Med Chem Res (2011) 20:854–864
DOI 10.1007/s00044-010-9399-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of in vitro antitubercular activity and antimicrobial activity of some novel 4*H*-chromeno[2,3-*d*] pyrimidine via 2-amino-4-phenyl-4*H*-chromene-3-carbonitriles

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Received: 21 February 2010 / Accepted: 22 July 2010 / Published online: 24 August 2010

Abstract Novel 5-phenyl-1,5-dihydro-2*H*-chromeno[2,3-*d*]pyrimidine-2,4(3*H*)-dithiones, 5-phenyl-3,5-dihydro-4*H*-chromeno[2,3-*d*]pyrimidin-4-ones, 7-phenyl-7,9-dihydro-8*H* pyrimido[5′,4′:5,6]pyrano[3,2-*h*]quinolin-8-ones, and 4-amino-5-phenyl-1,5-dihydro-2*H*-chromeno[2,3-*d*]pyrimidine-2-thiones were synthesized form 2-amino-4-phenyl-4*H*-chromene-3-carbonitrile. The newly synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectra, and Elemental analysis. The compounds were evaluated for their in vitro antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and antibacterial activity against *Staphylococcus Aureus* [ATCC-25923] and *Streptococcus pyogenes* [MTCC-443] as Gram-positive, *Escherichia coli* [ATCC-25922], and *Pseudomonas aeruginosa* [MTCC-441] as Gram-negative bacterial strains and antifungal activity against *Aspergillus niger* [MTCC-282]. Some of these derivatives exhibited pronounced antitubercular and antimicrobial activities.

Keywords 4*H*-chromeno[2,3-*d*]pyrimidines · Antitubercular activity · Antimicrobial activity

Saurabh K. Patel is a research guide.

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Introduction

Mycobacterium tuberculosis, the leading causative agent of tuberculosis (TB), is responsible for the morbidity and mortality of a large population worldwide, which is the leading cause of death (Dye *et al.* 1999; Frieden *et al.* 2003). According to the facts published by WHO, 9.4 million new TB cases were reported, and 1.8 million people died from TB in 2008. (Tuberculosis Facts, 2009). It is also estimated that between 2002 and 2020, approximately a billion people will be newly infected, and 36 million will die from TB. This is also a leading cause of death among people who are HIV-positive (13% of AIDS deaths worldwide) (Smith and Moss, 1994). The synergy with the HIV-1 epidemic led TB to increase, in particular, in some parts of the world, such as african region, where a large part of new TB cases were attributable to HIV (Human Immunodeficiency Virus) coinfection (Espinal, 2003). Furthermore, the emergence of *Mycobacterium tuberculosis* strains resistant to all of the first line drugs lead to the multi drug-resistant TB (MDR-TB) (Smith and Moss, 1994). Resistance to Isoniazid, Rifampin, any Fluoroquinolone, and at least one of three injectable second-line drugs (i.e., Amikacin, Kanamycin or Capreomycin) is causing serious concern in some countries, as patients could become virtually untreatable because microbes have resisted prophylaxis or therapy longer than any other form of life. In recent decades, problems of multidrug-resistant micro organisms have reached an alarming level in many countries around the world. Resistance to a number of antimicrobial agents (β-lactam antibiotics, Macrolides, Quinolones, and Vancomycin) among a variety of clinically significant species of bacteria is becoming an increasingly important global problem. Also a number of recent clinical reports describe the

increasing occurrence of Methicillin Resistant Staphylococcus Aureus (MRSA) which is the most disturbing cause of nosocomial infections in the developed countries (Francis *et al.* 2005; Kruszewska *et al.* 2004). Infections caused by these micro organisms pose a serious challenge to the medicinal community, and the need for an effective therapy has led to the search for novel anti-microbial agents. In particular, increasing drug resistance among Gram-positive bacteria such as *Staphylococci, Enterococci*, and *Streptococci* are concerns of significant health matter (Chu *et al.* 1996). This has stimulated the scientists for the development of novel molecules for combating these illnesses.

Chromenes constitute a group among the major classes of naturally occurring compounds, and interest in their chemistry continues unabated because of their usefulness as biologically active agents (Ellis, 1977). Some of the biological activities attributed to such derivatives include anticoagulant, spasmolytic (Green *et al.* 1995), cytotoxic (anticancer) (Doshi *et al.* 2007), antiananaphylactic (Bonsignore *et al.* 1993), hypoglycemic (Dell and Williams, 1995), hypotensive (Ohnishi *et al.* 1982), antimicrobial (Eid *et al.* 2004), antifungal (Bedair *et al.* 2001), and antioxidant activity (Pietta, 2000). Due to their abundance in plants and their low mammalian toxicity, these derivatives are present in large amounts in the diet of humans (Hoult *et al.* 1994).

The pyrimidine ring, an integral part of various natural products of therapeutic importance, plays a pivotal role in catalyzing both biological and chemical reactions. Pyrimidine nucleotide, the prosthetic group of many enzymes, is involved in various oxidation–reduction processes in living organisms. On the other hand, pyrimidine derivatives constitute an important class of compounds possessing diverse type of pharmacological activities viz. antimalarial (Agarwal *et al.* 2005; Stocks *et al.* 2002); antifilerial (Katiyar *et al.* 2005); fungicidal (Ren *et al.* 2007); antibacterial (Ali *et al.* 2003; Gangjee *et al.* 1997); antitoxoplasma (Saudi *et al.* 2008); and antileismanial (Sunduru *et al.* 2006; Torrence *et al.* 2006).

This study aims to develop new simpler methods for the synthesis of functionally substituted heterocycles with anticipated broad spectrum of biological activity. Consequently, we have examined several synthetic strategies to facilitate alteration of these biologically interesting scaffolds. While our primary research was based on synthetic approaches for the facile fusion of the pyrimidines at the pyran nucleus of chromene, we extended it to include the investigation of the pharmacological aspects of the newly synthesized heterocycles based on the finding that some heterocycles can achieve pharmacological activity. The combination of these approaches would obviously allow access to pharmaceutical interest-customized molecules.

Results and discussion

Chemistry

Pyrimidine and chromene derivatives have recently received considerable attention due to their pharmacological and biological interest. In this study, we explore the synthetic potential of chromene-fused pyrimidine from 2-amino-4-phenyl-4*H*-substituted chromene-3-carbonitrile **4(a–e)** via cyclization with carbon disulphide **5(a-e)**, formic acid **6(a–e)**, urea **7(a–e)**, and thiourea **8(a–e)** under reflux condition (Table 1).

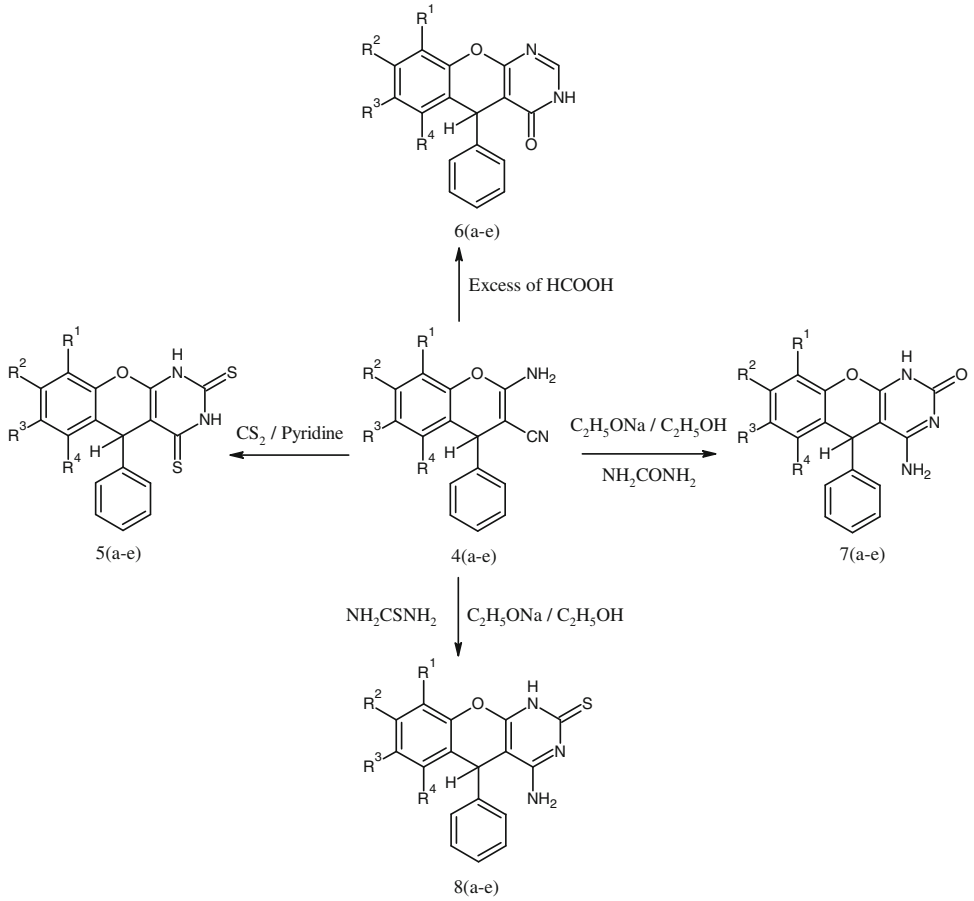
The intermediate compounds 2-amino-4-phenyl-4*H*-substitutedchromene-3-carbonitrile **4(a–e)** were prepared by one-pot three-component reaction of various phenol **1(a-e)**, malononitrile **(2)**, benzaldehyde **(3)**, and catalytic amount of cetyltrimethylammoniumchloride in aqueous media refluxed with continuous stirring for 6 h. (Roberto *et al.* 2001). The IR spectrum of these compounds showed in each case, absorption bands at ν (cm^{-1}): 3450–3200 cm^{-1} and 2200 cm^{-1} corresponding to $-NH_2$, and –CN groups, respectively. The ^{1}H-NMR spectra of **4(a–e)** revealed a sharp singlet pyran proton at 4.83 δ ppm, a broad singlet at 6.8 δ ppm assigned to the amine protons, and a multiplet at 7.3–8.1 δ ppm assigned to aromatic protons.

The target compounds 5-phenyl-1,5-dihydro-2*H*-substitutedchromeno[2,3-*d*]pyrimidine-2,4(3*H*)-dithiones **5(a–e)** were prepared by reacting 2-amino-4-(substitutedphenyl)-4*H*-(substitutedchromene)-3-carbonitriles **4(a–e)** with carbon disulphide in the presence of dry pyridine (Scheme 1). Spectroscopic methods confirm the structure of the new compounds. IR spectra of **5(a–e)** exhibited characteristic bands of –NH stretching vibration near 3400–3300 cm^{-1} and bending vibration near 1600 cm^{-1}, respectively. The band near 1310 cm^{-1} confirms the >C=S group in synthesized compounds. The ^{1}H-NMR spectra of **5(a–e)** showing a singlet at 4.81 δ ppm of methylene proton (–CH) and another singlet near at 8.60 δ ppm confirms the

Table 1 Structures of 4*H*-chromeno[2,3-*d*]pyrimidine derivatives for R^1, R^2, R^3, and R^4

1,4,5,6,7,8	R^1	R^2	R^3	R^4
a	–H	–H	–H	–H
b	–H	–OH	–H	–H
c	–CH=CHCH=CH–-(α-naphthol)		–H	–H
d	–H	–H	–CH=CHCH=CH–-(β-naphthol)	
e	–CH=CHCH=N–-(Quinoline)		–H	–H

Scheme 1 Synthetic route of compounds **5**, **6**, **7**, **8** (**a**–**e**)



secondary amine protons (–NH pyrimidine) common for all compounds at expected regions.

In addition, the treatment of **4(a–e)** with excess of formic acid afforded the 5-phenyl-3,5-dihydro-4*H*-substitutedchromeno[2,3-*d*]pyrimidin-4-ones **6(a–e)** (Scheme 1). The structural assignments of these compounds were based on their elemental analyses and spectral data. Their IR spectra showed absorption bands near 3420–3300 cm^{-1}, assignable to the –NH-stretching vibrations and a characteristic band near 3372 cm^{-1}, corresponding to a –C=N linkage at 1610 cm^{-1}. Their ^{1}H-NMR spectra showed a characteristic signal near 7.90 δ ppm, assignable to the –NH protons of pyrimidine ring.

The condensations of compounds **4(a–e)** with urea and thiourea in the presence of sodium ethoxide at reflux temperature afforded the corresponding 4-amino-5-phenyl-1,5-dihydro-2*H*-chromeno[2,3-*d*]pyrimidin-2-one **7(a–e)** and 4-amino-5-phenyl-1,5-dihydro-2*H*-chromeno[2,3-*d*]pyrimidine-2-thione **8(a–e)** derivatives, respectively (Scheme 1). The IR spectra of the products confirmed the disappearance of the nitrile absorption bands. The ^{1}H-NMR spectra also showed in each case a signal at 8.15 δ ppm, corresponding to the pyrimidine proton. The confirmations of the all the compounds were based on their elemental analysis and spectral data.

Biological activity

All the compounds prepared herein were screened for their potential biological activities such as antitubercular, antibacterial, and antifungal activities. Antitubercular activity was carried out against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC-27294). Bacterial strains, *Staphylococcus aureus* [ATCC-25923] and *Streptococcus pyogenes* [MTCC-443], as Gram positive; *Escherichia coli* [ATCC-25922], and *Pseudomonas aeruginosa* [MTCC-441] as Gram negative; and *Aspergillus niger* [MTCC-282] as fungal strains, were used for the in vitro studies.

The MIC values for the in vitro antitubercular studies of the compounds **5**, **6**, **7**, **8**, **(a–e)** and the standard are represented in Table 2. The results imply that compounds **5d**, **6c**, **6d**, **7b**, and **7e** show better activity against *Mycobacterium tuberculosis* $H_{37}Rv$; predominantly pyrimidin-4-one **6b** showed the highest activity **(62.5 µg/ml)** against mycobacteria. Encouraging activity was found for the chromeno[2,3-*d*]

Table 2 The in vitro antitubercular activity of the synthesized compounds against *M. tuberculosis* $H_{37}Rv$

Comp. no	MIC (μg/ml)	Comp. no	MIC (μg/ml)
5a	500	7a	500
5b	200	7b	125
5c	500	7c	1000
5d	100	7d	250
5e	200	7e	125
6a	500	8a	500
6b	62.5	8b	500
6c	125	8c	1000
6d	125	8d	1000
6e	500	8e	500
		Rifampicin	40

pyrimidine-dithione **5(a–e)**, chromeno[2,3-*d*]pyrimidin-4-ones **6(a–e)**, and chromeno[2,3-*d*]pyrimidin-2-one **7(a–e)** derivatives; however, the chromeno[2,3-*d*]pyrimidine-2-thione **8(a–e)** derivatives did not show any considerable activity.

MIC values for the in vitro antibacterial studies of the compounds **5**, **6**, **7**, **8 (a–e)** and the standard are represented in Table 3. The screening results indicated that the most of the synthesized compounds showed significant antimicrobial activity from moderate-to-good activity. Compounds **5b**, **5e**, **6c**, and **7d** were found to be more active than other compounds against *Streptococcus pyogenes* and *Staphylococcus aureus* with compared to Ampicilin except a moderate-to-poor potency against *Aspergillus niger*. Compounds pyrimidine-2-thione **8(e)** and pyrimidin-2-one **7(a)** have been found to be more active than the other compounds against *Escherichia coli* and *Pseudomonas aeruginosa*.

MIC values for the in vitro antifungal studies of the compounds **5**, **6**, **7**, **8 (a-e)** and the standard are represented in Table 3. Among the tested compounds, more interesting activities were found for compounds pyrimidine-2-thione **8a** and **8b** against *Aspergillus niger* compared to Nystatin and Greseofulvin, while the compound **8a** and **8b** were promising with MIC values of 125 μg/ml, respectively, against the fungi.

Table 3 The antimicrobial activity (MIC, μg/ml) of the synthesized compounds

Comp. no	The minimum inhibitory concentration (MIC)				
	Gram –ve		Gram +ve		Fungal
	Escherichia coli [ATCC-25922]	*Pseudomonas aeruginosa* [MTCC-441]	*Streptococcus pyogenes* [MTCC-443]	*Staphylococcus aureus* [ATCC-25923]	*Aspergillus niger* [MTCC-282]
5a	250	250	500	500	1000
5b	125	250	62.5	62.5	1000
5c	250	250	250	125	500
5d	250	125	250	125	500
5e	125	125	62.5	62.5	500
6a	250	250	500	250	500
6b	62.5	250	500	500	1000
6c	125	250	62.5	62.5	1000
6d	125	125	125	125	500
6e	250	125	250	125	500
7a	62.5	62.5	125	125	500
7b	250	125	250	125	250
7c	500	250	125	125	500
7d	250	500	62.5	62.5	250
7e	500	500	125	125	250
8a	125	125	250	250	125
8b	250	125	250	500	125
8c	500	125	500	500	1000
8d	500	500	500	250	1000
8e	62.5	62.5	250	125	500
Ciprofloxacin	25	25	50	50	–
Ampicilin	100	100	250	100	–
Nystatin	–	–	–	–	100
Greseofulvin	–	–	–	–	100

The preliminary in vitro antitubercular, antibacterial, and antifungal screening results show that the novel chromeno[2,3-*d*]pyrimidine derivatives reported here have the potential to act as antitubercular, antibacterial, and antifungal agents. The possible improvements in the activity can be further achieved by slight modifications in the substituents on the basic chromene nucleus. In view of the above findings, and to identify new candidates that may be of value in designing new, selective, less toxic antitubercular, and antimicrobial agents which might serve as new templates, we report the development of potent therapeutics in the field of chromeno[2,3-*d*]pyrimidine.

Experimental

General

Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded on a Hitachi 270-50 double beam spectrophotometer, using KBr pallets. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance II 400 spectrometer using TMS as the internal standard in $CDCl_3$ as solvent. The chemical shifts are expressed as δ values (ppm). Electron impact MS spectra were obtained on a Jeol SX-102 at 70 eV. The progress of the reaction was monitored by silica gel 60 F254 (Merck, 0.25 mm thick)-coated TLC plates (Mobile phase: toluene/ethyl acetate [7.5:2.5]), and visualization was accomplished by UV light or iodine vapour. Column chromatography was performed on silica gel (Merck N° 9385) using suitable mixture of solvents as eluents. The reagent grade chemicals were purchased from the commercial sources and purified by either distillation or recrystallization.

General preparation of 2-amino-4-phenyl-4H-substitutedchromene-3-carbonitrile 4(a–e)

2-amino-4-phenyl-4*H*-substitutedchromene-3-carbonitrile **4(a–e)** were synthesized as described by Roberto Ballini *et al.* (2001). A mixture of phenol **1(a–e)** (2 mmol), malononitrile **(2)** (2 mmol), benzaldehyde **(3)** (2 mmol), and catalytic amount of cetyltrimethylammoniumchloride in water (50 ml) was refluxed with continuous stirring for 6 h. The resulting mixture was cooled to room temperature; the precipitated product was collected by filtration, washed with water and dried. The crude product was purified by crystallization from absolute ethanol and by column chromatography (silica gel) using hexane:ethylacetate (3:1) as the eluent to afford the corresponding product **4(a–e).**

2-Amino-4-phenyl-4H-chromene-3-carbonitrile (4a) 210°C (ethanol), White solid, Yield 80%, IR (KBr) ν (cm^{-1}): 3420 (–NH_2), 2200 (–CN), 1254 (C–O–C at pyran ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 5.16 (s, 1H, pyran –CH), 6.48–7.22 (m, 9H, Ar–H.), 6.80 (s, 2H, –NH_2); m/z: 248.09 (100.0%), 249.10 (17.5%), 250.10 (1.8%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 15.7 and 131.0 (C=C at pyran ring), 180.4 (C–NH_2), 30.68 (–CH 1H at pyran ring), 80.20 (C–CN), 110.22 (C=N), 119.4, 120.2, 129.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2 (10 aromatic carbons); Anal. Calcd. for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.28; O, 6.44. Found: C, 77.36; H, 4.88; N, 11.30; O, 6.40.

2-Amino-7-hydroxy-4-phenyl-4H-chromene-3-carbonitrile (4b) 218°C (ethanol), White solid, Yield 80%, IR (KBr) ν (cm^{-1}): 3340 (–NH_2 stretching), 3220 (-OH), 2200 (-CN) 1646, 1650 (-NH_2 bending), 1442, 1254 (C–O-C at pyran ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 5.14 (s, 1H pyran), 6.80 (s, 2H, -NH_2), 7.02-7.66 (m, 9H aromatic H and OH); m/z: 264.09 (100.0%), 265.09 (18.1%), 266.10 (1.4%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 151.25 (C–OH), 145 and 121 (C=C at pyran ring), 177.4 (C-NH_2), 29.68 (-CH 1H at pyran ring), 79.5 (C–CN), 111.22 (C=N), 114.2, 114.5, 119.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4 (9 aromatic carbons); Anal. Calcd. for $C_{16}H_{12}N_2O_2$: C, 72.72; H, 4.58; N, 10.60; O, 12.11. Found: C, 72.76; H, 4.60; N, 10.58; O, 12.9.

2-Amino-4-phenyl-4H-benzo[h]chromene-3-carbonitrile (4c) 215°C (ethanol), White solid, Yield 90%, IR (KBr) ν (cm^{-1}): 3200, 2200; ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 5.13 (s, 1H, pyran), 6.08 (s 2H, –NH_2), 7.25–7.66 (m, 9H, aromatic); m/z: 298.11 (100.0%), 299.11 (22.4%), 300.12 (2.3%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 151.0 and 122.0 (C=C at pyran ring), 178.4 (C–NH_2), 30.1 (–CH 1H at pyran ring), 79.4 (C–CN), 109.20 (C=N), 118.0, 119.5, 121.2, 123.5, 125.9, 127.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2, 140.2 (14 aromatic carbons); Anal. Calcd. for $C_{20}H_{14}N_2O$: C, 80.52; H, 4.73; N, 9.39; O, 5.36. Found: C, 80.55; H, 4.74; N, 9.40; O, 5.41.

3-Amino-1-phenyl-1H-benzo[f]chromene-2-carbonitrile (4d) 278°C (ethanol), Yellow solid, Yield 74%, IR (KBr) ν (cm^{-1}): 3200, 2190; ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 5.31 (s, 1H, pyran –CH), 7.2–8.10 (m, 11H, Ar–H.), 6.9 (s, 2H, –NH_2); m/z: 298.11 (100.0%), 299.11 (22.4%), 300.12 (2.3%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 151.0 and 122.0 (C=C at pyran ring), 178.4 (C–NH_2), 30.1 (–CH 1H at pyran ring), 79.4 (C–CN), 109.20 (C=N), 118.0, 119.5, 121.2, 123.5, 125.9, 127.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2, 140.2 (14 aromatic

carbons); Anal. Calcd. for $C_{20}H_{14}N_2O$: C, 80.52; H, 4.73; N, 9.39; O, 5.36. Found: C, 80.55; H, 4.74; N, 9.40; O, 5.40.

2-Amino-4-phenyl-4H-pyrano[3,2-h]quinoline-3-carbonitrile (4e) 270°C (ethanol), Yellow solid, Yield 85%, IR (KBr) ν (cm^{-1}): 3200, 2200; ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 5.18 (s, 1H pyran -CH), 7.34–8.87 (m, 10H, Ar–H),. 6.9 (s, 2H, -NH_2), 7.30–7.56 (m, 3H, Ar–H at pyridine ring); m/z: 299.11 (100.0%), 300.11 (20.7%), 301.11 (2.4%), 300.10 (1.1%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 151.0 and 122.0 (C=C at pyran ring), 178.4 (C-NH_2), 30.1 (CH 1H at pyran ring), 79.4 (C–CN), 109.20 (C=N), 45.0 (-CH at pyran ring), 120.1, 126.2, 133.9, 135.0 (carbons at pyridine ring), 150.0 (C=N at pyridine ring), 129.6, 128.2, 129.8, 130.9, 132.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{19}H_{13}N_3O$: C, 76.24; H, 4.38; N, 14.04; O, 5.35. Found: C, 76.25; H, 4.40; N, 14.06; O, 5.39.

General preparation of 5-phenyl-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-2,4(3H)-dithione 5(a–e)

Mixture of Compound **4(a–e)** (2 mmol) and carbon disulphide (2 mmol) in pyridine (10 ml) were refluxed on water bath for 6 h, (monitored by TLC toluene:methanol; (7:3)). After completion of the reaction, the reaction mixture was cooled at room temperature, then poured into ice cold water, and neutralized with hydrochloricacid (1:1). The precipitated product was filtered off, washed and recrystallized from ethanol, and purified by column chromatography (silica gel) using hexane and ethylacetate (1:4) as the eluent to afford the corresponding product **5(a–e)**.

5-Phenyl-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-2,4(3H)-dithione (5a) 162–163°C (ethanol), Yellow solid, Yield 74%, IR (KBr) ν (cm^{-1}): 3337 (–NH stretching), 1508, 1646, 1622 (–NH bending), 1442, 1254 (C–O–C at pyran ring), 1307 (>C=S pyrimidine dithione ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.71 (s, 1H, pyran –CH), 6.48-7.22 (m, 9H, Ar–H.), 8.56 (s, 1H, –NH); m/z: 324.04 (100.0%), 325.04 (20.8%), 326.03 (9.0%), 327.04 (1.7%), 326.05 (1.6%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 150.7 and 166.0 (C–O–C at pyran ring), 45.0 (–CH at pyran ring), 177.0 and 194.9 (>C=S at pyrimidine dithione ring), 118.0, 119.4, 120.2, 129.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{17}H_{12}N_2OS_2$: C, 62.90; H, 3.70; N, 8.65. Found: C, 62.96; H, 3.74; N, 8.62.

8-Hydroxy-5-phenyl-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-2,4(3H)-dithione (5b) 180–182°C (ethanol), Yellow solid, Yield 68%, IR (KBr) ν (cm^{-1}): 3428, 3299 (–NH stretching), 1510, 1637, 1625 (–NH bending), 3200 (–OH), 1448, 1246 (C–O–C at pyran ring), 1315 (>C=S at pyrimidine dithione ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.74 (s, 1H, pyran –CH), 6.58–7.85 (m, 8H, Ar–H.), 8.58 (s, 1H, –NH), 8.98 (s, 1H, –OH); m/z: 340.03 (100.0%), 341.04 (18.6%), 342.03 (9.2%), 342.04 (2.3%), 341.03 (2.3%), 343.03 (1.8%). ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 151.1 and 168.0 (C–O–C at pyran ring), 160.0 (C–OH), 43.1 (–CH, at pyran ring), 177.5 and 194.7 (>C=S at pyrimidine dithione ring), 119.4, 120.2, 129.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2 (aromatic carbons); Anal. Calcd. For $C_{17}H_{12}N_2O_2S_2$: C, 60.02; H, 3.50; N, 8.20. Found: C, 59.98; H, 3.55; N, 8.24.

7-Phenyl-7H-benzo[7,8]chromeno[2,3-d]pyrimidine-8,10(9H,11H)-dithione (5c) 173–174°C (ethanol), Yellow solid, Yield 66%, IR (KBr) ν (cm^{-1}): 3343 (–NH stretching), 1513, 1648, 1620 (–NH Bending), 1434, 1249 (C–O–C at pyran ring), 1303 (>C=S at pyrimidine dithione ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ: 4.77 (s, 1H, pyran –CH), 7.01–7.89 (m, 10H Ar–H.), 8.54 (s, 1H, –NH); m/z: 374.05 (100.0%), 375.06 (22.9%), 376.05 (9.1%), 376.06 (3.2%), 375.05 (2.3%), 377.05 (2.2%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 152.1 and 170.0 (C–O–C at pyran ring) 44.9 (–CH, at pyran ring), 177.5 and 194.7 (>C=S at pyrimidine dithione ring), 119.4, 120.2, 123.9, 155, 129.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{21}H_{14}N_2OS_2$: C, 67.30; H, 3.78; N, 7.45. Found: C, 67.36; H, 3.83; N, 7.49.

12-Phenyl-8,12-dihydro-9H-benzo[5,6]chromeno[2,3-d]pyrimidine-9,11(10H)-dithione (5d) 185–187°C (ethanol), Cream solid, Yield 67%, IR (KBr) ν (cm^{-1}): 3339 (–NH stretching), 1503 1639, 1627 (–NH Bending), 1436, 1266 (C–O–C at pyran ring), 1292 (>C=S at pyrimidine dithione ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.74 (s, 1H, pyran –CH), 7.24-8.27 (m, 11H Ar–H), 8.61 (s, 1H, –NH); m/z: 374.05 (100.0%), 375.06 (22.9%), 376.05 (9.1%), 376.06 (3.2%), 375.05 (2.3%), 377.05 (2.2%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 152.1 and 170.0 (C–O–C at pyran ring) 44.9 (–CH, at pyran ring), 177.5 and 194.7 (>C=S at pyrimidine dithione ring), 119.6, 120.3, 123.9, 125.0, 129.1, 128.4, 129.4, 130.0, 132.2, 134.7, 135.5, 136.4 (aromatic carbons); Anal. Calcd. for $C_{21}H_{14}N_2OS_2$: C, 67.33; H, 3.80; N, 7.45. Found: C, 67.33; H, 3.77; N, 7.51.

7-Phenyl-7H-pyrimido[5′,4′:5,6]pyrano[3,2-h]quinoline-8,10(9H,11H) dithione (5e) 192–194°C(ethanol), Cream solid, Yield 61%, IR (KBr) ν (cm^{-1}): 3329 (–NH stretching), 1519, 1640, 1631 (–NH Bending), 1447, 1249 (C–O–C at pyran ring), 1313 (>C=S at pyrimidine dithione ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.78 (s, 1H pyran –CH),

7.34-8.87 (m, 10H, Ar–H), 8.26 (s, 1H, –NH), 7.30–7.56 (m, 3H, Ar–H at pyridine ring); m/z: 375.05 (100.0%), 376.05 (24.4%), 377.05 (9.9%), 377.06 (2.3%), 378.05 (2.1%). ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 151.1 and 169.0 (C–O–C at pyran ring) 44.0 (CH at pyran ring), 178.2 and 194.7 (>C=S at pyrimidine dithione ring), 120.1, 126.2, 133.9, 135.0 (carbons at pyridine ring), 150.0 (C=N at pyridine ring), 129.6, 128.2, 129.8, 130.9, 132.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{20}H_{13}N_3OS_2$: C, 64.00; H, 3.45; N, 11.15. Found: C, 63.97; H, 3.49; N, 11.19.

General preparation of 5-phenyl-3,5-dihydro-4H-substitutedchromeno[2,3-d]pyrimidin-4-ones 6(a–e)

Mixture of Compound **4(a–e)** (2 mmol) and excess of formic acid were refluxed on sand bath for 12 h (monitored by TLC toluene:methanol; (7:3)) under inert condition. After completion of the reaction, the solvent was distilled off under reduced pressure, and the solid thus obtained was purified by recrystallization from absolute ethanol and purified by column chromatography (silica gel) using benzene and ethylacetate (1:3) as the eluent to give compounds **6(a–e).**

5-Phenyl-3,5-dihydro-4H-chromeno[2,3-d]pyrimidin-4-one (6a) 154–156°C (ethanol), Cream solid, Yield 71%, IR (KBr) ν (cm^{-1}): 3372 (–NH stretching), 1690 (>C=O), 1610 (C=N at pyrimidine ring), 1515 (–NH bending), 1441, 1256 (C–O–C at pyran ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.74 (s, 1H, pyran –CH), 7.92 (d, 1H, –NH at pyrimidine ring), 6.83–7.37 (m, 9H, Ar–H), 7.64 (d, 1H, –CH at pyrimidine ring); m/z: 276.09 (100.0%), 277.09 (19.2%), 278.10 (1.6%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 155.5 and 152.9 (C–O–C at pyran ring), 39.3 (–CH at pyran ring), 150.2 (C=N at pyrimidine ring), 162.1.0 (>C=O), 106.1, 108.5, 110.5, 113.6, 120.2, 123.3, 125.9, 128.4, 129.2,130.3 (aromatic carbons); Anal. Calcd. for $C_{17}H_{12}N_2O_2$: C, 73.90; H, 4.38; N, 10.14; Found: C, 73.86; H, 4.33; N, 10.18.

8-Hydroxy-5-phenyl-3,5-dihydro-4H-chromeno[2,3-d]pyrimidin-4-one (6b) 169–172°C (ethanol), Yellow solid, Yield 77%, IR (KBr) ν (cm^{-1}): 3381 (–NH stretching), 1697 (>C=O stretching), 1616 (C=N at pyrimidine ring), 1511 (–NH bending), 1457, 1239 (C–O–C at pyran ring), 3200 (–OH); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.81 (s, 1H, pyran –CH), 8.12 (d, 1H, –NH at pyrimidine ring), 6.77–7.30 (m, 8H, Ar–H), 7.50 (d, 1H, –CH at pyrimidine ring), 9.0 (s, 1H, –OH); m/z: 292.08 (100.0%), 293.09 (18.6%), 294.09 (2.4%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 156.5 and 154.5 (C–O–C at pyran ring), 40.0 (–CH, at pyran ring), 150.6 (C=N at pyrimidine ring), 161.3 (>C=O), 162.6 (C–OH), 101.1, 108.5, 111.5, 123.6, 123.2, 125.3, 125.9, 128.4, 129.2, 130.3, 131.0 (aromatic carbons); Anal. Calcd. for $C_{17}H_{12}N_2O_3$: C, 69.86; H, 4.14; N, 9.58; Found: C, 69.82; H, 4.18; N, 9.64.

7-Phenyl-7,9-dihydro-8H-benzo[7,8]chromeno[2,3-d]pyrimidin-8-one (6c) Brown solid, 163–166°C (ethanol), Yield 64%, IR (KBr) ν (cm^{-1}): 3364 (–NH stretching), 1705 (>C=O), 1602 (C=N at pyrimidine ring), 1523 (–NH bending), 1450, 1266 (C–O–C at pyran ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.80 (s, 1H, pyran –CH), 8.10 (d, 1H, –NH at pyrimidine ring), 6.50 to 7.25 (m, 11H, Ar–H), 7.55 (d, 1H, –CH at pyrimidine ring); m/z: 326.11 (100.0%), 327.11 (23.0%), 328.11 (3.1%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 144.0 and 151.0, (C–O–C at pyran ring) 37.9 (–CH at pyran ring), 151.8 (C=N at pyrimidine ring), 161.2 (>C=O), 118.2, 120.7, 121.8, 122.6, 123.2, 125.8, 126.9, 127.4, 128.6, 129.9, 130.4, 130.2 (aromatic carbons); Anal. Calcd. for $C_{21}H_{14}N_2O_2$: C, 77.29; H, 4.32; N, 8.58; Found: C, 77.37; H, 4.39; N, 8.51.

12-Phenyl-10,12-dihydro-11H-benzo[5,6]chromeno[2,3-d]pyrimidin-11-one (6d) 202–204°C (ethanol), Brown solid, Yield 68%, IR (KBr) ν (cm^{-1}): 3389 (–NH stretching), 1725 (>C=O), 1618 (C=N at pyrimidine ring), 1522 (–NH bending), 1460, 1221 (C–O–C at pyran ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.85 (s, 1H, pyran –CH), 8.15 (d, 1H, –NH at pyrimidine ring), 6.77–7.45 (m, 11H, Ar–H), 7.52 (d, 1H, –CH at pyrimidine ring); m/z: 326.11 (100.0%), 327.11 (23.0%), 328.11 (3.1%). ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 145.0 and 151.0 (C–O–C at pyran ring) 37.6 (–CH at pyran ring), 151.2 (C=N at pyrimidine ring), 161.4 (>C=O), 118.2, 120.7, 121.8, 122.6, 123.2, 125.8, 126.9, 127.4, 128.6, 129.9, 130.4, 130.2 (aromatic carbons). Anal. Calcd. for $C_{21}H_{14}N_2O_2$: C, 77.29; H, 4.32; N, 8.58; Found: C, 77.23; H, 4.37; N, 8.54.

7-Phenyl-7,9-dihydro-8H-pyrimido[5′,4′:5,6]pyrano[3,2-h]quinolin-8-one (6e) 146–150°C (ethanol), Brown solid, Yield 63%, IR (KBr) ν (cm^{-1}): 3376 (–NH stretching), 1730 (>C=O), 1601 (C=N at pyrimidine ring), 1504 (–NH bending), 1448, 1247 (C–O–C at pryan ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.92 (s, 1H, pyran –CH), 8.10 (d, 1H, –NH at pyrimidine ring), 6.83–7.55 (m, 7H, Ar–H), 7.56 (d, 1H, –CH at pyrimidine ring), 7.34–7.49 (m, 3H, Ar–H at pyridine ring); m/z: 327.10 (100.0%), 328.10 (22.8%), 329.11 (2.7%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 162.2 and 151.4 (C–O–C at pyran ring), 39.3 (–CH at pyran ring), 150.0 (C=N at pyrimidine ring), 162.6 (>C=0), 120.1, 127.2, 135.2, 137.6 (carbons at pyridine ring), 150.7 (C=N at pyridine ring), 128.6, 131.2, 131.8, 132.9, 133.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal.

Calcd. for $C_{20}H_{13}N_3O_2$: C, 73.38; H, 4.00; N, 12.84; Found: C, 73.43; H, 3.95; N, 12.90.

General preparation of 4-amino-5-phenyl-1,5-dihydro-2H-substitutedchromeno[2,3-d]pyrimidin-2-one 7(a–e)

Mixture of compound **4(a–e)** (2 mmol) and urea (2 mmol) with catalytic amount of sodium ethoxide in ethanol was refluxed on sand bath for 6–7 h (monitored by TLC toluene:methanol; (7:3)). After completion of the reaction, the reaction mixture was poured in crushed ice and neutralized with diluted hydrochloric acid. The precipitated product was collected by the filtration and washed with water. The crude product was purified by crystallization from absolute ethanol and purified by column chromatography (silica gel) using hexane and ethylacetate (2:5) as the eluent to afford the corresponding product. **7(a–e).**

4-Amino-5-phenyl-1,5-dihydro-2H-chromeno[2,3-d]pyrimidin-2-one (7a) 178–182°C (ethanol), Yellow solid, Yield 58%, IR (KBr) ν (cm^{-1}): 3446 (–NH stretching at NH_2), 3301 (–NH stretching at pyrimidine ring), 1653 (>C=O), 1605, 1509 (–NH bending at NH_2), 1579 (C=N at pyrimidine ring), 1454, 1400 (C–N at pyrimidine ring), 1448, 1260 (C–O–C at pyran ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.84 (s, 1H, pyran –CH), 8.15 (s, 2H, $–NH_2$), 8.21 (s, 1H, –NH at pyrimidine ring), 6.78–7.45 (m, 9H, Ar–H); m/z: 291.10 (100.0%), 292.10 (19.6%), 293.11 (2.0%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 150.3 and 148.9 (C–O–C at pyran ring), 33.8 (–CH at pyran ring), 163.3 (C–NH_2), 162.5 (>C=O), 122.5, 122.5, 123.6, 124.2, 125.3, 127.9, 128.4, 129.2, 130.3 131.0 (aromatic carbons); Anal. Calcd. for $C_{17}H_{13}N_3O_2$: C, 70.09; H, 4.50; N, 14.42; Found: C, 70.14; H, 4.46; N, 14.48.

4-Amino-8-hydroxy-5-phenyl-1,5-dihydro-2H-chromeno[2,3-d]pyrimidin-2-one (7b) 190–193°C (ethanol), Orange solid, Yield 61%, IR (KBr) ν (cm^{-1}): 3450 (–NH stretching at NH_2), 3306 (–NH stretching at pyrimidine ring), 3215 (–OH), 1667 (>C=O), 1613, 1509 (–NH stretching at NH_2), 1580 (C=N pyrimidine ring), 1456, 1407 (C=N pyrimidine ring), 1437, 1265 (C–O–C at pyran ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.76 (s, 1H, pyran –CH), 8.0 (s, 2H, $–NH_2$), 8.12 (s, 1H, –NH at pyrimidine ring), 6.85–7.30 (m, 8H, Ar–H), 9.09 (s, 1H, –OH); m/z: 307.10 (100.0%), 308.10 (18.7%), 309.10 (2.4%), 308.09 (1.1%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 154.5 and 136.2 (C–O–C at pyran ring), 34.4 (–CH at pyran ring), 164.6 (C–NH_2), 161.3 (>C=O), 162.8 (C–OH), 100.2, 110.5, 111.5, 123.6, 123.2, 125.3, 125.9, 128.4, 129.2, 130.3, 131.0 (aromatic carbons); Anal. Calcd. for $C_{17}H_{13}N_3O_3$: C, 66.44; H, 4.26; N, 13.67; Found: C, 66.38; H, 4.31; N, 13.72.

8-Amino-7-phenyl-7,11-dihydro-10H-benzo[7,8]chromeno[2,3-d]pyrimidin-10-one (7c) 182–185°C (ethanol), Brown solid, Yield 55%, IR (KBr) ν (cm^{-1}): 3448 (–NH stretching at NH_2), 3311 (–NH stretching at pyrimidine ring), 1657 (>C=O stretching), 1605, 1510 (–NH bending at NH_2), 1575 (C=N pyrimidine ring), 1444, 1416 (C–N at pyrimidine ring), 1264 (C–O–C at pyran ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.75 (s, 1H, pyran –CH), 8.05 (s, 2H, $–NH_2$), 8.16 (s, 1H, –NH at pyrimidine ring), 7.03–7.89 (m, 11H, Ar–H); m/z: 341.12 (100.0%), 342.12 (23.0%), 343.12 (3.1%), 342.11 (1.1%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 136.0 and 151.0 (C–O–C at pyran ring), 32.7 (–CH at pyran ring), 164.6 (C–NH_2), 148.4 (>C=O), 123.2, 122.7, 123.8, 123.6, 124.2, 125.8, 126.9, 127.4, 128.6, 129.9, 130.4, 130.2 (aromatic carbons); Anal. Calcd. for $C_{21}H_{15}N_3O_2$: C, 73.89; H, 4.43; N, 12.31; Found: C, 73.85; H, 4.48; N, 12.36.

11-Amino-12-phenyl-8,12-dihydro-9H-benzo[5,6]chromeno[2,3-d]pyrimidin-9-one (7d) 240–244°C (ethanol), Brown solid, Yield 74%, IR (KBr) ν (cm^{-1}): 3446 (–NH stretching at NH_2), 3322 (–NH stretching at pyrimidine ring), 1643 (>C=O), 1615, 1505 (–NH bending at NH_2), 1579 (C=N at pyrimidine ring), 1452, 1419 (C–N at pyrimidine ring), 1267 (C–O–C at pyran ring); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.78 (s, 1H, pyran –CH), 7.95 (s, 2H, $–NH_2$), 8.05 (s, 1H, –NH at pyrimidine ring), 6.89–7.35 (m, 11H, Ar–H); m/z: 341.12 (100.0%), 342.12 (23.0%), 343.12 (3.1%), 342.11 (1.1%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 136.0 and 151.0 (C–O–C at pyran ring), 32.7 (–CH at pyran ring), 164.6 (C–NH_2), 158.4 (>C=O), 123.2, 122.7, 123.8, 123.6, 124.2, 125.8, 126.9, 127.4, 128.6, 129.9, 130.4, 130.2 (aromatic carbons); Anal. Calcd. for $C_{21}H_{15}N_3O_2$: C, 73.89; H, 4.43; N, 12.31; Found: C, 73.93; H, 4.38; N, 12.28.

8-Amino-7-phenyl-7,11-dihydro-10H-pyrimido[5′,4′:5,6]pyrano[3,2-h]quinolin-10-one (7e) 188–190°C (ethanol), Green solid, Yield 78%, IR (KBr) ν (cm^{-1}): 3446 (–NH stretching at NH_2), 3311 (–NH stretching at pyrimidine ring), 1649 (>C=O stretching), 1609, 1511 (–NH bending at NH_2), 1580(C=N Pyrimidine ring), 1454, 1405 (C–N at pyrimidine ring), 1250 (C–O–C); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.84 (s, 1H, pyran –CH), 8.24 (s, 2H, $–NH_2$), 8.11 (s, 1H, –NH at pyrimidine ring), 6.83–7.64 (m, 7H, Ar–H), 7.33–7.57 (m, 3H, Ar–H at pyridine ring); m/z: 342.11 (100.0%), 343.12 (21.9%), 344.12 (2.7%), 343.11 (1.5%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 136.9 and 151.4 (C–O–C at pyran ring) 34.8 (–CH at pyran ring), 164.8 (C–NH_2), 160.9 (C=0), 119.6, 128.0, 135.8, 137.2 (carbons at pyridine ring), 150.0 (C=N at pyridine ring), 128.6, 131.2, 131.8, 132.9, 133.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{20}H_{14}N_4O_2$: C,

70.17; H, 4.12; N, 16.37; Found: C, 70.20; H, 4.08; N, 16.42.

General preparation of 4-amino-5-phenyl-1,5-dihydro-2H-chromeno[2,3-d]Pyrimidine-2-thione 8(a–e)

Mixture of compound **4(a–e)** (2 mmol) and thiourea (2 mmol) with catalytic amount of sodium ethoxide in ethanol was refluxed on sand bath for 6–7 h (monitored by TLC toluene:methanol; (7:3)). After completion of reaction, the reaction mixture was poured in crushed ice and neutralized by the diluted hydrochloric acid. The precipitated product was collected by the filtration and washed with water. The crude product was purified by crystallization from absolute ethanol and purified by column chromatography (silica gel) using hexane and ethylacetate (1:3) as the eluent to afford the corresponding product **8(a–e)**.

4-Amino-5-phenyl-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-2-thione (8a) 185–187°C (ethanol), Cream solid, Yield 65%, IR (KBr) ν (cm^{-1}): 3430 (–NH stretching at NH_2), 3331 (–NH stretching at pyrimidien ring), 1607 (–NH bending at NH_2), 1573 (C=N pyrimidine ring), 1505 (–NH bending at pyrimidien ring), 1454, 1250 (C–O–C at pyran ring), 1180 (>C=S stretching); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.74 (s, 1H, pyran –CH), 8.14 (s, 2H, –NH_2), 8.15 (s, 1H, –NH at pyrimidine ring), 6.83–7.37 (m, 9H, Ar–H); m/z: 307.08 (100.0%), 308.08 (19.4%), 309.07 (4.5%), 309.08 (2.2%), 308.07 (1.1%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 153.3 and 149.9 (C–O–C at pyran ring), 34.4 (–CH at pyran ring), 164.6 (C–NH_2), 182.5 (>C=S), 121.5, 122.2, 123.4, 125.1, 125.6, 128.2, 128.8, 129.3, 131.0, 131.3 (aromatic carbons); Anal. Calcd. for $C_{17}H_{13}N_3OS$: C, 66.08; H, 4.20; N, 13.62; Found: C, 66.11; H, 4.26; N, 13.67.

4-Amino-8-hydroxy-5-phenyl-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-2-thione (8b) 196–198°C (ethanol), Orange solid, Yield 61%, (KBr) ν (cm^{-1}): 3438 (–NH stretching at NH_2), 3316 (–NH stretching at pyrimidien ring), 3210 (–OH), 3030, 1610 (–NH bending at NH_2), 1567 (C=N Pyrimidine ring), 1525 (–NH bending at pyrimidien ring), 1452, 1250 (C–O–C at pyran ring), 1179 (>C=S Stretching); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.80 (s, 1H, pyran –CH), 8.09 (s, 2H, –NH_2), 8.22 (s, 1H, –NH at pyrimidine ring), 7.10–8.10 (m, 9H, Ar–H), 9.05 (s, 1H, –OH); m/z: 323.07 (100.0%), 324.08 (18.6%), 325.07 (4.7%), 325.08 (2.2%), 324.07 (1.9%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 154.5 and 149.9 (C–O–C at pyran ring), 34.0 (–CH at pyran ring), 164.6 (C–NH_2), 180.4 (>C=S), 162.9 (C–OH), 100.4, 100.5, 112.2, 123.2, 123.5, 125.8, 126.0, 128.4, 129.2, 130.3, 131.5 (aromatic carbons); Anal. Calcd. for $C_{17}H_{13}N_3O_2S$: C, 63.19; H, 4.00; N, 12.95; Found: C, 63.23; H, 4.05; N, 12.99.

8-Amino-7-phenyl-7,11-dihydro-10H-benzo[7,8]chromeno[2,3-d]pyrimidine-10-thione (8c) 188–189°C (ethanol), Yellow solid, Yield 65%, (KBr) ν (cm^{-1}): 3430 (–NH stretching at NH_2), 3315 (–NH stretching at pyrimidien ring), 3050, 1610 (–NH bending at NH_2), 1570 (C=N pyrimidine ring), 1505 (–NH bending at pyrimidien ring), 1450, 1250 (C–O–C at pyran ring), 1185 (>C=S Stretching); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.80 (s, 1H, pyran –CH), 8.17 (s, 2H, –NH_2), 8.10 (s, 1H, –NH at pyrimidine ring), 6.90–8.10 (m, 10H, Ar–H); m/z: 357.09 (100.0%), 358.10 (22.9%), 359.09 (4.8%), 359.10 (2.9%), 358.09 (1.9%), 360.09 (1.1%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 143.0 and 149.2 (C–O–C at pyran ring), 34.8 (–CH at pyran ring), 165.6 (C–NH_2), 181.4 (>C=S), 119.2, 120.5, 123.5, 123.6, 123.2, 125.3, 125.9, 126.4, 126.6, 127.9, 128.4, 129.2, 130.3, 131.0 (aromatic carbons); Anal. Calcd. for $C_{21}H_{15}N_3OS$: C, 70.56; H, 4.24; N, 11.76; Found: C, 70.59; H, 4.26; N, 11.79.

11-Amino-12-phenyl-8,12-dihydro-9H-benzo[5,6]chromeno[2,3-d]pyrimidine-9-thione (8d) 256–258°C (ethanol), Cream solid, Yield 68%, (KBr) ν (cm^{-1}): 3446 (–NH stretching at NH_2), 3301 (–NH stretching at pyrimidien ring), 3050, 1605 (–NH bending at NH_2) 1573 (C=N pyrimidine ring), 1515 (–NH bending at pyrimidien ring), 1445, 1250 (C–O–C at pyran ring), 1180 (>C=S Stretching); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.80 (s, 1H, pyran –CH), 8.18 (s, 2H, –NH_2), 8.10 (s, 1H, –NH at pyrimidine ring), 6.60–7.90 (m, 10H, Ar–H); m/z: 357.09 (100.0%), 358.10 (22.9%), 359.09 (4.8%), 359.10 (2.9%), 358.09 (1.9%), 360.09 (1.1%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 149.0 and 150.2 (C–O–C at pyran ring), 35.9 (–CH at pyran ring), 163.9 (C–NH_2), 180.4 (>C=S), 123.2, 122.7, 128.8, 129.6, 130.2, 131.8, 132.9, 133.4, 133.6, 134.9, 135.4, 135.2, 135.3, 136.0 (aromatic carbons); Anal. Calcd. for $C_{21}H_{15}N_3OS$: C, 70.56; H, 4.24; N, 11.76; Found: C, 70.57; H, 4.25; N, 11.80.

8-Amino-7-phenyl-7,11-dihydro-10H-pyrimido[5′,4′:5,6]pyrano[3,2-h]quinoline-10-thione (8e) 200–202°C (ethanol), Brown solid, Yield 69%, (KBr) ν (cm^{-1}): 3438 (–NH stretching at NH_2), 3329 (–NH stretching at pyrimidien ring), 3043, 1612 (–NH bending at NH_2), 1576 (C=N pyrimidine ring), 1506 (–NH bending at pyrimidien ring), 1454, 1260 (C–O–C at pyran ring), 1175 (>C=S Stretching); ^{1}H-NMR (300MHz, $CDCl_3$) δ (ppm): 4.81 (s, 1H, pyran –CH), 8.10 (s, 2H, –NH_2), 8.12 (s, 1H, –NH at pyrimidine ring), 6.82–8.97 (m, 10H, Ar–H), 7.28–7.47 (m, 3H, Ar–H at pyridine ring); m/z: 342.11 (100.0%), 343.12 (21.9%), 344.12 (2.7%), 343.11 (1.5%); m/z: 358.09

(100.0%), 359.09 (23.9%), 360.08 (4.5%), 360.10 (2.3%), 361.09 (1.0%); ^{13}C-NMR (300MHz, $CDCl_3$) δ (ppm): 149.0 and 150.2 (C–O–C at pyran ring), 36.8 (–CH at pyran ring), 164.9 (C–NH_2), 180.7 (>C=S), 120.1, 122.2, 130.3, 134.5 (carbons at pyridine ring), 149.4 (C=N at pyridine ring), 129.6, 130.2, 131.8, 132.9, 133.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{20}H_{14}N_4OS$: C, 67.02; H, 3.94; N, 15.63; Found: C, 67.00; H, 3.97; N, 15.59.

The minimum inhibitory concentration

A definition of the minimum inhibitory concentration (MIC) is "the lowest concentration which resulted in maintenance or reduction of inoculums viability." The determination of the MIC involves a semi-quantitative test procedure which gives an approximation to the least concentration of antimicrobial agent needed to prevent microbial growth. The serial dilution technique (Collee *et al.* 1989) was applied for the determination of MIC of the tested compounds against four species of bacterial strains: *Staphylococcus aureus* [ATCC-25923] and *Streptococcus pyogenes* [MTCC-443] as Gram-positive, *Escherichia coli* [ATCC-25922], and *Pseudomonas aeruginosa* [MTCC-441] as Gram-negative; and one species of fungal strain *Aspergillus niger* [MTCC-282]. Dilution series were set up with 62.5, 125, 250, 500, and 1000 µg/ml of nutrient broth medium to each tube, 1000 µl of standardized suspension of the test microbes (107 cell/ml) were added and incubated at 37°C for 24 h.

Agar micro dilution method

The in vitro activity of the compounds against *Mycobacterium tuberculosis* $H_{37}Rv$ was determined by agar micro dilution technique (Siddiqi, 1992). Twofold dilutions of each test compound were added to 7H10 agar and *Mycobacterium tuberculosis* $H_{37}Rv$ was used as test organism. MIC is the concentration of the compound that completely inhibits the growth and colony-forming ability of *Mycobacterium tuberculosis*. In 24-well plates, 3-ml middle brook 7H11 agar medium with OADC (oleic acid–albumin–dextrose–catalase) supplement was dispensed in each well. The test compound was added to the middle brook medium agar before in duplicate so that the final concentration of the test compound in each well was 1000, 500, 250, 125, and 62.5 µg/ml, respectively. The known CFU (colony-forming unit) of the $H_{37}Rv$ culture was dispensed on top of agar in each well in a negative pressure biosafety hood. The plates were then incubated at 37°C in CO_2 incubator. The concentration at which complete inhibition of colonies was observed was taken as MIC of test drug.

Acknowledgments The authors are thankful to the Department of Chemistry, VNSGU, Surat, for providing the laboratory facilities, and D. Rajani, Microcare Laboratory, Surat, for antitubercular and antimicrobial activity. The authors also thank SAIF., Chandigarh, for spectral analysis.

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