental analysis (± 0.4% of theoretical values).

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

Compound	Minimum cytotoxic concentration (μg/mL)[b]	Minimum inhibitory concentration (μ/mL)[a]				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK KOS ACV
3	≥16	>16	>16	>16	>16	>16
4	16	>3.2	>3.2	>3.2	>3.2	>3.2
5	80	>16	>16	>16	>16	>16
6	80	>16	>16	>16	>16	>16
7	>400	>400	>400	80	>400	240
8	>400	>400	>400	240	>400	240
9	≥400	>400	>400	>400	>400	>400
12	≥16	>16	>16	>16	>16	>16
13	80	>16	>16	>16	>16	>16
14	80	>16	>16	>16	>16	>16
15	80	>16	>16	>16	>16	>16
16	≥80	>80	>80	>80	>80	>80
17	≥80	>80	>80	>80	>80	>80
18	≥400	>400	>400	>400	>400	>400
21	80	>16	>16	>16	>16	>16
22	80	>16	>16	16	>16	>16
23	80	>16	>16	>16	>16	>16
24	80	>16	>16	>16	>16	>16
25	16	>3.2	>3.2	>3.2	>3.2	>3.2
26	400	>80	>80	>80	>80	>80
27	400	>80	>80	>80	>80	>80
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%.

Table 3. *In vitro* antitubercular activity of 4-(4′-substituted phenyl)-6-(4′-nitrophenyl)-2-(substituted imino) pyrimidines.

Compound	Concentration (μg/mL)[a]	Percentage Inhibition
3	6.25	98
4	6.25	97
5	6.25	100
6	6.25	100
7	6.25	82
9	6.25	80
15	6.25	78
26	6.25	65
27	6.25	70
Rifampicin	0.25	98
Isoniazid	0.031	95
Tobramycin	10.0	99
Ethionamide	1.17	99
PAS	2.31	99

[a] The concentration represents their MIC's.

result of antituberculostic activity (Table 3) indicates that the pyrimidines with chloro and *p*-methoxyphenyl **5**, trimethoxyphenyl **6** showed more potent activity than the reference drugs. Pyrimidines with chloro and chlorophenyl **3**, and *p*-*N*-dimethylaminophenyl **4** substitutions showed equipotent activity in comparison with the reference drugs. The rest of compounds showed comparable activity. In antibacterial screening (Table 4), the screened compounds exhibited mild to moderate activity against *Klebsiella pneumoniae*. Against other bacterial strains, only few compounds showed activity comparable to that of the standard drugs and especially against *Staphylococcus aureus*; only few compounds were shown to have mild to moderate activity.

In summary, the synthesis of a new series of 4-(4-substituted phenyl)-6-(4-nitrophenyl)-2-substituted imino) pyrimidines derivatives is described here. These derivatives exhibit moderate antiviral, antituberculostic, and antibacterial activity. However, QSAR studies should be carried out to predict the structure-activity relationship for all the compounds.

The authors are grateful to Rega Institute for Medical Research, Katholieke University, Leuven, Belgium for screening antiviral activity and to National Institute of Allergy and Infectious Diseases, Tuberculosis antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham (USA) for screening antituberculostic activity. The authors are also thankful to Hamdard University, New Delhi for providing facilities to carry out the research work.

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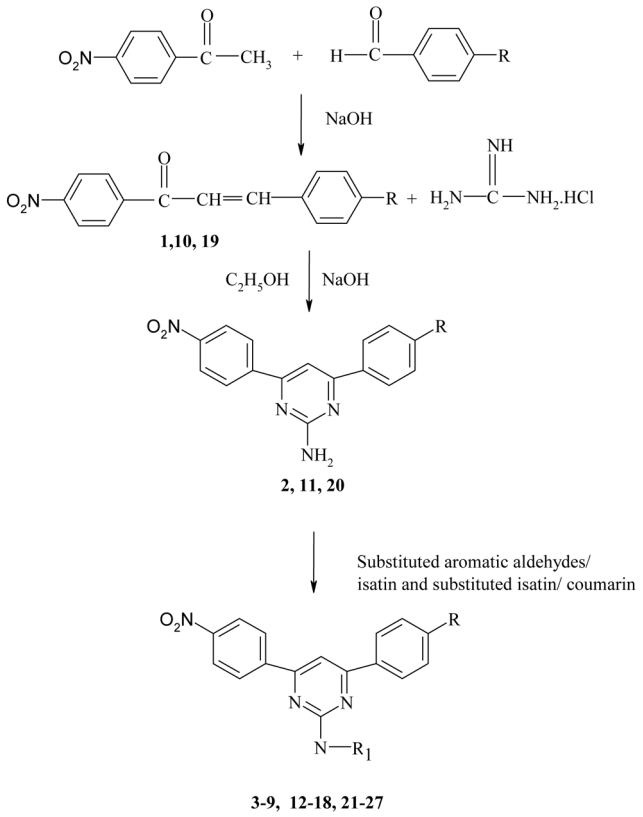

Scheme 1. Synthesis of 4-(4′-substituted phenyl)-6-(4′-nitrophenyl)-2-(substituted imino) pyrimidines.

Experimental

Chemistry

Melting points were determined in open capillaries on a Veego VMP-1 melting point apparatus (Veego Instruments, Mumbai, India) and are uncorrected. IR spectra were recorded in KBr on a Perkin Elmer IR spectrophotometer (values in cm^{-1}; Perkin Elmer), NMR spectra (in DMSO) on a JEOL Fx-100 FT-NMR spectrophotometer using TMS as an internal standard and Mass spectra on a JEOL Bx 102/DA-6000 spectrometer (JEOL, Tokyo, Japan).

The preparation of 4-(4-substitutedphenyl)-6-(4-nitrophenyl)-2-(substituted imino) pyrimidines involves three steps.

Preparation of 3-(4-chlorophenyl)-1-(4-nitrophenyl)-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-nitroacetophenone (0.02 mol) in ethanol (6 mL). The reaction mixture was stirred overnight at room temperature and then poured into water (100 mL). After neutralization with HCl (10% w/v), a pale yellow solid, which separated, was recrystallized from ethanol to obtain a pale-yellow crystalline compound. Yield: 85%, mp 101°C; IR (KBr) cm^{-1}: 2888 (CH), 1681.05 (C=O), 1606 (C=C), 1343 (NO_2); ^{1}H-NMR (DMSO-d_6) δ: 6.82 (d, 1H, J = 8.7 H_Z, C*H*-Ar), 7.51 – 8.02 (m, 8H, Ar-*H*), 8.12 (d, 1H, J = 8.7 Hz, CO=C*H*); MS *(m/z)* 288 [M^+ + 1]; Anal. Calcd. for $C_{15}H_{10}NO_3Cl$: C, 62.62; H, 3.50; N, 4.86. Found: C, 62.65; H, 3.48; N, 4.89. Adopting this procedure, starting materials **10** and **19** were prepared by reported procedure [18].

Table 4. Antibacterial activity of 4-(4′-substituted phenyl)-6-(4′-nitrophenyl)-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*
5	100	–	–	–	–
	200	–	–	–	18
	500	–	18	–	20
6	100	16	–	–	–
	200	19	–	–	–
	500	21	–	–	–
7	100	–	–	–	15
	200	–	–	–	18
	500	–	–	–	20
8	100	–	–	–	–
	200	–	–	17	–
	500	–	–	20	–
9	100	–	–	–	–
	200	–	16	–	–
	500	–	19	–	17
12	100	–	–	–	–
	200	18	–	15	–
	500	20	–	19	–
17	100	–	15	–	–
	200	–	18	–	–
	500	–	20	–	–
18	100	15	17	–	16
	200	18	19	–	19
	500	20	20	–	21
24	100	–	–	–	15
	200	–	–	–	18
	500	–	–	–	20
26	100	–	15	–	17
	200	–	17	–	20
	500	–	22	–	22
Ampicillin	50	23	21	25	21
Chloramphenicol	50	22	25	23	20

(–) Denotes no activity

Preparation of 4-(4-chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine ***(2)***

A mixture of 3-(4′-chlorophenyl)-1-(4′-nitrophenyl)-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 10 h and poured onto crushed ice. The resultant solid was washed with dilute ethanol, dried, and recrystallized from ethanol-chloroform mixture to yield a yellow crystalline solid. Yield: 78.5%, mp 125°C; IR (KBr) cm^{-1}: 3418 (NH_2), 1595.07 (C=N), 1412 (NO_2), 817 (C-Cl); ^{1}H-NMR (DMSO-d_6) δ: 4.80 (s, 2H, N*H*$_2$), 6.63 – 8.21 (m, 8H, Ar-*H*), 8.45 (s, 1H, Pyr-C-5); MS *(m/z)* 326 [M^+]; Anal. Calcd. for $C_{16}H_{10}N_4O_2Cl$: C, 58.99; H, 3.09; N, 17.20. Found: C, 58.95; H, 3.12; N, 17.16. Adopting this

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procedure, intermediates **11** and **20** were prepared by reported procedure [19].

Preparation of (4-chlorobenzylidene)-[4-(4-chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]amine ***(3)***

Equimolar quantities of 4-(4-cholorophenyl)-6-(4-nitrophenyl)-2-aminopyrimidine **2** (0.002 mol) and 3,4,5-trimethoxybenzaldehyde (0.002 mol) were dissolved in warm ethanol (75 mL) containing glacial acetic acid (1 mL). The reaction mixture was refluxed for 11 h and poured onto crushed ice. The resultant pale-yellow solid was dried and recrystallized from ethanol to get a pale-yellow crystalline solid. Yield: 65%, mp 92 – 94°C; IR (KBr) cm^{-1}: 2972 (CH), 1582 (C=N), 1390 (NO_2), 845.3 (C-Cl); ^{1}H-NMR (DMSO-d_6): δ: 6.38 (s, 1H, C*H*), 6.52 – 8.69 (m, 13H, Ar-*H*); ^{13}C-NMR ($CDCl_3$): d 115.4, 121.1 (2C), 127.2 (2C), 128.2 (2C), 129.4 (2C), 129.7 (2C), 129.9, 130.6 (2C), 132.8, 135.4, 139.6, 143.7, 149.4, 165.6, 166.8 (2C), 182.4; MS *(m/z)* 450 [M^+ +1]; Anal. Calcd. for $C_{23}H_{14}N_4O_2Cl_2$: C, 61.48; H, 3.14; N, 12.47. Found: C, 61.40; H, 3.00; N, 12.13. Adopting this procedure, compounds **3** – **9**, **12** – **18**, and **21** – **27** were prepared.

[4-(4-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]-(4-dimethylamino-benzylidene)amine ***(4)***

IR (KBr) cm^{-1}: 2970 (CH), 1585 (C = N), 1388 (NO_2), 845.7 (C-Cl); ^{1}H-NMR (DMSO-d_6): δ: 3.11 (s, 6H, N(CH_3)$_2$), 6.34 (s, 1H, C*H*), 6.52 – 8.72 (m, 13H, Ar-*H*); MS *(m/z)* 459 [M^+ +1]; Anal. Calcd. for $C_{25}H_{20}N_5O_2Cl$: C, 65.57; H, 4.40; N, 15.29. Found: C, 65.52; H, 4.35; N, 15.13.

[4-(4-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]-(4-methoxy-benzylidene)amine ***(5)***

IR (KBr) cm^{-1}: 2972 (CH), 1582 (C=N), 1390 (NO_2), 845.3 (C-Cl); ^{1}H-NMR (DMSO-d_6): δ: 3.86 (s, 3H, OCH_3), 6.35 (s, 1H, C*H*), 6.49-8.67 (m, 13H, Ar-*H*); MS *(m/z)* 445 [M^+]; Anal. Calcd. for $C_{24}H_{17}N_4O_3Cl$: C, 64.80; H, 3.85; N, 12.59. Found: C, 64.62; H, 3.95; N, 12.23.

[4-(4-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]-(3,4,5-trimethoxy-benzylidene)amine ***(6)***

IR (KBr) cm^{-1}: 2968 (CH), 1589 (C=N), 1398 (NO_2), 850 (C-Cl); ^{1}H-NMR (DMSO-d_6): δ: 3.86 (s, 9H, OCH_3), 6.38 (s, 1H, C*H*), 6.47 – 8.59 (m, 11H, Ar-*H*); MS *(m/z)* 504 [M^+ +1]; Anal. Calcd. for $C_{26}H_{21}N_4O_5Cl$: C, 61.84; H, 4.19; N, 11.09. Found: C, 61.80; H, 4.16; N, 11.13.

[4-(4-Chlorophenyl)-6-(4-nitrophenyl-pyrimidin-2-yl)furan-2-ylmethylene-amine ***(7)***

IR (KBr) cm^{-1}: 2976 (CH), 1594 (C=N), 1388 (NO_2), 850 (C-Cl); ^{1}H-NMR (DMSO-d_6): δ: 6.38 (s, 1H, C*H*), 6.27 – 8.39 (m, 12H, Ar-*H*); MS *(m/z)* 406 [M^+ +]; Anal. Calcd. for $C_{21}H_{13}N_4O_3Cl$: C, 62.31; H, 3.24; N, 13.84. Found: C, 62.52; H, 3.35; N, 13.63.

[4-(4-Chlorophenyl)-6-(4-nitrophenyl)-pyrimidin-2-yl]-chromen-2-ylidene-amine ***(8)***

IR (KBr) cm^{-1}: 3028 (CH), 1700 (Cyclic C-O-C), 1617 (C = N), 1375 (NO_2), 828.6 (C-Cl); ^{1}H-NMR (DMSO-d_6): δ: 6.48 (d, 1H, *J* = 10.2 Hz, C*H*), 6.72 (d, 1H, *J* = 10.2 Hz, C*H*), 6.92-8.48 (m, 13H, Ar-*H*); MS *(m/z)* 454 [M^++1]; Anal. Calcd. for $C_{25}H_{15}N_4O_3Cl$: C, 66.01; H, 3.32; N, 12.31. Found: C, 66.05; H, 3.31; N, 12.35.

5-Chloro-3-{[4-(4-chlorophenyl)-6-(4-nitrophenyl)-pyrimidin-2-ylimino]-methyl}-1,3-dihydro-indol-2-one ***(9)***

IR (KBr) cm^{-1}: 3354 (NH), 1600 (CO), 1522 (C = N), 1370 (NO_2), 776 (Ar-Cl); ^{1}H-NMR (DMSO-d_6) δ: 3.91 (s, 1H, C*H*), 6.36 (s, 1H, C*H*), 6.55 – 7.78 (m, 12H, Ar-*H*), 8.25 (brs, 1H, N*H*); MS *(m/z)* 491 [M^+ + 1]; Anal. Calcd. for $C_{24}H_{13}N_5O_3Cl_2$: C, 58.79; H, 2.67; N, 14.28. Found: C, 58.52; H, 2.55; N, 14.13.

(4-Chloro-benzylidene)-[4-(4-dimethylamino-phenyl)-6-(4-nitro-phenyl)-pyrimidin-2-yl]-amine ***(12)***

(KBr) cm^{-1}: 2920 (CH), 1590 (C=N), 815 (Ar-Cl); ^{1}H-NMR (DMSO-d_6) δ: 3.02 (s, 6H, N(CH_3)$_2$), 6.62 (s, 1H, C*H*), 7.41-8.45 (m, 13H, Ar-*H*); MS *(m/z)* 458 [M^+ + 1]; Anal. Calcd. for $C_{25}H_{20}N_5O_2Cl$: C, 65.57; H, 4.40; N, 15.29. Found: C, 65.61; H, 4.43; N, 15.27.

(4-Dimethylaminobenzylidene)-[4-(4-dimethylaminophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]amine ***(13)***

IR (KBr) cm^{-1}: 2924 (CH), 1596 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 3.00 (s, 12H, 2-N(CH_3)$_2$, 6.63 (s, 1H, C*H*), 7.67 – 8.41 (m, 13H, Ar-*H*); MS *(m/z)* 443 [M^+ + 1]; Anal. Calcd. for $C_{27}H_{26}N_6O_2$: C, 69.51; H, 5.61; N, 18.01. Found: C, 69.55; H, 5.63; N, 18.00.

[4-(4-Dimethylaminophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]-(4-methoxy-benzylidene)amine ***(14)***

IR (KBr) cm^{-1}: 2972 (CH), 1582 (C=N); ^{1}H-NMR (DMSO-d_6): δ: 3.08 (s, 6H, N(CH_3)$_2$) 3.89 (s, 3H, OCH_3), 6.39 (s, 1H, C*H*), 7.52 – 8.62 (m, 13H, Ar-*H*); MS *(m/z)* 454 [M^+ + 1]. Anal. Calcd. for $C_{26}H_{23}N_5O_3$: C, 68.86; H, 5.11; N, 15.44. Found: C, 68.52; H, 5.25; N, 15.13.

[4-(4-Dimethylaminophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]-(3,4,5-trimethoxy-benzylidene)amine ***(15)***

IR (KBr) cm^{-1}: 2968 (CH), 1589 (C=N); ^{1}H-NMR (DMSO-d_6): δ: 3.04 (s, 6H, N(CH_3)$_2$), 3.84 (s, 9H, OCH_3), 6.34 (s, 1H, C*H*), 7.47 – 8.59 (m, 11H, Ar-*H*); ^{13}C-NMR ($CDCl_3$): δ 39.4 (2C), 54.7 (3C), 104.3 (2C), 127.6 (2C), 128.9 (2C), 130.4 (2C), 130.6 (2C), 131.2 (2C), 134.9, 139.2, 142.7, 143.1, 148.9 (2C), 164.6, 165.9 (2C), 182.4; MS *(m/z)* 502 [M^+ + 1]; Anal. Calcd. for $C_{27}H_{27}N_5O_5$: C, 64.66; H, 5.43; N, 13.96. Found: C, 64.52; H, 5.25; N, 14.00.

[4-(4-Dimethylaminophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]furan-2-ylmethylene-amine ***(16)***

IR (KBr) cm^{-1}: 2979 (CH), 1598 (C = N); ^{1}H-NMR (DMSO-d_6): δ 3.10 (s, 6H, N(CH_3)$_2$), 6.42 (s, 1H, C*H*), 7.27 – 8.39 (m, 12H, Ar-*H*); MS *(m/z)* 414 [M^+ + 1]; Anal. Calcd. for $C_{23}H_{19}N_5O_3$: C, 66.82; H, 4.63; N, 16.94. Found: C, 66.62; H, 4.35; N, 16.90.

Chromen-2-ylidene-[4-(4-dimethylaminophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]amine ***(17)***

IR (KBr) cm^{-1}: 2920 (CH), 1700 (Cyclic C-O-C), 1596 (C=N), 1370.5 (NO_2); ^{1}H-NMR (DMSO-d_6) δ 3.02 (s, 6H, N(CH_3)$_2$), 6.48 (d, 1H, *J* = 10.4 Hz, C*H*), 6.64 (d, 1H, *J* = 10.4 Hz, CH), 7.35 – 8.42 (m, 13H, Ar-*H*); MS *(m/z)* 463 [M^+ + 1]; Anal. Calcd. for $C_{27}H_{21}N_5O_3$: C, 69.96; H, 4.56; N, 15.11. Found: C, 69.93; H, 4.60; N, 15.13.

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5-Chloro-3-{[4-(4-dimethylaminophenyl)-6-(4-nitrophenyl)pyrimidin-2-ylimino]-methyl}-1,3-dihydro-indol-2-one ***(18)***

IR (KBr) cm^{-1}: 3354 (NH), 1600 (CO), 1522 (C=N), 1370 (NO_2), 776 (Ar-Cl); ^{1}H-NMR (DMSO-d_6) δ 3.03 (s, 6H, $N(CH_3)_2$), 6.55 – 7.78 (m, 12H, Ar-*H*), 8.45 (s, 1H, N*H*); MS *(m/z)* 498 [M^+]; Anal. Calcd. for $C_{26}H_{19}N_6O_3Cl$: C, 62.59; H, 3.83; N, 16.84. Found: C, 62.58; H, 3.87; N, 16.82

(4-Chlorobenzylidene)-[4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]amine ***(21)***

IR (KBr) cm^{-1}: 2924 (CH), 1603 (C=N), 1366 (NO_2), 824 (Ar-Cl); ^{1}H-NMR (DMSO-d_6) δ 3.81 (s, 3H, OCH_3), 6.33 (s, 1H, C*H*), 6.66 – 8.73 (m, 13H, Ar-*H*); MS *(m/z)* 445 [$M^+ + 1$]; Anal. Calcd. for $C_{24}H_{17}N_4O_3Cl$: C, 64.79; H, 3.85; N, 12.59. Found: C, 64.70; H, 3.84; N, 12.63.

(4-Dimethylaminobenzylidene)-[4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]amine ***(22)***

IR (KBr) cm^{-1}: 2924 (CH), 1596 (C = N), 1391 (NO_2); ^{1}H-NMR (DMSO-d_6) δ 3.06 (s, 6H, $N(CH_3)_2$), 3.84 (s, 3H, OCH_3), 6.65 (s, 1H, CH), 7.67 – 8.41 (m, 13H, Ar-H); MS (m/z) 455 [$M^+ + 1$]; Anal. Calcd. for $C_{26}H_{23}N_5O_3$: C, 68.86; H, 5.11; N, 15.44. Found: C, 68.52; H, 5.25; N, 15.13.

3.1.19 (4-Methoxybenzylidene)-[4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]amine ***(23)***

IR (KBr) cm^{-1}: 2924 (CH), 1596 (C=N), 1391 (NO_2); ^{1}H-NMR (DMSO-d_6) δ 3.89 (s, 6H, 2-OCH_3), 6.67 (s, 1H, CH), 7.67-8.41 (m, 13H, Ar-H); MS (m/z) 441 [M^+ +1]; Anal. Calcd. for $C_{25}H_{20}N_4O_4$: C, 68.18; H, 4.58; N, 12.72. Found: C, 68.22; H, 4.25; N, 12.53.

[4-(4-Methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]-(3,4,5-trimethoxy-benzylidene)amine ***(24)***

IR (KBr) cm^{-1}: 2940 (CH), 1586 (C=N), 1391 (NO_2), 1015, 823; ^{1}H-NMR (DMSO-d_6) δ 3.86 (s, 12H, 4-OCH_3), 6.32 (s, 1H, C*H*), 6.66 – 8.23 (m, 11H, Ar-*H*); MS *(m/z)* 500 [M^+]; Anal. Calcd. for $C_{27}H_{24}N_4O_6$: C, 64.79; H, 4.83; N, 11.19. Found: C, 64.71; H, 4.80; N, 11.17.

Furan-2-ylmethylene-[4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]amine ***(25)***

IR (KBr) cm^{-1}: 2928 (CH), 1604 (C = N), 1370 (NO_2), 845; ^{1}H-NMR (DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 6.32 (s, 1H, C*H*), 6.61-8.47 (m, 12H, Ar-*H*); MS *(m/z)* 400 [M^+]; Anal. Calcd. for $C_{22}H_{16}N_4O_4$: C, 65.99; H, 4.02; N, 13.99. Found: C, 65.96; H, 4.07; N, 13.98.

Chromen-2-ylidene-[4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]amine ***(26)***

IR (KBr) cm^{-1}: 2920 (CH), 1700 (Cyclic C-O-C), 1596 (C=N), 1370.5 (NO_2); ^{1}H-NMR (DMSO-d_6) δ 3.82 (s, 3H, OCH_3), 6.38 (d, 1H, *J* = 10.2 Hz, C*H*), 6.61 (d, 1H, *J* = 10.4 Hz, C*H*), 6.72 – 8.47 (m, 13H, Ar-*H*); MS *(m/z)* 449 [M^+]; Anal. Calcd. for $C_{26}H_{18}N_4O_4$: C, 69.32; H, 4.02; N, 12.43. Found: C, 69.32; H, 4.04; N, 12.40.

5-Chloro-3-{[4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-ylimino]methyl}-1,3-dihydro-indol-2-one ***(27)***

IR (KBr) cm^{-1}: 3355 (NH), 2928 (CH), 1661 (CO), 1602 (C =N), 1368 (NO_2), 823.4 (Ar-Cl); ^{1}H-NMR (DMSO-d_6) δ 3.51 (s, 3H, OCH_3), 6.65 – 8.45 (m, 12H, Ar-H); ^{13}C-NMR ($CDCl_3$): d 35.9, 54.7, 113.9(2C), 115.9, 127.2 (2C), 127.9 (2C), 128.2 (2C), 129.3 (2C), 129.9 (2C), 131.1, 139.7, 142.8, 147.6, 161.2, 163.2, 165.4 (2C), 167.7, 179.8; MS (m/z) 484 [$M^+ - 1$]; Anal. Calcd. for $C_{25}H_{16}N_5O_4Cl$: C, 61.79; H, 3.31; N, 14.41. Found: C, 61.78; H, 3.35; N, 14.45.

Biological evaluation

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

Cytotoxicity assay

Each synthesized compound was separately dissolved in 1 mL of distilled dimethyl sulphoxide (DMSO) and the volume was brought up to 10 mL with maintenance medium to obtain a stock solution of 1 mg/mL concentration. It was 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, India). Fresh medium containing different concentrations of the test sample was added 24 h after the seeding. Control cells were incubated without the 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. 16 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} (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 [20].

Antiviral assay (MIC)

Confluent cell cultures in 96-well microtiter 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 a 1 – 2 h period of virus adsorption, 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 microscopically 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. 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 [21, 22].

Antitubercular activity (Alamar blue susceptibility test (MABA))

Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, CT, USA) in order to minimize

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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 de-ionized 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 the compounds. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37°C. Starting at day 4 of the incubation, 20 μL of 10 × Alamar blue solution (Alamar Biosciences/Accumed, Westlake, OH, USA) and 12.5 mL of 20% Tween 80 were added to one B well and one M well, and plates were re-incubated 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, MA, USA.) 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 were 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 inhibitions of bacterial growth at 6.25 μg/mL of all the selected synthesized compounds are depicted in Table 3 along with the MIC's of standard drugs [23, 24]. The compounds which were shown 90 and above of percentage inhibition have been taken up for second phase of screening to determine their actual MIC's by TAACF, Birmingham, USA.

Antibacterial activity

The synthesized compounds were tested for their antibacterial activity by the cup-plate method [25]. 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 was compared with that of the reference standard antibiotics namely ampicilin (50 μg/mL) and chloramphenicol (50 μg/mL).

References

[1] V. Alagarsamy, U. S. Pathak, V. R. Solomon, S. Meena, *et al*., *Indian J. Heterocycl. Chem.* **2004**, *13*, 347–352.

[2] A. Gangjee, Y. Jianming, L. K. Roy, W. H. Haile, *et al*., *J. Med. Chem.* **2003**, *46*, 591–600.

[3] E. B. Skibo, X. Huang, R. Martinez, R. H. Lemus, W. A. Craigo, *J. Med. Chem.* **2002**, *45*, 5543–5555.

[4] A. Gangjee, Y. Zeng, J. J. Mc Gurie, R. L. Kisliuk, *J. Med. Chem.* **2002**, *45*, 1942–1948.

[5] A. Holy, I. Ivan, M. Masojidkova, G. Andrei, *et al*., *J. Med. Chem.* **2002**, *45*, 1918–1929.

[6] R. Kumar, M. Nath, D. Lorne, J. Tyrrell, *J. Med. Chem.* **2002**, *45*, 2032–2040.

[7] A. Gueiffier, M. Lhassani, A. Elhaxmaouri, R. Snoeck, *et al*., *J. Med. Chem.* **1996**, *39*, 2856–2859.

[8] A. Mai, M. Artico, G. Sbardella, S. Massa, *et al*., *J. Med. Chem.* **1995**, *38*, 3258–3263.

[9] A. Atlas, *Jpn. Kokai Tokkyo Koho Jp, 01,151*, **1989**; 595, [89,151,595] (Cl. CO7H19/067).

[10] T. S. Lin, J. Y. Guo, R. F. Schinazi, C. K. Chu, W. H. Prusoff, *J. Med. Chem.* **1988**, *31*, 336–340.

[11] K. Keppeler, G. Kiefer, E. D. Clerq, *Arch. Pharm.* **1986**, *319,* 360–364.

[12] D. Sriram, P. Yogeeswari, S. N. Pandeya, S. Ananthan, *Sci Pharm* **2002**, *70*, 39–48.

[13] Y. M. Abou-Zeid, M. M. El-Kerdawy, M. M. El-Enany, *Bull. Fac. Pharm. Cairo. Univ.* **1970**, *9*, 99–106.

[14] D. V. Singh, A. R. Mishra, R. M. Mishra, A. K. Pandey, *et al*., *Indian J. Heterocycl. Chem*. **2005**, *14*, 319–332.

[15] O. A. E. Sayed, F. M. E. Bieh, S. I. E. Aqeel, B. A. Bassam, *et al*., *Indian J. Heterocycl. Chem.* **2005**, *14*, 327–332.

[16] D. Jagmohan, S. Goel, P. Anupama, *Indian J. Heterocycl. Chem.* **2005**, *14*, 369 – 372.

[17] Y. A. Ammar, M. M. F. Ismail, M. S. A. EL-Gaby, M. A. Zahran, *Indian J. Heterocycl. Chem.* **2002**, *41*, 1486–1490.

[18] R. Bardia, J. T. Rao, *Asian. J. Chem.* **2004**, *16*, 1194–1196.

[19] R. Bardia, J. T. Rao, *J. Indian. Chem. Soc.* **1988**, *65*, 798–799.

[20] D. Francis, K. L. Rita, *J. Immunol. Methods* **1986**, *89*, 271–277.

[21] E. D. Clercq, J. Descamps, G. Verhelst, R. T. Walker, *et al*., *J. Infect. Dis.* **1980,** *141*, **563–564.**

[22] E. D. Clercq, T. Sakuma, M. Babu, R. Pauwels, *et al*., *Antiviral Res.* **1987**, *8*, 261–272.

[23] C. B. Inderleid, K. A. Nash, V. Lorian in *Antibiotics in Laboratory Medicin*, 4th Ed., William & Wilkins, Baltimore **1996**, pp. 127–175.

[24] C. B. Inderleid, M. Salfinger, P. R. Murray, E. J. Baron, *et al*., in *Manual of Clinical Microbiology*, 6th Ed., ASM Press, Washington, DC, **1995**, pp. 1385–1404.

[25] D. Greenwood, R. C. B. Slack, J. F. Peutherer in *Medical Microbiology*, 14th Ed., Churchill Livingstone **1992**, pp. 1–4.

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