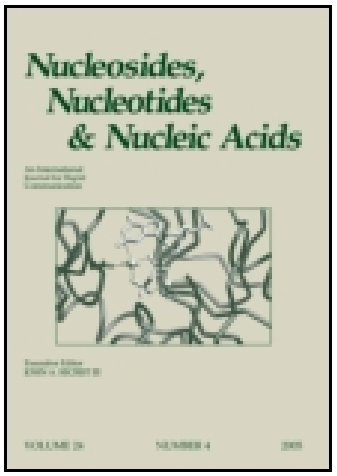


Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Chromone-Fused Cytosine Analogues: Synthesis, Biological Activity, and Structure-Activity Relationship

Dhaval D. Haveliwala[a], Nimesh R. Kamdar[a], Prashant T. Mistry[a] & Saurabh K. Patel[a]

[a] Department of ChemistryVeer Narmad South Gujarat University, Surat, Gujarat, India

Published online: 24 Mar 2014.

To cite this article: Dhaval D. Haveliwala, Nimesh R. Kamdar, Prashant T. Mistry & Saurabh K. Patel (2014) Chromone-Fused Cytosine Analogues: Synthesis, Biological Activity, and Structure-Activity Relationship, Nucleosides, Nucleotides and Nucleic Acids, 33:2, 80-91, DOI: 10.1080/15257770.2013.873128

To link to this article: http://dx.doi.org/10.1080/15257770.2013.873128

Nucleosides, Nucleotides and Nucleic Acids, 33:80–91, 2014

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2013.873128

Taylor & Francis
Taylor & Francis Group

CHROMONE-FUSED CYTOSINE ANALOGUES: SYNTHESIS, BIOLOGICAL ACTIVITY, AND STRUCTURE–ACTIVITY RELATIONSHIP

Dhaval D. Haveliwala, Nimesh R. Kamdar, Prashant T. Mistry, and Saurabh K. Patel

Department of Chemistry, Veer Narmad South Gujarat University, Surat, Gujarat, India

□ *The preparation of a series of novel chromone-fused cytosine analogues, i.e., chromeno[2,3-d]pyrimidines has been carried out from substituted 2-amino-4-oxo-4H-chromene-3-carbonitriles with urea, thiourea, and guanidine under different reaction conditions. These chromone-fused cytosine analogues were evaluated for their in vitro activity against Mycobacterium tuberculosis $H_{37}Rv$ strain and different microbial pathogenic strains in cell culture for their structure–activity relationships, respectively. Among the synthesized compounds,* ***2d****,* ***3a****, and* ***4e*** *showed better results against Mycobacterium tuberculosis $H_{37}Rv$. The compounds* ***2a****,* ***2b****, and* ***3a*** *showed potential antibacterial activity against E. coli and P. aeruginosa, while the majority of compounds were found to be active against S. aureus as compared to ampicillin. The synthesized cytosine analogues having an imine (–C=NH) have been less sensitive to the bacterial and fungal strains but have a more beneficial effect on Mycobacterium tuberculosis $H_{37}Rv$.*

Keywords Antibacterial nucleosides; synthetic methodology; nucleoside synthesis; base modification

INTRODUCTION

The study of the chemistry and biochemistry of nucleoside and nucleotides has been of enormous importance; not only because of the relevance to nucleic acid chemistry and molecular biology but also because of a number of nucleosides (adenosine, guanosine, uridine, cytidine, and thymidine) have been found to be a part of useful antibiotic and antitumor scaffolds. Chemical modifications in the structure of nucleobases are widely studied in the search of important bioactive molecules. Nevertheless, the search for new bioactive compounds that lead to the formation of modified components of nucleobases having useful physicochemical properties still

Received 20 July 2012; accepted 3 December 2013.

The author wish to thank the Head, Department of Chemistry, V.N.S.G.U., Surat for providing the laboratory facility; Microbiologist, Microcare Laboratory for pharmacological essay and instrumental facility SAIF, Chandigarh for analytical data.

Address correspondence to Saurabh K. Patel, Department of Chemistry, Veer Narmad South Gujarat University, Udhana-Magdalla Road, Surat 395007, Gujarat, India. E-mail: saurabh@vnsgu.ac.in

remains as an urgent task. Pyrimidine-based derivatives have a prestigious position in medicinal chemistry and are important due to their being versatile building blocks for the synthesis of nitrogen-containing heteroaromatic species of biological importance. Recently, many chemical structures containing a pyrimidine moiety have been reported to exhibit diverse biological and pharmaceutical activities such as antibacterial,[1] glycogen inhibitory,[2] antimicrobial,[3,4] kinase Inhibitors,[5] and anti molluscicidal[6] agents. Moreover, pyrimidine-2 ones have been used as anti-inflammatory and analgesic agents,[7] anti mycobacterium tuberculosis and anti-mycobacterial[8], antiviral[9] and imino pyrimidine were reported to have antiviral[10] activity. Furthermore, imino pyrimidines have been used as antileishmanial agents [11], anti-histaminic activity[12], and antagonists of the human A2A receptor.[13] In addition, pyrimidine-2-thiones were reported as antimicrobial[14,15] and antiviral agent.[16]

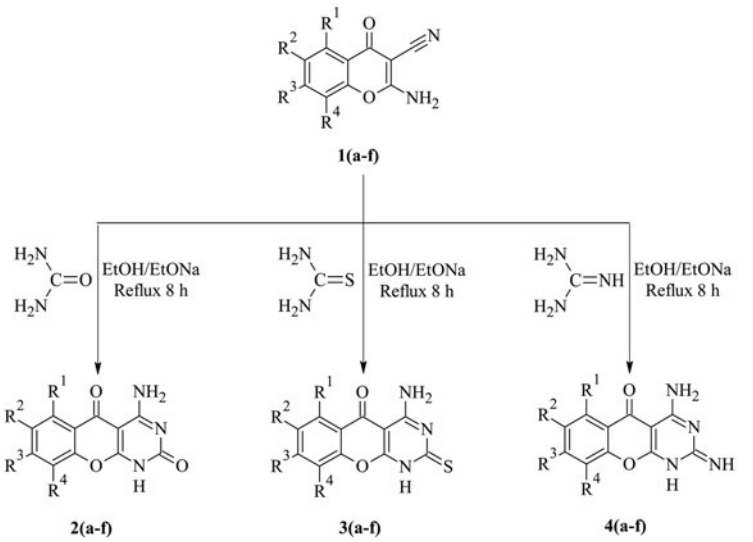
The natural compounds containing chromone constitute an interesting class because of their usefulness as biologically active agents.[17,18] Due to their abundance in plants and their low mammalian toxicity, chromone derivatives are present in large amounts in the diet of humans.[19]

We recently initiated that the structural modification of nucleobases with the prospect of yielding new classes of therapeutically active agents. The synthesis of these types of molecules can be made by several protocols and different reaction conditions. Although various known methods are reported concerning the synthesis of some fused pyrimidine derivatives we exploited the o-amino nitrile functionality in this study to design new routes for the preparation of biologically active heterocyclic compounds.[20–23] We herein disclose the synthesis of natural product chromone-containing structurally related cytosine fused systems and their antitubercular and antimicrobial profile to provide some structure–activity relationships (SAR).

RESULTS AND DISCUSSION

Eighteen chromone-fused cytosine analogues were synthesized and screened for their antimicrobial and antitubercular activity. The synthetic procedures adopted to obtain the target compounds are depicted in Scheme 1. The synthesis of precursor 2-amino-4-oxo-4*H*-chromene-3-carbonitriles **1(a–f)** derivatives were carried out by the chlorination of 2-(acetyloxy)benzoic acids with thionyl chloride followed by the treatment of malononitrile.[24]

The reactive amino and nitrile functionality of the precursor 2-amino-4-oxo-4*H*-chromene-3-carbonitriles **1(a–f)** was efficiently transformed into various cytosine analogue **2(a–f)**, **3(a–f)** and **4(a–f)** by treatment of urea, thiourea, and guanidine respectively in an ethanolic solution containing catalytic amount of sodium ethoxide. The structures of compounds **2(a–f)**,



SCHEME 1 Synthetic route to chromeno[2,3-*d*]pyrimidines.

3(a–f) and **4(a–f)** were established through spectroscopic and elemental analyses data. The IR spectra of the final compounds **2(a–f)**, **3(a–f),** and **4(a–f)** revealed that the cyclocondensation occurs at their o-amino nitrile functionality of the chromone nucleus with the disappearance of –C≡N band in each case. The ^{1}H-NMR spectra of all the compounds also show characteristic signals for the protons of the cytosine nucleus. Some representative ^{13}C-NMR spectra of compounds further confirm the proposed structures.

Antimycobacterial Assay

Antimycobacterial evaluation was carried out at the Microcare Laboratory, Surat, Gujarat, India. Primary screening of all compounds was performed against the *Mycobacterium tuberculosis H$_{37}$Rv* strain. All the compounds were tested for their antitubercular activity by the L. J. method[21] and compared with standard drug rifampicin (40 μg/mL). The percent inhibition activity of synthesized compounds is summarized in Fig. 1.

Compounds **2d**, **3a,** and **4e** were the most effective against *Mycobacterium tuberculosis* H$_{37}$Rv with 99% of inhibition, while compounds **2c**, **2b**, **4d,** and **4f** showed transitional antitubercular activity between 50% and 85% of inhibition. Among the unsubstituted cytosine analogues, those with a thio group (>C=S) show almost 100% inhibition of mycobacterial tubercular species, but the introduction of other substituents decreases the activity; typically a 7-chloro substitution at chromone nucleus diminishes activity completely. In contrast, a 7-chloro substitution containing a keto group (>C=O) shows the maximum inhibition of the tubercular pathogen. The introduction of electron withdrawing groups shows more pronounced activity than electron

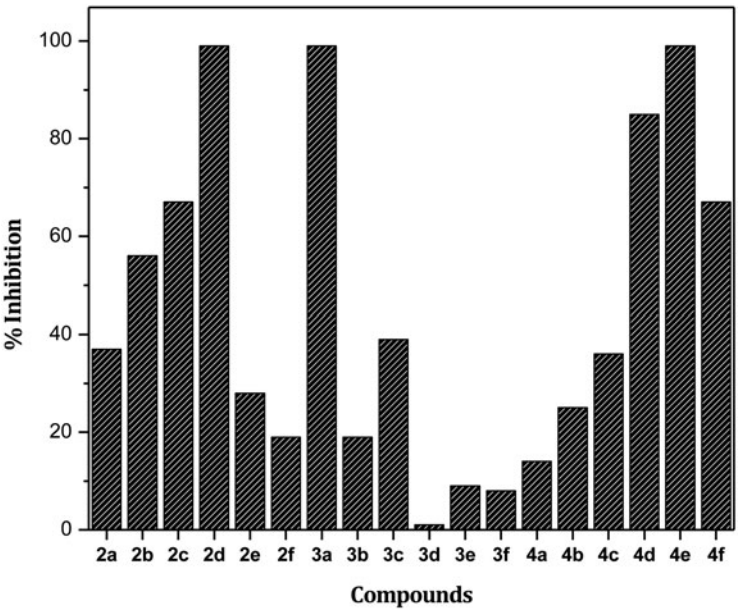


FIGURE 1 Antitubercular activity of chromeno[2,3-*d*]pyrimidines.

donating groups. In imino cytosine analogues, the introduction of methyl group ($-CH_3$) at eighth position shows maximum inhibition, while a change in the position of the methyl group decreases the activity. The 7-chloro substitution also shows appreciable inhibition, while the other substitutions reduce the inhibition of tubercular species.

Antimicrobial Assay

In vitro antibacterial activity of synthesized compounds **2**,**3**,**4(a–f)** was determined against isolated species of bacterial strains, i.e., *Staphylococcus aureus* [MTCC-96] and *Streptococcus pyogenes* [MTCC – 442] as gram positive, *Escherichia coli* [MTCC-443] and *Pseudomonas aeruginosa* [MTCC-1688] as gram negative and antifungal activity against two fungal strain *Candida albicans* [MTCC-227] and *Aspergillus niger* [MTCC-282]. The minimum inhibitory concentration (MIC), i.e., the lowest concentration of compound that completely inhibited microbial growth was determined by the broth dilution technique.[25] Ampicillin and greseofulvin were used as standard drugs for bacterial and fungal strains, respectively. The procedure was performed with two-fold dilution of the compounds in RPMI 1640 medium buffered to pH 7.0. The MICs were determined after 24 hours and 48 hours of static incubation at 35°C. The results of all compounds in vitro tested against bacterial as well as fungal strains are summarized in Table 1.

The MIC values (Table 1) show that the chromone ring containing different substituents on the aromatic ring elicited an interesting activity

TABLE 1 Antimicrobial assay of chromeno[2,3-*d*]pyrimidines

	Minimal Bactericidal Concentrations (μg/mL)				Minimal Fungicidal Concentrations (μg/mL)	
Code No.	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenus*	*C. albicans*	*A. niger*
2a	62.5	100	150	250	1000	500
2b	62.5	100	150	250	500	1000
2c	250	250	250	500	500	>1000
2d	200	100	200	250	250	1000
2e	250	250	200	200	250	1000
2f	200	200	200	250	>1000	>1000
3a	62.5	100	150	250	1000	500
3b	200	250	250	500	500	1000
3c	250	250	100	250	500	>1000
3d	150	150	250	250	250	1000
3e	250	250	100	200	250	500
3f	100	200	200	250	>1000	>1000
4a	500	500	1000	1000	500	500
4b	150	200	200	500	>1000	>1000
4c	250	250	500	500	>1000	>1000
4d	100	200	500	500	500	>1000
4e	250	250	500	500	1000	>1000
4f	250	250	500	500	>1000	>1000
A	100	100	250	100	—	—
B	—	—	—	—	500	100

Ampicillin (A) was used as standard drugs for bacterial strains, while greseofulvin (B) was used as standard drugs for fungal strains.

against gram negative bacteria. The observed activities are particularly good against *E. coli*. Compounds **2a**, **2b,** and **3a** show highest activity against *E. coli* and the values are even better than the standard drug ampicillin. Only the strain *S. pyogenus* is resistant to most of the synthesized compounds. Among the unsubstituted derivatives, **2a** and **3a** are found as be more sensitive than ampicillin against *E. coli*, *P. aeruginosa*, and *S. aureus*. In fact $-NO_2$ derivatives are totally inactive on both gram negative bacteria, while dibromo and monochloro compounds possess similar MIC value as ampicillin against *P. aeruginosa*. More interestingly, the compounds **2a**, **2b**, **2d**, **2e**, **2f**, **3a**, **3c**, **3e**, **3f,** and **4b** show MIC value in range of 100–200 μg/mL against *S. aureus*; in particular, the chloro and methyl derivatives (**3c** and **3e)** having sulfur-containing cytosine nucleus show remarkable (100 μg/mL) MIC value than ampicillin.

The antifungal activity data support the specific observation that compounds **2d** (7-Cl) and **2e** (8-CH_3) possess better antifungal activity than greseofulvin (B) against *C. albicans*. Similar activity is also observed in compounds **3d** (7-Cl) and **3e** (8-CH_3) which contain a sulfur-based cytosine nucleus. The replacement of a keto (–C=O) or thio (–C=S) group on cytosine with an

imine (–C=NH) group results in the complete loss of antifungal activity. Most of the compounds were insensitive toward *A. niger*.

Among the synthesized cytosine analogues, imine (–C=NH) group compounds in general reduce the potency against all the tested micro-organisms as compared to keto (–C=O) and thio (–C=S) group. The unsubstituted chromeno[2,3-*d*]pyrimidines containing keto (–C=O) and thio (–C=S) groups show very potent activity against *E. coli*. bacteria.

CONCLUSIONS

Novel chromone fused cytosine analogues have been successfully synthesized by simple condensation with urea, thiourea, and guanidine, respectively. A majority of the compounds were found to be active against *S. aureus*. Compounds **2a**, **2b,** and **3a** show higher antimicrobial potency against *E. coli*, while compounds **2d**, **2e**, **3d**, and **3e** were found to be more potent against *C. albicans* as compared to the standard drug. The antimicrobial study shows that the synthesized compounds have been found to be more active toward bacteria than fungi. The SAR of the title compounds showed that the presence of an imino functionality on cytosine and groups like 8-methyl, 7-chloro, and 9-methyl on the chromone ring are responsible for the good antitubercular activity.

EXPERIMENTAL

Chemistry

All organic solvents used for the synthesis were of analytical grade. The solvents were dried and freshly distilled under moisture free atmosphere. TLC (Merck, 0.25 mm thick) was performed on coated silica gel plates in the toluene–ethyl acetate (3:1) solvents system. The plates were detected in UV (254 nm). Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded using KBr pellets on Nicolet 400 IR-spectrophotometer (Thermo Fisher). ^{1}H and ^{13}C-NMR spectra were recorded on a Varian 400 MHz spectrometer using DMSO-d_6 or $CDCl_3$ as the solvent and TMS as the internal standard.

General Procedure for Synthesis of Compounds 2(a–f)

A mixture of **1(a–f)** (0.05 mol) and urea (0.05 mol) in absolute ethanol (20 mL) containing sodium ethoxide (0.05 mol) was refluxed for 8 hours. The reaction mixture was left to cool to room temperature, then poured into ice cold water (50 mL) and neutralized with dilute hydrochloric acid; the separated precipitates were filtered and recrystallized from methanol to give compounds **2(a–f)**.

4-amino-2H-chromeno[2,3-d]pyrimidine-2,5(1H)-dione (2a)

Solid brown; Yield 68%; m. p. 246–249°C; IR (KBr, ν, cm^{-1}): 3299 ($-NH_2$), 3126 (>NH), 1738 (>C=O pyrimidine ring), 1665 (chromone ring >C=O), 1615 (–C=N–); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): δ 6.66–6.73 (2H, multiplet, Ar–H_9/H_7), 7.17 (1H, ddd, Ar–H_8, J = 8.28, 2.14, 1.80 Hz), 7.67 (1H, dd, Ar–H_6, J = 8.28, 1.80 Hz), 8.09 (2H, broad s, $-NH_2$, D_2O exchangeable), 8.35 (1H, s, >NH); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 116, 123, 125, 129 135, 157 (aromatic carbons), 172.9 (C–NH_2), 177.5 (chromone >C=O), 148.5 (pyrimidone >C=O), 76 and 173 (C=C at pyrimidine ring); Anal. Calcd. for $C_{11}H_7N_3O_3$: C, 57.65, H, 3.08, N, 18.33, Found: C, 57.64, H, 3.10, N, 18.34.

4-amino-7,9-dibromo-2H-chromeno[2,3-d]pyrimidine-2,5(1H)-dione (2b)

Solid brown; Yield 74%; m. p. 272–276°C; IR (KBr, ν, cm^{-1}): 3281 ($-NH_2$), 3112 (>NH), 1728 (>C=O pyrimidine ring), 1668 (chromone ring >C=O), 1624 (–C=N–), 726 (C–Br); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 7.85 (1H, d, Ar–H_8, J = 2.0 Hz), 8.26 (1H d, Ar–H_6, J = 2.0 Hz), 8.36 (2H, broad s, $-NH_2$, D_2O exchangeable), 8.44 (1H, s, >NH); Anal. Calcd. for $C_{11}H_5N_3O_3Br_2$: C, 34.14, H, 1.28, N, 10.86, Found: C, 34.16, H, 1.30, N, 10.84.

4-amino-7-nitro-2H-chromeno[2,3-d]pyrimidine-2,5(1H)-dione (2c)

Solid brown; Yield 69%; m. p. 285–287°C; IR (KBr, ν, cm^{-1}): 3295 ($-NH_2$), 3120 (>NH), 1736 (>C=O pyrimidine ring), 1666 (chromone ring >C=O), 1618 (–C=N–), 1520 (C–NO_2); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 7.31 (1H, d, Ar–H_9, J = 9.16 Hz),), 8.32 (1H dd, Ar–H_8, J = 9.10, 2.56 Hz), 8.64 (1H, dd, Ar–H_6, J = 2.56 Hz), 8.23 (2H, broad s, $-NH_2$, D_2O exchangeable), 8.48 (1H, s, >NH); Anal. Calcd. for $C_{11}H_6N_4O_5$: C, 48.18, H, 2.21, N, 20.43, Found: C, 49.19, H, 2.22, N, 20.44.

4-amino-7-chloro-2H-chromeno[2,3-d]pyrimidine-2,5(1H)-dione (2d)

Pale yellow; Yield 72%; m. p. 258–261°C; IR (KBr, ν, cm^{-1}): 3272 ($-NH_2$), 3100 (>NH), 1724 (>C=O pyrimidine ring), 1669 (chromone ring > C=O), 1621 (–C=N–), 812 (C–Cl); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 7.23 (1H, d, Ar–H_9, J = 8.8 Hz), 7.55 (1H, dd, Ar–H_8, J = 8.8, 2.6 Hz), 7.84 (1H, d, Ar–H_6, J = 2.6 Hz), 8.26 (1H, s, >NH), 8.56 (2H, broad s–NH_2, D_2O exchangeable); Anal. Calcd. for $C_{11}H_6N_3O_3Cl$: C, 50.11, H, 2.29, N, 15.94, Found: C, 50.09, H, 2.27, N, 15.96.

4-amino-8-methyl-2H-chromeno[2,3-d]pyrimidine-2,5(1H)-dione (2e)

Brown; Yield 71%; m. p. 275–278°C; IR (KBr, ν, cm^{-1}): 3301 ($-NH_2$), 3127 (>NH), 1742 (>C=O pyrimidine ring), 1665 (chromone ring >C=O), 1614 (–C=N–), 1360 (C–CH_3); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 2.45

(3H, s, Ar–CH_3), 7.00 (1H, d, Ar–H_9, $J = 1.64$), 7.16 (1H dd, Ar–H_7, $J = 1.64$, 7.88 Hz), 7.94 (1H, d, Ar–H_6, $J = 7.88$ Hz), 8.17 (2H, broad s, –NH_2, D_2O exchangeable), 8.08 (1H, s, >NH); Anal. Calcd. for $C_{12}H_9N_3O_3$: C, 59.26, H, 3.73, N, 17.28, Found: C, 59.28, H, 3.71, N, 17.27.

4-amino-9-methyl-2H-chromeno[2,3-d]pyrimidine-2,5(1H)-dione (2f)

Solid brown; Yield 74%; m. p. 262–264°C; IR (KBr, ν, cm^{-1}): 3302 (–NH_2), 3135 (>NH), 1738 (>C=O pyrimidine ring), 1667 (chromone ring >C=O), 1625 (–C=N–), 1356 (C–CH_3); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 2.38 (3H, s, Ar–CH_3), 6.87 (1H, t, Ar–H_7, $J = 7.28$ Hz), 7.26 (1H dd, Ar–H_8, $J = 7.24$, 2.82 Hz), 7.75 (1H, dd, Ar–H_6, $J = 7.28$, 2.82 Hz), 8.33 (2H, broad s, –NH_2, D_2O exchangeable), 8.17 (1H, s, >NH); Anal. Calcd. for $C_{12}H_9N_3O_3$: C 59.26, H, 3.73, N, 17.28, Found: C, 59.25, H, 3.73, N, 17.28.

General Procedure for Synthesis of Compounds 3(a–f)

A mixture of **1(a–f)** (0.05 mol) and thiourea (0.05 mol) in absolute ethanol (20 mL) containing sodium ethoxide (0.05 mol) was refluxed for 8 hours. The reaction mixture was left to cool to room temperature, then poured into ice cold water (50 mL) and neutralized with dilute hydrochloric acid; the separated precipitates were filtered and recrystallized from methanol to give compounds **3(a–f)**.

4-amino-2-thioxo-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (3a)

Yellow, Yield 66%, m.p. > 300 °C; IR (KBr, ν, cm^{-1}): 3308 (–NH_2), 3144 (>NH), 1668 (>C=O), 1622 (–C=N–), 1338 (>C=S); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): δ 6.78–6.93 (2H, multiplet, Ar–H_9/H_7), 7.22 (1H, ddd, Ar–H_8, $J = 8.20$, 2.12, 1.78 Hz), 7.73 (1H, dd, Ar–H_6, $J = 8.20$, 1.78 Hz), 8.35 (2H, broad s, –NH_2, D_2O exchangeable), 8.56 (1H, s, >NH); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 115, 121, 125, 129 140, 158 (aromatic carbons), 175.1 (>C=O), 180.4 (>C=S), 167.5 (C–NH_2), 77 and 177 (C=C at pyrimidine ring); Anal. Calcd. for $C_{11}H_7N_3O_2S$: C, 53.87, H, 2.88, N, 17.13, S, 13.07; Found: C, 53.88, H, 2.89, N, 17.15, S, 13.09.

4-amino-7,9-dibromo-2-thioxo-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (3b)

Colorless, Yield 67%, m.p. 295- 297 °C; IR (KBr, ν, cm^{-1}): 3359 (–NH_2), 3100 (>NH), 1684 (>C=O), 1601 (–C=N–), 1325 (>C=S), 731 (C–Br); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 7.97 (1H, d, Ar–H_8, $J = 2.2$ Hz), 8.31 (1H d, Ar–H_6, $J = 2.2$ Hz), 8.56 (2H, br s, NH_2, D_2O exchangeable), 8.74 (1H, s, >NH); Anal. Calcd. for $C_{11}H_5N_3O_2SBr_2$: C, 32.38, H, 1.25, N, 10.43, S, 7.96; Found: C, 32.37, H, 1.24, N, 10.42, S, 8.00.

4-amino-7-nitro-2-thioxo-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (3c)

Brown, Yield 73%, m.p. > 300 °C; IR (KBr, ν, cm^{-1}): 3294 (–NH_2), 3138 (>NH), 1673 (>C=O), 1617 (–C=N–), 1345 (>C=S), 1525 (C–NO_2); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 7.54 (1H, d, Ar–H_9, $J = 9.04$ Hz), 8.19 (1H, dd, Ar–H_8, $J = 9.04$, 2.84 Hz), 8.70 (1H, dd, Ar–H_6, $J = 2.84$ Hz), 9.01 (2H, broad s–NH_2, D_2O exchangeable), 8.61 (1H, s, >NH); Anal. Calcd. for $C_{11}H_6N_4O_4S$: C, 45.48, H, 2.12, N, 19.28, S, 11.00, Found: C, 45.46, H, 2.15, N, 19.25, S, 11.00.

4-amino-7-chloro-2-thioxo-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (3d)

Brown, Yield 69%, m.p. 290–292°C; IR (KBr, ν, cm^{-1}): 3312 (–NH_2), 3153 (–NH), 1675 (>C=O), 1632 (–C=N–), 1329 (>C=S), 816 (C–Cl); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 7.29 (1H, d, Ar–H_9, $J = 8.8$ Hz), 7.42 (1H, d d, Ar–H_8, $J = 8.8$, 2.68 Hz), 7.88 (1H, d, Ar–H_6, $J = 2.68$ Hz), 8.28 (2H, broad s–NH_2, D_2O exchangeable), 8.17 (1H, s, >NH); Anal. Calcd. for $C_{11}H_6N_3O_2SCl$: C, 47.24, H, 2.16, N, 15.02, S, 11.46; Found: C, 47.26, H, 2.15, N, 15.05, S, 11.48.

4-amino-8-methyl-2-thioxo-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (3e)

Light Brown, Yield 71%, m.p. > 300°C; IR (KBr, ν, cm^{-1}): 3297 (–NH_2), 3115 (–NH), 1670 (>C=O), 1627 (–C=N–), 1331 (>C=S), 1367 (C–CH_3); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 2.37 (3H, s, Ar–CH_3), 7.17 (1H, d, Ar–H_9, $J = 1.62$ Hz) 7.29 (1H dd, Ar–H_7, $J = 1.62$ Hz, 7.88 Hz), 8.04 (1H, d, Ar–H_6, $J = 7.88$ Hz), 8.35 (2H, broad s, –NH_2, D_2O exchangeable), 8.21 (1H, s, >NH); Anal. Calcd. for $C_{12}H_9N_3O_2S$: C, 55.59, H, 3.50, N, 16.21, S, 12.37; Found: C, 55.50, H, 3.51, N, 16.24, S, 12.38.

4-amino-9-methyl-2-thioxo-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (3f)

Light Brown, Yield 70%, m.p. 291–293°C; IR (KBr, ν, cm^{-1}): 3307 (–NH_2), 3132 (–NH), 1668 (>C=O), 1621 (–C=N–), 1324 (>C=S), 1361 (C–CH_3); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 2.39 (3H, s, Ar–CH_3), 6.72 (1H, t, Ar–H_7, $J = 7.28$ Hz), 7.27 (1H, dd, Ar–H_8, $J = 7.24$, 2.81 Hz), 7.57 (1H, dd, Ar–H_6, $J = 7.28$, 2.81 Hz), 8.38 (2H, broad s, –NH_2, D_2O exchangeable), 8.25 (1H, s, >NH); Anal. Calcd. for $C_{12}H_9N_3O_2S$: C, 55.59, H 3.50, N, 16.21, S, 12.37; Found: C, 55.60, H 3.51, N, 16.24, S, 12.38.

General Procedure for Synthesis of Compounds 4(a–f)

A mixture of **1(a–f)** (0.05 mol) and guanidine nitrate (0.05 mol) in absolute ethanol (20 mL) containing sodium ethoxide (0.05 mol) was refluxed for 8 hr. The reaction mixture was left to cool to room temperature,

then poured into ice cold water (50 mL) and neutralized with dilute hydrochloric acid; the separated precipitates were filtered and recrystallized from methanol **4(a–f)**.

4-amino-2-imino-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (4a)

Solid beige; Yield 73%; m. p. 234–236°C; IR (KBr, ν, cm^{-1}): 3483, 3303 ($-NH_2$), 1665 (>C=O), 1609 (–C=N–); ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 6.62–6.87 (2H, multiplet, Ar–H_9/H_7), 7.07 (1H, ddd, Ar–H_8, J = 8.18, 2.14, 1.80 Hz), 7.48 (1H, dd, Ar–H_6, J = 8.18, 1.80 Hz), 8.47 (2H, broad s, $-NH_2$, D_2O exchangeable), 8.66 (1H, s, >NH); ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 118, 121, 124, 130, 137, 156 (aromatic carbons), 153.6 (>C=NH), 167.2 (C–NH_2), 176.5 (>C=O), 74 and 183 (C=C at pyrimidine ring); Anal. Calcd. for $C_{11}H_8N_4O_2$: C, 57.89, H, 3.53, N, 24.55; Found: C, 57.91, H, 3.51, N, 24.55.

4-amino-7,9-dibromo-2-imino-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (4b)

Solid beige (methanol); Yield 70%; m. p. 223–225°C; IR (KBr, ν, cm^{-1}): 3467, 3315 ($-NH_2$), 1669 (>C=O), 1621 (–C=N–), 728 (C–Br); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 7.87 (1H, d, Ar–H_8, J = 2.0 Hz), 8.23 (1H d, Ar–H_6, J = 2.0 Hz), 8.47 (2H, br s, NH_2, D_2O exchangeable), 8.68 (1H, s, >NH); Anal. Calcd. for $C_{11}H_6N_4O_2Br_2$: C, 34.23, H, 1.57, N, 14.51; Found: C, 34.25, H, 1.59, N, 14.51.

4-amino-7-nitro-2- imino-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (4c)

Solid beige (methanol); Yield 66%; m. p. 256–258°C; IR (KBr, ν, cm^{-1}): 3473, 3308, ($-NH_2$), 1673 (>C=O), 1616 (–C=N–), 1528 (C–NO_2); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 7.66 (1H, d, Ar–H_9, J=9.2 Hz), 8.36 (1H, dd, Ar–H_8, J = 9.2, 2.84 Hz), 8.69 (1H, dd, Ar–H_6, J = 2.84 Hz), 8.89 (2H, broad s–NH_2, D_2O exchangeable), 8.79 (2H, s–NH); Anal. Calcd. for $C_{11}H_7N_5O_4$: C, 48.36, H, 2.58, N, 25.63; Found: C, 48.38, H, 2.56, N, 25.64.

4-amino-7-chloro-2-imino-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (4d)

Solid yellow (methanol); yield 71%; m. p. 201–203°C; IR (KBr, ν, cm^{-1}): 3453, 3313 ($-NH_2$), 1667 (>C=O), 1613 (–C=N–), 810 (C–Cl); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 7.19 (1H, d, Ar–H_9, J = 8.8 Hz), 7.39 (1H, dd, Ar–H_8, J = 8.8, 2.68 Hz), 7.82 (1H, d, Ar–H_6, J = 2.68 Hz), 8.34 (2H, broad s–NH_2, D_2O exchangeable), 8.21 (1H, s, >NH); Anal. Calcd. for $C_{11}H_7N_2O_4Cl$: C, 50.30, H 2.59, N, 21.33; Found: C, 50.32, H 2.62, N, 21.33.

4-amino-2-imino-8-methyl-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (4e)

Solid light brown (methanol); yield 66%; m. p. 218–220°C; IR (KBr, ν, cm^{-1}): 3462, 3303 (–NH_2), 1669 (>C=O), 1623 (–C=N–), 1355 (C–CH_3); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 2.36 (3H, s, Ar–CH_3), 7.01(1H, d, Ar–H_9, $J = 1.63$ Hz) 7.24(1H dd, Ar–H_7, $J = 1.63$, 7.88 Hz), 7.86 (1H, d, Ar–H_6, $J = 7.88$ Hz), 8.26 (2H, broad s, –NH_2, D_2O exchangeable), 8.14 (1H, s, >NH); Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50, H, 4.16, N, 23.13; Found: C, 59.53, H, 4.13, N, 23.14.

4-amino-2-imino-9-methyl-1,2-dihydro-5H-chromeno[2,3-d]pyrimidin-5-one (4f)

Solid light brown (methanol); yield 68%; m. p. 207–209°C; IR (KBr, ν, cm^{-1}): 3482, 3299 (–NH_2), 1666 (>C=O), 1614 (–C=N–), 1358 (C–CH_3); ^{1}H-NMR: (400 MHz, $CDCl_3$:DMSO-d_6): 2.45 (3H, s, Ar–CH_3), 6.66 (1H, t, Ar–H_7, $J = 7.28$ Hz), 7.26 (1H, dd, Ar–H_8, $J = 7.24$, 2.80 Hz), 7.84 (1H, dd, Ar–H_6, $J = 7.28$, 2.80 Hz), 8.10 (2H, broad s, –NH_2, D_2O exchangeable), 8.08 (1H, s, >NH); Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50, H, 4.16, N, 23.13; Found: C, 59.51, H, 4.15, N, 23.15.

REFERENCES

1. Prasad, M.R.; Prashanth, J.; Shilpa, K.; Kishore, D.P. Synthesis and antibacterial activity of some novel triazolothienopyrimidines. *Chem. Pharm. Bull.* **2007**, 55(4), 557–560.
2. Testard, A.; Logé, C.; Léger, B.; Robert, J.M.; Lozach, O.; Blairvacq, M.; Méijer, L.; Thiéry, V.; Besson, T. Thiazolo[5,4-f]quinazolin-9-ones, inhibitors of glycogen synthase kinase-3. *Bioorg. Med. Chem. Lett.* **2006**, 16(13), 3419–3423.
3. Bhuiyan, M.M.H.; Rahman, K.M.M.; Hossain, M.I.; Naser, M.A.; Shumi, W. Fused pyrimidines. III. Synthesis and antimicrobial activity of some furopyrimidines and imidazopyrazolopyrimidine. *J. App. Sci. Res.* **2005**, 1(2), 218–222.
4. Vaghasia, S.J.; Shah, V.H. Microwave assisted synthesis and antimicrobial activity of some novel pyrimidine derivatives, *J. Serb. Chem. Soc.* **2007**, 72(2) 109–117.
5. Kim, S.Y.; Kim, D.J.; Yang, B.S.; Yoo, K.H. Synthesis and biological evaluation of furo[2,3-*d*]pyrimidines as Akt1 kinase inhibitors. *Bull. Kore. Chem. Soc.* **2007**, 28(7), 1114–1118.
6. Abdelrazek, F.M.; Michael, F.A.; El-Mahrouky, S.F. Synthesis and molluscicidal activity of some new substituted-furan and furo[2,3-*d*]pyrimidine derivatives. *Int. J. Phys. Sci.* **2007**, 2(8), 212–216.
7. Sondhi, S.M.; Jain, S.; Dinodia, M.; Shukla, R.; Raghubir, R. One pot synthesis of pyrimidine and bispyrimidine derivatives and their evaluation for anti-inflammatory and analgesic activities. *Bioorg. Med. Chem.* **2007**, 15(10), 3334–3344.
8. Gasse, C.; Douguet, D.; Huteau, V.; Marchal, G.; Lehmann, H.M.; Pochet, S. Substituted benzyl-pyrimidines targeting thymidine monophosphate kinase of Mycobacterium tuberculosis: synthesis and in vitro anti-mycobacterial activity. *Bioorg. Med. Chem.* **2008**, 16(11), 6075–6085.
9. Kifli, N.; Clercq, E.D.; Balzarini, J.; Simonsa, C. Novel imidazo[1,2-*c*]pyrimidine base-modified nucleosides: synthesis and antiviral evaluation. *Bioorg. Med. Chem.* **2004**, 12(15), 4245–4252.
10. Deshmukh, M.B.; Salunkhe, S.M.; Patil, D.R.; Anbhule, P.V. A novel and efficient one step synthesis of 2-amino-5-cyano-6-hydroxy-4-aryl pyrimidines and their anti-bacterial activity. *Eur. J. Med. Chem.* **2009**, 44(6), 2651–2654.
11. Agarwal, A.; Ramesh; Ashutosh; Goyal, N.; Chauhan, P.M.S.; Gupta, S. Dihydropyrido[2,3-d]pyrimidines as a new class of antileishmanial agents. *Bioorg. Med. Chem.* **2005**, 13(24), 6678–6684.

12. Rahaman, Sk. A.; Pasad, Y.R.; Kumar, P.; Kumar, B. Synthesis and anti-histaminic activity of some novel pyrimidines. *Saudi Pharma. J.* **2009**, 17(3), 255–258.
13. Gillespie, R.J.; Bamford, S.J.; Clay, A.; Gaur, S.; Haymes, T.; Jackson, P.S.; Jordan, A.M.; Klenke, B.; Leonardi, S.; Liu, J.; Mansell, H.L.; Ng, S.; Saadi, M.; Simmonite, H.; Stratton, G.C.; Todd, R.S.; Williamson, D.S.; Yule, I.A. Antagonists of the human A2A receptor. Part 6: further optimization of pyrimidine-4-carboxamides. *Bioorg. Med. Chem.* **2009**, 17(18), 6590–6605.
14. Shamroukh, A.H.; Rashad, A.E.; Sayed, H.H. Synthesis of some pyrazolo[3,4]pyrimidine derivatives for biological evaluation. *Phosph. Sulfur Silicon Relat. Elem.* **2005**, 180(10), 2347–2360.
15. Abdelhamid, A.O.; Elghandour, A.H.; Ahmed, S.A.; Zaki, Y.H. Reaction of hydrazonoyl halides 49 [1]: synthesis and antimicrobial activity of some new pyrimido[1,2-*b*][1,2,4,5]tetrazin-6-one, tetrazino[3,2-*b*]quinazolin-5-one, pyrimidino[1,2-*b*]1,2,4,5-tetrazin-5-one and triazolo[4,3-*a*]pyrimidine derivatives. *J. Sulfur Chem.* **2005**, 26(4–5), 405–410.
16. Rashad A.E.; and Ali, M.A. Synthesis and antiviral screening of some thieno[2,3-*d*]pyrimidine nucleosides. *Nucleosides Nucleotides Nucleic Acids.* **2006**, 25(1), 17–28.
17. Silva, A.M.S.; Pinto, D.C.G.A.; Cavaleiro, J.A.S.; Levai, A.; Patonay, T. Synthesis and reactivity of styrylchromones. *Arkivoc* **2004**, 7(vii), 106–123.
18. Levai, A. Synthesis of exocyclic α,β-unsaturated ketones. *Arkivoc* **2004**, 12(vii), 15–33.
19. Valenti, P.; Rampa, A.; Budriesi, R.; Bisi, A.; Chiarini, A. Coumarin 1,4-dihydropyridine derivatives. *Biorg. Med. Chem.* **2000**, 6(6), 803–810.
20. Kamdar, N.; Haveliwala, D.; Mistry, P.; Patel, S. Design, synthesis and in vitro evaluation of antitubercular and antimicrobial activity of some novel pyranopyrimidines. *Eur. J. Med. Chem.* 45(11), 5056–5063.
21. Kamdar, N.; Haveliwala, D.; Mistry, P.; Patel, S. Synthesis and evaluation of in vitro antitubercular activity and antimicrobial activity of some novel 4H-chromeno[2,3-*d*]pyrimidine via 2-amino-4-phenyl-4*H*-chromene-3-carbonitriles. *Med. Chem. Res.* **2011**, 20(7), 854–864.
22. Mistry, P.; Kamdar, N.; Haveliwala, D.; Patel, S. Concise and facile synthesis of novel pyrano[2,3-*d*]pyrimidine-7-one/thione derivatives as in vitro antimicrobial and antitubercular agents. *Lett. Drug Des. Discov.* **2011**, 8(8), 750–757.
23. Haveliwala, D.; Kamdar, N.; Mistry, P.; Patel, S. Synthesis of novel thiopyrimidines: an investigation of anti-tubercular and antimicrobial activity. *J. Sulfur Chem.* **2011**, 32(5), 451–162.
24. Brown, R.E.; Lustgarten, D.M. Substituted 2-amino chromones and process for the preparation thereof. U. S. Patent 3,932,466' January **1976.**
25. Collee, J.G.; Duguid, J.P.; Fraser, A.G.; Marmion, B.P. in *Practical Medicinal Microbiology*, eds., Mackie and McCartney, 13th ed., Churchill Livingstone, **1989**, p. 87.
26. Siddiqi, S. *Clinical Microbiology Handbook*, ASM Press, Washington, DC, **1992**.