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

[a] Department of Chemistry, Veer Narmad South Gujarat University, Surat, 395007, Gujarat, India

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Synthesis of novel thiopyrimidines: an investigation of anti-tubercular and antimicrobial activity

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

Department of Chemistry, Veer Narmad South Gujarat University, Surat 395007, Gujarat, India

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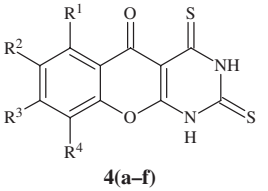
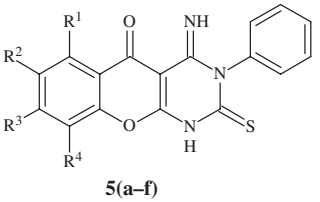
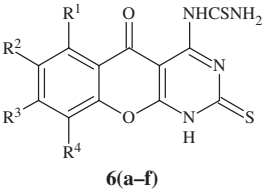
A variety of novel sulfur-containing tricyclic pyrimidine derivatives have been synthesized via the reaction of 2-amino-4-oxo-4*H*-chromene-3-carbonitriles **3(a–f)** with different reagents and characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analysis. The anti-tubercular and antimicrobial activities of the synthesized compounds were investigated.



4(a–f)



5(a–f)



6(a–f)

Keywords: thiopyrimidines; dithiopyrimidines; chromone; anti-tubercular activity; antimicrobial activity

1. Introduction

Condensed heterocyclic systems, especially those linked to a thiopyrimidine ring, play an important role as potential medicinal agents in cancer and virus research (*1*, *2*). Recent studies reported the synthesis of some new fused thiopyrimidine candidates which showed antimicrobial (*3*), anticonvulsant (*4*, *5*), antiarrhythmic (*6*), anti-inflammatory (*7*) and anti-tumor (*8–10*) activities.

On the other hand, a wide range of biological activities have been attributed to compounds containing chromone ring systems (*11–17*). If the chromone is fused with the thiopyrimidine ring, the linked tricyclic compounds are expected to display interesting biological activities. So our interest in the tricyclic compounds containing thiopyrimidine moieties leads us to search for novel bio-active scaffold based on the use of aspirin as the starting material.

*Corresponding author. Emails: saurabh@vnsgu.ac.in; saurabh.silvina@gmail.com

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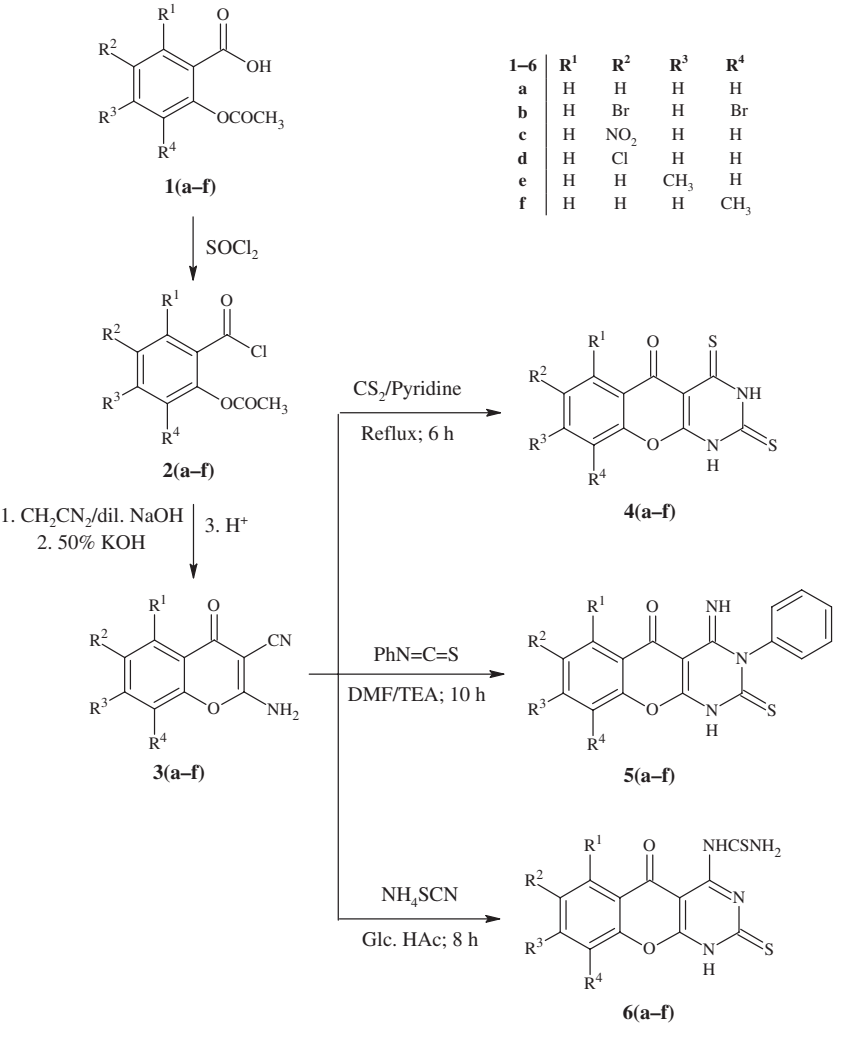
2. Results and discussion

2.1. *Chemistry*

An *o*-aminonitrile is one of the routes for the synthesis of the fused systems including thiopyrimidine (*18–21*). With the aim of obtaining thiopyrimidine-fused chromone derivatives, cyclization was carried out by the reaction of substituted *o*-aminonitrile of chromones **3**(**a**–**f**) and each of carbon disulphide, ammonium thiocyanate and phenylisothiocyanate, respectively. Aspirins **1**(**a**–**f**) were used as the starting material to synthesize functionalized *o*-aminonitrile of chromones **3**(**a**–**f**) followed by thionylation.

Reaction of acid chloride of aspirins **2**(**a**–**f**) with malononitrile in the presence of diluted 10% NaOH solution at 0–5 °C followed by treatment with 50% KOH solution and subsequent acidification afforded substituted 2-amino-4-oxo-4*H*-chromene-3-carbonitriles **3**(**a**–**f**) (*22*) (Scheme 1). The IR spectra of **3**(**a**–**f**) exhibited a sharp peak in the region of 3230–3295 cm^{-1} due to $-NH_2$ stretching vibrations and a sharp peak in the range of 2220–2240 cm^{-1} and 1660–1670 cm^{-1},



1–6	R^1	R^2	R^3	R^4
a	H	H	H	H
b	H	Br	H	Br
c	H	NO_2	H	H
d	H	Cl	H	H
e	H	H	CH_3	H
f	H	H	H	CH_3

Scheme 1. Synthetic route of compounds **4**, **5**, **6**(**a**–**f**).

suggesting the presence of −C≡N and >C=O groups. ^{1}H NMR spectra exhibited a broad signal in the region 8.95–9.30 δ ppm due to the $-NH_2$ proton. In ^{13}C NMR, −C≡N and >C=O carbon signals appeared in the region of 119.8–124.8 and 173.4–178.8 δ ppm, respectively.

The target ring system **4**(**a**–**f**) was synthesized by the reaction of **3**(**a**–**f**) with carbon disulfide through several intermediates whose subsequent rearrangement leads to fused pyrimidinedithiones **4**(**a**–**f**) (Scheme 1). The IR spectra of the products **4**(**a**–**f**) confirmed the disappearance of the primary amine ($-NH_2$) and nitrile (−C≡N) absorption bands. The ^{1}H NMR spectrum of the products **4**(**a**–**f**) showed in each case the disappearance of the broad amine signal. Cyclization reaction was further confirmed by the ^{13}C NMR spectra of compounds **4**(**a**–**f**). The reaction products **5**(**a**–**f**) were obtained in a one-step reaction through prolonged heating of **3**(**a**–**f**) with phenylisothiocyanate in DMF containing a catalytic amount of TEA (Scheme 1). Meanwhile, compounds **3**(**a**–**f**) in glacial acetic acid were treated with ammonium thiocyanate to give 1-(5-oxo-2-thioxo-1,5-dihydro-2*H*-chromeno[2,3-*d*]pyrimidin-4-yl)thiourea derivatives **6**(**a**–**f**) under refluxed temperature (Scheme 1). The structures of the reaction products were established based on its elemental analysis and spectral data displayed in Section 5.

3. Biological activity

3.1. *Anti-tubercular activity*

Anti-tubercular activity was carried out against *Mycobacterium tuberculosis H$_{37}$Rv* [MTCC-200] at the concentration of 62.5 μg/ml, and the values are shown in Table 1. Rifampicin was used as the standard drug. All the compounds were tested for their anti-tubercular activity by the agar micro-dilution technique (*23*). The halogen group containing substituted derivatives, **6b** and **6d**, displayed a relatively higher inhibitory activity in general. However, substitution at chromone with a methyl group reduces the anti-tubercular activity. The presence of a dibromo group on chromone caused a remarkable improvement in the anti-tubercular activity (Table 1).

3.2. *Antibacterial activity*

All the compounds were tested for their *in vitro* antibacterial activity against *Staphylococcus aureus* [MTCC-96] and *Streptococcus pyogenes* [MTCC-442] as Gram-positive and *Escherichia coli* [MTCC-443] and *Pseudomonas aeruginosa* [MTCC-1688] as Gram-negative bacterial species. Ampicillin and chloramphenicol were used as standard drugs. Minimum inhibitory concentration (MIC, μg/ml) values are displayed in Table 2. All the compounds were tested for their antibacterial activity by the two-fold serial dilution method (*24*). It is evident from the results that, in general, the generation of thiopyrimidines ring system enhances the activity against

Table 1. The *in vitro* anti-tubercular activity of the synthesized compounds against *M. tuberculosis H$_{37}$Rv*.

Compound	% Inhibition	Compound	% Inhibition	Compound	% Inhibition
4a	5	**5a**	6	**6a**	41
4b	23	**5b**	29	**6b**	99
4c	6	**5c**	21	**6c**	57
4d	13	**5d**	8	**6d**	99
4e	15	**5e**	14	**6e**	24
4f	11	**5f**	9	**6f**	20

Note: Rifampicin: 99% inhibition at 40 μg/ml.

Table 2. The *in vitro* antimicrobial activity (MIC, μg/ml) of the synthesized compounds.

	Minimum inhibition concentration (μg/ml)						
	Gram negative		Gram positive		Fungal species		
Compound	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenes*	*C. albicans*	*A. niger*	*A. clavatus*
3a	250	200	250	200	1000	1000	1000
3b	250	200	200	250	500	>1000	1000
3c	500	250	250	250	>1000	1000	>1000
3d	250	500	250	500	500	1000	>1000
3e	250	200	150	250	500	>1000	1000
3f	500	500	200	250	500	>1000	>1000
4a	100	125	250	250	>1000	>1000	>1000
4b	100	200	200	200	150	500	500
4c	100	250	250	250	500	>1000	>1000
4d	200	500	250	500	250	>1000	>1000
4e	150	150	200	250	250	1000	>1000
4f	250	250	100	200	500	1000	>1000
5a	62.5	125	100	200	1000	500	500
5b	125	200	150	250	500	1000	1000
5c	100	100	200	250	1000	500	1000
5d	250	500	100	200	500	1000	>1000
5e	125	100	200	250	250	1000	>1000
5f	100	100	200	250	500	>1000	>1000
6a	500	500	125	200	1000	1000	>1000
6b	200	250	62.5	200	500	1000	>1000
6c	125	250	150	250	>1000	>1000	500
6d	50	125	150	200	500	1000	500
6e	200	250	62.5	200	250	>1000	>1000
6f	250	250	200	250	500	>1000	>1000
A	100	100	250	100	–	–	–
B	50	50	50	50	–	–	–
C	–	–	–	–	100	100	100
D	–	–	–	–	500	100	100

Note: Ampicillin (A) and chloramphenicol (B) were used as standard drugs for bacterial strains while nystatin (C) and greseofulvin (D) were used as standard drugs for fungal strains.

Gram-negative as well as Gram-positive species except *S. pyogenes*. Among the synthesized compounds, **5a** and **6d** display significant antibacterial activity compared to chloramphenicol against *E. coli* in the range of 62.5 and 50 μg/ml, respectively. Dithiopyrimidine **4a** has a similar potency compared to ampicillin but replacement of the thio group at the fourth position by a thiourea linkage decreased the antibacterial potency against *E. coli*. The imino compounds bearing 7-nitro as well as 8 and 9-methyl derivatives show relatively higher potency than ampicillin against *P. aeruginosa*. Similarly, dithiopyrimidine and 4-imino *N*-phenyl thiopyrimidine showed exceptional potency while 7,9-dibromo and 8-methyl derivatives are comparable to chloramphenicol against *S. aureus*. The activity results reveal that on *E. coli* species the activity is exceptionally enhanced than their precursors, while on other species, the results are random (Table 2).

3.3. *Antifungal activity*

The antifungal activity of all the compounds was studied against the fungal strains viz. *Aspergillus niger* [MTCC-282], *Candida albicans* [MTCC-227] and *Aspergillus clavatus* [MTCC-1323] by two-fold serial dilution. Nystatin and greseofulvin were used as standard drugs. MIC values in μg/ml are displayed in Table 2. The antifungal activity results indicated that the synthesized compounds showed more fungicidal potency against *C. albicans* rather than against *A. niger* and *A. clavatus* when compared with the standard drug. None of the compounds shows similar or

high fungicidal potency against *A. niger* as well as *A. clavatus*. However, the compounds show varied activity against *C. albicans* when compared with the standard drug greseofulvin. Even, compound **4b** showed a high potency comparable to the standard drug nystatin. Compounds **4d**, **4e**, **5e** and **6e** possess remarkably higher fungicidal potency while compounds **3b**, **3d–f**, **4c**, **4f**, **5b**, **5d**, **5f**, **6b**, **6d** and **6f** have similar potency as greseofulvin against *C. albicans*. The unsubstituted and nitro-substituted thiopyrimidines were inactive, whereas the 8-methyl thiopyrimidines were found to be more potent than the 9-methyl thiopyrimidines. Among the halo-substituted thiopyrimidines, mono halo-substituted thiopyrimidines showed consistently higher potency while the dithiopyrimidine-bearing dibromo substitution showed remarkable fungicidal potency (Table 2).

4. Conclusion

We report the successful synthesis and anti-tubercular and antimicrobial activities of new tricyclic chromone-fused thiopyrimidines. The assumed structures are confirmed by the IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. The anti-tubercular studies revealed that **6b** and **6d** showed promising anti-tubercular activity against *M. tuberculosis* $H_{37}Rv$. From the biological activity of the newly synthesized compounds, it is evident that the fused thiopyrimidines containing tricyclic chromone derivatives showed, in general, good antibacterial potency which suggests that the introduction of sulfur is valuable for its activity.

5. Experimental

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. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer using TMS as the internal standard with DMSO-d_6 or $CDCl_3$ as the solvent. 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 vapor. All the chemicals were purchased from the commercial sources and purified by either distillation or recrystallization.

5.1. *General procedure for the synthesis of compound 3(a–f)*

A mixture of acetylated acids **1**(**a**–**f**) (10.0 mmol) and thionyl chloride (15.0 mmol) was heated on a water bath for 2 h till no more hydrogen chloride is evolved. The reaction mixture was left to cool to room temperature and the excess of thionyl chloride was distilled out under anhydrous condition, and the gummy solid product was used as early as possible in the next step. A mixture of acid chlorides and malononitrile (5.0 mmol) was stirred vigorously for 10 min in the presence of ice (5 g) and of 20% (w/v) NaOH solution (1 ml). Second portion of malononitrile (5.0 mmol) and 20% (w/v) NaOH solution (1 ml) was added; the mixture was stirred for another 10 min, and then warmed to 40 °C with stirring. After completion of the reaction, 50% of KOH solution was added in 5 ml increments till the solution becomes clear. The solution was cooled, acidified with conc. HCl and the solid product was separated by filtration, washed with water and dried.

5.1.1. *2-Amino-4-oxo-4H-chromene-3-carbonitrile (**3a**)*

Yield 74%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3264 ($-NH_2$), 2225 ($-C{\equiv}N$), 1666 (>C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.43–7.93 (m, 4H, Ar−H), 8.94 (2H, br s, $-NH_2$);

^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 120.2, 130.2, 132.0, 134.4, 135.4, 136.2 (aromatic carbons), 191.5 (C−NH_2), 120.8 (−C≡N), 90.8 (>C−CN), 175.8 (>C=O); *m/z*: 186.04 (100.0%), 187.05 (11.0%). Anal. Calcd for $C_{10}H_6N_2O_2$: C, 64.52; H, 3.25; N, 15.05. Found: C, 64.46; H, 3.23; N, 14.96.

5.1.2. *2-Amino-6,8-dibromo-4-oxo-4H-chromene-3-carbonitrile (**3b**)*

Yield 78%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3277 (−NH_2), 2228 (−C≡N), 1666 (>C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.78 and 8.75 (s, 2H, Ar−H), 9.01 (2H, br s, −NH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 109, 115 (2C–Br), 128, 135, 140, 152 (aromatic carbons), 190.5 (C−NH_2), 120.9 (−C≡N), 91.8 (>C−CN), 175.1 (>C=O); *m/z*: 343.86 (100.0%), 341.86 (51.4%), 345.86 (48.7%), 344.87 (11.0%), 346.86 (5.7%), 342.87 (5.6%). Anal. Calcd for $C_{10}H_4N_2O_2Br_2$: C, 34.92; H, 1.17; N, 8.14. Found: C, 34.86; H, 1.24; N, 8.26.

5.1.3. *2-Amino-6-nitro-4-oxo-4H-chromene-3-carbonitrile (**3c**)*

Yield 71%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3295 (−NH_2), 2225 (−C≡N), 1661 (>C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.64–8.78 (m, 3H, Ar-H), 9.27 (br s 2H, −NH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 142.5 (−C−NO_2), 114, 120, 125, 144, 152 (aromatic carbons), 187.6 (C−NH_2), 124.8 (−C≡N), 90.8 (>C−CN), 174.4 (>C=O); *m/z*: 231.03 (100.0%), 232.03 (12.1%), 233.03 (1.5%). Anal. Calcd for $C_{10}H_5N_3O_4$: C, 51.96; H, 2.18; N, 18.18. Found: C, 52.01; H, 2.24; N, 18.06.

5.1.4. *2-Amino-6-chloro-4-oxo-4H-chromene-3-carbonitrile (**3d**)*

Yield 75%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3237 (−NH_2), 2239 (−C≡N), 1667 (>C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.49–7.89 (3H, m, Ar−H), 9.03 (2H, br s, −NH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 129.5 (C–Cl), 119, 122, 125, 140, 155 (aromatic carbons), 191.5 (C−NH_2), 120.8 (−C≡N), 90.8(>C−CN), 175.8 (>C=O); *m/z*: 220.00 (100.0%), 222.00 (32.0%), 221.01 (10.9%), 223.00 (3.7%). Anal. Calcd for $C_{10}H_5N_2O_2Cl$: C, 54.44; H, 2.28; N, 12.70. Found: C, 54.51; H, 2.34; N, 12.86.

5.1.5. *2-Amino-7-methyl-4-oxo-4H-chromene-3-carbonitrile (**3e**)*

Yield 77%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3230 (−NH_2), 2222 (−C≡N), 1662 (>C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.36 (3H, s, Ar−CH_3), 7.51–7.93 (3H, m, Ar−H), 9.12 (2H, br s, −NH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 31.5 (C−CH_3), 113, 122, 125, 129 130, 155 (aromatic carbons), 185.5 (C−NH_2), 119.8 (−C≡N), 90.8 (−C−CN), 178.8 (>C=O); *m/z*: 200.06 (100.0%), 201.06 (12.8%). Anal. Calcd for $C_{11}H_8N_2O_2$: C, 66.00; H, 4.03; N, 13.99. Found: C, 66.08; H, 3.97; N, 14.07.

5.1.6. *2-Amino-8-methyl-4-oxo-4H-chromene-3-carbonitrile (**3f**)*

Yield 76%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3253 (−NH_2), 2226 (−C≡N), 1667 (>C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.38 (3H, s, Ar−CH_3), 7.73–8.12 (3H, m, Ar−H), 9.08 (2H, br s, −NH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 32.5 (C−CH_3), 114, 120, 123, 129 135, 158 (aromatic carbons), 191.5 (C−NH_2), 122.8 (−C≡N), 92.8 (−C−CN), 173.4 (>C=O); *m/z*: 200.06 (100.0%), 201.06 (12.8%). Anal. Calcd for $C_{11}H_8N_2O_2$: C, 66.00; H, 4.03; N, 13.99. Found: C, 66.11; H, 3.95; N, 14.05.

5.2. *General procedure for synthesis of compounds 4(a–f)*

A mixture of compound **3**(**a**–**f**) (5.0 mmol) and carbon disulphide (5.0 mmol) in dry pyridine (20 ml) was refluxed on a water bath for 6 h (monitored by TLC). After completion of the reaction, the reaction mixture was left to cool to room temperature, poured into ice-cold water (50 ml) and neutralized with dil. HCl (1:1). The separated product was filtered off, washed with water and recrystallized from acetic acid to give compounds **4**(**a**–**f**).

5.2.1. *2,4-Dithioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d]pyrimidine-5-one (**4a**)*

Yield 65%; mp 293–295 °C; IR (KBr) υ (cm^{-1}): 3139 (−NH), 1661 (>C=O), 1325 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.24–7.38 (4H, m, Ar−H), 8.18 (1H, s, −NH), 9.08 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 114, 120, 125, 135, 144, 152 (aromatic carbons), 170.5 (>C=O), 190.3 (2 >C=S), 78 and 172 (C=C at pyrimidine ring); m/z: 261.99 (100.0%), 262.99 (13.6%), 263.98 (9.1%), 263.99 (1.4%), 264.99 (1.2%). Anal. Calcd for $C_{11}H_6N_2O_2S_2$: C, 50.37; H, 2.31; N, 10.68; S, 24.45. Found: C, 50.45; H, 2.37; N, 10.76; S, 24.50.

5.2.2. *7,9-Dibromo-2,4-dithioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d]pyrimidine-5-one (**4b**)*

Yield 63%; mp 282–284 °C, IR (KBr) υ (cm^{-1}): 3072 (−NH), 1676 (>C=O), 1318 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.58–7.94 (2H, s, Ar−H), 8.16 (1H, s, −NH), 9.00 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 110.5 and 116 (2 C−Br), 119, 122, 125, 155 (aromatic carbons), 169.2 (>C=O), 195.4 (2 >C=S), 79 and 176 (C=C at pyrimidine ring); m/z: 419.81 (100.0%), 421.80 (57.4%), 417.81 (51.0%), 420.81 (14.1%), 418.81 (7.3%), 422.81 (7.0%), 423.80 (4.7%), 419.80 (4.6%), 421.81 (1.4%), 422.80 (1.3%). Anal. Calcd for $C_{11}H_4N_2O_2Br_2S_2$: C, 31.45; H, 0.96; N, 6.67; S, 15.27. Found: C, 31.41; H, 1.05; N, 6.61; S, 15.30.

5.2.3. *7-Nitro-2,4-dithioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d]pyrimidine-5-one (**4c**)*

Yield 57%; mp 298–300 °C; IR (KBr) υ (cm^{-1}): 3152 (−NH), 1667 (>C=O), 1325 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.92–8.75 (3H, m, Ar−H), 8.12 (1H, s, −NH), 9.05 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 142.5 (C–NO_2), 114, 120, 125, 144, 152 (aromatic carbons), 171.4 (>C=O), 199.8 (2 >C=S), 71 and 172 (C=C at pyrimidine ring); m/z: 306.97 (100.0%), 307.98 (12.1%), 308.97 (9.4%), 307.97 (2.7%), 308.98 (1.5%), 309.97 (1.2%). Anal. Calcd for $C_{11}H_5N_3O_4S_2$: C, 42.99; H, 1.64; N, 13.67; S, 20.87. Found: C, 43.06; H, 1.57; N, 13.71; S, 20.81.

5.2.4. *7-Chloro-2,4-dithioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d]pyrimidine-5-one (**4d**)*

Yield 66%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3169 (−NH), 1672 (>C=O), 1333 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.12–7.40 (3H, m, Ar−H), 8.15 (1H, s, −NH), 9.06 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 125.9 (C−Cl), 111, 120, 125, 144, 152 (aromatic carbons), 176.9 (>C=O), 198.8 (2 >C=S), 71 and 172 (C=C at pyrimidine ring); m/z: 295.95 (100.0%), 297.95 (33.3%), 296.95 (14.4%), 297.94 (9.1%), 298.95 (5.0%), 299.94 (3.1%). Anal. Calcd for $C_{11}H_5N_2O_2ClS_2$: C, 44.52; H, 1.70; N, 9.44; S, 21.61. Found: C, 44.48; H, 1.73; N, 9.42; S, 21.57.

5.2.5. *8-Methyl-2,4-dithioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d]pyrimidine-5-one (**4e**)*

Yield 64%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3143 (−NH), 1678 (>C=O), 1323 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.36 (3H, s, $-CH_3$), 7.21–7.46 (3H, m, Ar−H), 8.15 (1H, s, −NH), 9.05 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 31.5 (C−$\underline{C}H_3$), 113, 122, 125, 129 130, 155 (aromatic carbons), 175.1 (>C=O), 196.2 (2 >C=S), 77 and 179 (C=C at pyrimidine ring); m/z: 276.00 (100.0%), 277.01 (13.1%), 278.00 (9.2%), 277.00 (2.3%), 278.01 (1.4%), 279.00 (1.3%). Anal. Calcd for $C_{12}H_8N_2O_2S_2$: C, 52.16; H, 2.92; N, 10.14; S, 23.21. Found: C, 52.17; H, 2.89; N, 10.17; S, 23.23.

5.2.6. *9-Methyl-2,4-dithioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d]pyrimidine-5-one (**4f**)*

Yield 63%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3168 (−NH), 1668 (>C=O), 1334 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.36 (3H, s, $-CH_3$), 7.25–7.66 (3H, m, Ar−H), 8.11 (1H, s, −NH), 9.00 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 35.6 (C−$\underline{C}H_3$), 114, 120, 126, 128 130, 150 (aromatic carbons), 176.1 (>C=O), 196.2 (2 >C=S), 77 and 180 (C=C at pyrimidine ring); m/z: 276.00 (100.0%), 277.01 (13.1%), 278.00 (9.2%), 277.00 (2.3%), 278.01 (1.4%), 279.00 (1.3%). Anal. Calcd for $C_{12}H_8N_2O_2S_2$: C, 52.16; H, 2.92; N, 10.14; S, 23.21. Found: C, 52.18; H, 2.87; N, 10.16; S, 23.27.

5.3. *General procedure for synthesis of compounds 5(a–f)*

A mixture of **3**(**a**–**f**) (5.0 mmol) and phenyl isothiocyanate (5.0 mmol) in dimethylformamide (20 ml) containing a catalytic amount of triethylamine (3–4 drops) was refluxed on a sand bath for 10 h (monitored by TLC). After completion of the reaction, the mixture was left to cool to room temperature and poured into ice-cold water (50 ml) for complete precipitation. Product was separated by filtration, washed with water, dried and recrystallized from methanol to give compounds **5**(**a**–**f**).

5.3.1. *4-Imino-3-phenyl-2-thioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d]pyrimidine-5-one (**5a**)*

Yield 67%; mp 154–156 °C; IR (KBr) υ (cm^{-1}): 3071 (−NH), 1666 (>C=O), 1316 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.18–8.15 (9H, m, Ar−H), 8.21 (1H, s, −NH), 9.02 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 114, 118, 120, 122, 125, 128, 130, 131, 136, 139, 144, 146 (aromatic carbons) 178.2 (>C=O), 181.3 (>C=S), 164.8 (>C=NH), 65 and 167 (C=C at pyrimidine ring); m/z: 321.06 (100.0%), 322.06 (19.4%), 323.05 (4.5%), 323.06 (2.4%), 322.05 (1.1%). Anal. Calcd for $C_{17}H_{11}N_3O_2S$: C, 63.54; H, 3.45; N, 13.08; S, 9.98. Found: C, 63.61; H, 3.41; N, 13.15; S, 10.01.

5.3.2. *7,9-Dibromo-4-imino-3-phenyl-2-thioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d] pyrimidine-5-one (**5b**)*

Yield 65%; mp 226–228 °C; IR (KBr) υ (cm^{-1}): 3115 (−NH), 1661 (>C=O), 1326 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.12–8.25 (7H, m, Ar−H), 8.27 (1H, s, −NH), 9.12 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 109 and 112 (C–Br), 119, 122, 123, 125, 129, 130, 135, 136, 139, 144, 147 (aromatic carbons), 178.2 (>C=O), 185.2 (>C=S), 164.4 (>C=NH), 67 and 169 (C=C at pyrimidine ring); m/z: 478.88 (100.0%), 480.87 (52.6%), 476.88 (50.8%), 479.88 (19.6%), 477.88 (10.4%), 481.88 (9.9%), 480.88 (2.4%), 482.87 (2.3%),

478.87 (2.3%), 482.88 (1.1%), 479.87 (1.1%). Anal. Calcd for $C_{17}H_9N_3O_2Br_2S$: C, 42.61; H, 1.89; N, 8.77; S, 6.69. Found: C, 42.66; H, 1.94; N, 8.81; S, 6.73.

5.3.3. *4-Imino-7-nitro-3-phenyl-2-thioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d]pyrimidine-5-one (**5c**)*

Yield 61%; mp 258–260 °C; IR (KBr) υ (cm^{-1}): 3163 (−NH), 1670 (>C=O), 1338 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.88–8.72 (8H, m, Ar−H), 8.10 (1H, s, −NH), 9.06 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 145.6 (C–NO_2) 118, 120, 122, 125, 128, 130, 131, 136, 139, 144, 146 (aromatic carbons), 178.2 (>C=O), 181.3 (>C=S), 164.8 (>C=NH), 65 and 167 (C=C at pyrimidine ring); *m/z*: 366.04 (100.0%), 367.05 (18.7%), 368.04 (4.8%), 368.05 (2.6%), 367.04 (2.3%). Anal. Calcd for $C_{17}H_{10}N_4O_4S$: C, 55.73; H, 2.75; N, 15.29; S, 8.75. Found: C, 55.76; H, 2.81; N, 15.33; S, 8.78.

5.3.4. *7-Chloro-4-imino-3-phenyl-2-thioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d] pyrimidine-5-one (**5d**)*

Yield 67%; mp 294–296 °C; IR (KBr) υ (cm^{-1}): 3216 (−NH), 1686 (>C=O), 1321 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.33–8.27 (8H, m, Ar−H), 8.68 (1H, s, −NH), 9.34 (H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 145.2 (C–NO_2), 117, 120, 122, 125, 128, 130, 135, 136, 139, 144, 146 (aromatic carbons), 179.2 (>C=O), 189.3 (>C=S), 166.8 (>C=NH), 60 and 165 (C=C at pyrimidine ring); *m/z*: 355.02 (100.0%), 357.02 (34.3%), 356.02 (20.5%), 358.02 (6.8%), 357.01 (4.5%), 359.01 (1.5%). Anal. Calcd for $C_{17}H_{10}N_3O_2ClS$: C, 57.39; H, 2.83; N, 11.81; S, 9.01. Found: C, 57.44; H, 2.85; N, 11.78; S, 9.08.

5.3.5. *4-Imino-8-methyl-3-phenyl-2-thioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d] pyrimidine-5-one (**5e**)*

Yield 64%; mp 276–278 °C; IR (KBr) υ (cm^{-1}): 3186 (−NH), 1667 (>C=O), 1326 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.33 (3H, s, $-CH_3$), 7.28–8.46 (8H, m, Ar−H), 8.24 (1H, s, −NH), 9.20 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 45.2 (C–$\underline{C}H_3$), 117, 120, 122, 125, 128, 130, 135, 136, 139, 144, 146 (aromatic carbons), 179.2 (>C=O), 189.3 (>C=S), 166.8 (>C=NH), 60 and 165 (C=C at pyrimidine ring); *m/z*: 335.07 (100.0%), 336.08 (19.7%), 337.07 (4.7%), 337.08 (2.4%), 336.07 (1.9%). Anal. Calcd for $C_{18}H_{13}N_3O_2S$: C, 64.46; H, 3.91; N, 12.53; S, 9.56. Found: C, 64.44; H, 3.88; N, 12.50; S, 9.53.

5.3.6. *4-Imino-9-methyl-3-phenyl-2-thioxo-1,2,3,4-tetrahydro-5H-chromeno[2,3-d] pyrimidine-5-one (**5f**)*

Yield 60%; mp 263–265 °C; IR (KBr) υ (cm^{-1}): 3142 (−NH), 1672 (>C=O), 1333 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.34 (3H, s, $-CH_3$), 7.12–8.33 (8H, m, Ar−H), 8.43 (1H, s, −NH), 9.10 (1H, s, −NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 40.9 (C−$\underline{C}H_3$), 117, 121, 122, 126, 128, 130, 134, 136, 139, 141, 146 (aromatic carbons), 175.2 (>C=O), 185.3 (>C=S), 165.8 (>C=NH), 68 and 167 (C=C at pyrimidine ring); *m/z*: 335.07 (100.0%), 336.08 (19.7%), 337.07 (4.7%), 337.08 (2.4%), 336.07 (1.9%). Anal. Calcd for $C_{18}H_{13}N_3O_2S$: C, 64.46; H, 3.91; N, 12.53; S, 9.56. Found: C, 64.49; H, 3.95; N, 12.58; S, 9.59.

5.4. *General procedure for synthesis of compounds 6(a–f)*

A solution of compound **3**(**a**–**f**) (5.0 mmol) and ammonium thiocyanate (15.0 mmol) in glacial acetic acid (20 ml) was refluxed on a sand bath for 8 h (monitored by TLC). After completion of the reaction, the reaction mixture was left to cool to room temperature and then poured into ice-cold water (50 ml) for complete precipitation. The product was separated by filtration and washed with water, dried well and recrystallized from methanol to give compounds **6**(**a**–**f**).

5.4.1. *1-(5-Oxo-2-thioxo-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-4-yl)thiourea (**6a**)*

Yield 75%; mp 284–286 °C; IR (KBr) υ (cm^{-1}): 3362 ($-NH_2$), 3140 ($-$NH), 1666 (>C=O), 1321 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.12–8.20 (4H, m, Ar$-$H), 8.45 (2H, s, $-$2NH), 9.31 (2H, br s, $-NH_2$); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 114, 120, 123, 125, 144, 152 (aromatic carbons), 180.2 (>C=S), 171 (>C=N at pyrimidine ring), 184 (>C=S at thiourea linkage), 178 (>C=O), 65 and 167 (>C=C at pyrimidine ring); m/z: 304.01 (100.0%), 305.01 (16.1%), 306.00 (9.0%), 307.01 (1.2%). Anal. Calcd for $C_{12}H_8N_4O_2S_2$: C, 47.36, H, 2.65, N, 18.41, S, 21.07. Found: C 47.42, H, 2.61, N, 18.34, S, 21.13.

5.4.2. *1-(7,9-Dibromo-5-oxo-2-thioxo-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-4-yl) thiourea (**6b**)*

Yield 78%; mp 219–221 °C; IR (KBr) υ (cm^{-1}): 3354 ($-NH_2$), 3157 ($-$NH), 1661 (>C=O), 1328 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.29–7.75 (2H, s, Ar$-$H), 8.02 (2H, s, $-$2NH), 9.02 (2H, br s, $-NH_2$); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 103 and 111 (C$-$Br), 115, 118, 125, 150 (aromatic carbon), 179.3 (>C=S), 169 (>C=N at pyrimidine ring), 185 (>C=S at thiourea linkage), 177 (>C=O), 67 and 170 (>C=C at pyrimidine ring); m/z: 461.83 (100.0%), 459.83 (48.9%), 463.83 (47.9%), 462.83 (14.6%), 463.82 (8.8%), 464.83 (8.0%), 460.83 (7.9%), 465.82 (4.5%), 462.82 (1.5%). Anal. Calcd for $C_{12}H_6N_4O_2Br_2S_2$: C, 31.19; H, 1.31; N, 12.12; S, 13.88. Found: C, 31.26; H, 1.34; N, 12.14; S, 13.83.

5.4.3. *1-(7-Nitro-5-oxo-2-thioxo-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-4-yl) thiourea (**6c**)*

Yield 67%; mp >300 °C; IR (KBr) υ (cm^{-1}): 3308 ($-NH_2$), 3149 ($-$NH), 1673 (>C=O) 1321 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.49–8.66 (3H, m, Ar$-$H), 8.00 (2H, s, $-$2NH), 8.98 (2H, br s, $-NH_2$); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 142.5 (C$-NO_2$), 114, 120, 125, 144, 152 (aromatic carbons), 181 (>C=S), 178 (>C=N at pyrimidine ring), 184 (>C=S at thiourea linkage), 175(C=O), 65 and 168 (C=C pyrimidine ring); m/z: 348.99 (100.0%), 350.00 (13.2%), 350.99 (9.3%), 349.99 (3.4%), 351.00 (1.8%), 351.99 (1.4%). Anal. Calcd for $C_{12}H_7N_5O_4S_2$: C, 41.26; H, 2.02; N, 20.05; S, 18.36. Found: C, 41.28; H, 2.07; N, 20.00; S, 18.34.

5.4.4. *1-(7-Chloro-5-oxo-2-thioxo-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-4-yl) thiourea (**6d**)*

Yield 74%; mp 279–281 °C; IR (KBr) υ (cm^{-1}): 3326 ($-NH_2$), 3206 ($-$NH), 1662 (>C=O) 1329 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.58–8.45 (3H, m, Ar$-$H), 8.43 (2H, s, –2NH), 9.45 (2H, br s, $-NH_2$); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 126.9 (C–Cl), 114, 118, 130, 135, 155 (aromatic carbon), 185 (>C=S), 180 (>C=N at pyrimidine ring), 185 (>C=S at

thiourea linkage), 178 (>C=O), 66 and 170 (>C=C at pyrimidine ring); *m/z*: 337.97 (100.0%), 339.97 (41.8%), 338.97 (16.1%), 340.97 (6.0%), 341.96 (3.1%). Anal. Calcd for $C_{12}H_7N_4O_2S_2Cl$: C, 42.54; H, 2.08; N, 16.54; S, 18.93. Found: C, 42.48; H, 2.14; N, 16.61; S, 18.87.

5.4.5. *1-(8-Methyl-5-oxo-2-thioxo-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-4-yl) thiourea (**6e**)*

Yield 77%; mp 265–267 °C; IR (KBr) υ (cm^{-1}): 3311 (−NH_2), 3193 (−NH), 1668 (>C=O) 1325 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.30 (3H, s, −CH_3), 7.66–7.87 (3H, m, Ar−H), 8.21 (2H, s, −2NH), 8.42 (2H, br s, −NH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 31.5 (C−$\underline{C}H_3$), 113, 122, 125, 129 130, 155 (aromatic carbons), 179.3 (>C=S), 169 (>C=N at pyrimidine ring), 185 (>C=S at thiourea linkage), 177 (>C=O), 67 and 170 (>C=C at pyrimidine ring); *m/z*: 318.02 (100.0%), 319.03 (14.3%), 320.02 (9.3%), 319.02 (3.1%), 320.03 (1.6%), 321.02 (1.5%). Anal. Calcd for $C_{13}H_{10}N_4O_2S_2$: C, 49.04; H, 3.17; N, 17.60; S, 20.14. Found: C, 48.96; H, 3.22; N, 17.56; S, 20.06.

5.4.6. *1-(9-methyl-5-oxo-2-thioxo-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-4-yl) thiourea (**6f**)*

Yield 71%; mp 247–249 °C; IR (KBr) υ (cm^{-1}): 3336 (−NH_2), 3207 (−NH), 1678 (>C=O) 1337 (>C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.33 (3H, s, −CH_3), 7.46–8.25 (3H, m, Ar−H), 8.65 (2H, s, −2NH), 8.96 (2H, br s, −NH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 32.5 (C−$\underline{C}H_3$), 114, 120, 123, 129, 135, (aromatic carbons), 181 (>C=S), 178 (>C=N at pyrimidine ring), 184 (>C=S at thiourea linkage), 175 (>C=O), 65 and 168 (C=C pyrimidine ring); *m/z*: 318.02 (100.0%), 319.03 (14.3%), 320.02 (9.3%), 319.02 (3.1%), 320.03 (1.6%), 321.02 (1.5%). Anal. Calcd for $C_{13}H_{10}N_4O_2S_2$: C, 49.04; H, 3.17; N, 17.60; S, 20.14. Found: C, 49.10; H, 3.13; N, 17.64; S, 20.17.

5.5. *Agar micro-dilution method*

The *in vitro* activity of the compounds against *M. tuberculosis H37Rv* [MTCC-200] was determined by the agar micro-dilution technique. Two-fold dilutions of each test compound were added to 7H10 agar and *M. tuberculosis H37Rv* was used as the test organism. MIC is the concentration of the compound that completely inhibits the growth and colony-forming ability of *M. tuberculosis*. In a 24-well plate, 3 ml of middle brook 7H11 agar medium with OADC 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 well was 62.5 μg/ml. The known colony-forming unit (CFU) of the *H37Rv* culture was dispensed on top of the agar in each well in a negative-pressure bio-safety hood. The plates were then incubated at 37 °C in a CO_2 incubator. The concentration at which the complete inhibition of colonies was observed was taken as the MIC of the test drug.

5.6. *The MIC*

The definition of the 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 the antimicrobial agent needed to prevent the microbial growth. The serial dilution technique was applied for the determination of MIC of the tested compounds against four species of bacterial strains *S. aureus* [MTCC-96] and

S. pyogenes [MTCC-442] as Gram-positive, *E. coli* [MTCC-443] and *P. aeruginosa* [MTCC-1688] as Gram-negative and three species of fungal strain *A. niger* [MTCC-282], *C. albicans* [MTCC-227] and A. Clavatus [MTCC-1323]. Dilution series was set up with 62.5, 100, 125, 150, 200, 250, 500 and 1000 μg/ml of the nutrient broth medium, and to each tube, 1000 μl of standardized suspension of the test microbes (107 cell/ml) was added and incubated at 37 °C for 24 h.

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