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ORIGINAL ARTICLE

Synthesis, antibacterial and antifungal activity of novel benzothiazole pyrimidine derivatives

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Abstract A new series of 5-amino-6-(benzo[*d*]thiazol-2-yl)-2-(2-(substitutedbenzylidene)hydrazinyl)-7-(4-chlorophenyl)pyrido[2,3-*d*]pyrimidin-4(3*H*)-one derivatives (**7a–k**) were synthesized. All the newly synthesized compounds were screened for their *in vitro* antibacterial activity, against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Streptococcus pyogenes* and for antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium marneffei* and *Mucor*. Compounds **7b, 7e, 7f, 7g, 7h** and **7j** showed excellent *in vitro* antibacterial activity and antifungal activity than the standard drugs. All the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, LCMS mass and C, H, N analyses.

1. Introduction

Pyrimidine which is an integral part of DNA and RNA imparts diverse pharmacological properties as effective bactericide and fungicide (Williams and Cline, 1936; Maddila and Jonnalagadda, 2012a,b). Many pyrimidine derivatives are known to exhibit analgesic (Regnier et al., 1972), antihypertensive (Winter et al., 1962), anti-tumour (Suguira et al., 1973), antimalarial (Brown and Evans, 1985), antioxidant (Stefani et al., 2006), antimitotic (Mayer et al., 1999), and anti-HIV activities (Okabe et al., 1991). Some dihydropyrimidines (DHPM) have emerged as integral backbones of calcium channel blockers, antihypertensive agents, adrenergic and neuropeptide antagonists (Pasha et al., 2005). Several alkaloids containing dihydropyrimidine isolated from marine sources such as batzelladine alkaloids are reported to be potent HIV-gp-120-CD4 inhibitors (Kappe et al., 2000; Kappe et al., 2000; Patil et al., 1995).

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In addition to their diverse biological activities, in association with other heterocyclics, pyrimidines are known to play a crucial role in several processes of chemical and pharmacological importance as therapeutics in clinical applications. A literature survey reveals that thiazole derivatives of pyrimidine have received much attention in recent years, due to their efficacy as analgesic, anti-inflammatory, ulcerogenic (Hafez and El-Gazzar, 2008), antifungal (Maddila et al., 2011a,b, 2016), antitubercular (Kapustina et al., 1991), antimalarial (Rosowsky et al., 1973), antitumour (Sasaki et al., 2003), cytotoxic (Suh et al., 2000), and anticancer agents (Skibo et al., 2002).

In the view of having a wide scope to find new potentially active agents, we have synthesized a new series of benzothiazole pyrimidine derivatives (**7a–k**) which is an extension of our previous reported work on biological studies of novel 2-(4-substitutedbenzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)pyrido[2,3-*d*]-pyrimidin-4(3*H*)-one (Maddila et al., 2011). All the new compounds were characterized by elemental and spectral analyses and screened for their antibacterial and anti- fungal abilities.

2. Results and discussion

2.1. Chemistry

For the synthesis of the new materials, initially, 2-(benzo[*d*]thiazol-2-yl)acetonitrile **(1)** was treated with cyanoacetic acid in the presence of acetic anhydride to give their corresponding 2-(benzo[*d*]thiazol-2-yl)-3-oxopentane dinitrile (**2**). Compound **2** was reacted with *p*-chlorobenzaldehyde in the presence of piperidine to obtain 2-(benzo[*d*]thiazol-2-yl)-3-(4-chlorophenyl)-acrylonitrile **(3)** (Saito et al., 1983). Compound **3** by cyclization with 6-aminothiouracil in the presence of few drops of piperidine, was converted into 5-amino-6-(benzo[*d*]thiazol-2-yl)-7-(4-chlorophenyl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one **(4)** with good yield. Compound **4** was treated with benzyl bromide in the presence of K_2CO_3 and DMF medium to give its benzylthio derivative (**5**) in good yield (Maddila et al., 2011b).

The benzylthio compound was successfully reacted with hydrazine hydrate to afford its hydrazinyl derivative (**6**). The hydrazinyl derivative was condensed with a range of selected substituted aldehydes in the presence of DMF solvent, to yield a new series of corresponding Schiff bases in good yields (**7a**−**k**) (Scheme 1).

All the newly synthesized compounds gave moderate to high yields. Products were purified and characterized by various spectroscopic techniques. The IR spectra of compounds (7a–k) showed characteristic absorption bands at 3460–3387 cm^{-1}, 1679–1609 cm^{-1}, 1623–1561, and 1548–1520 cm^{-1} corresponding to the $N–H_{2str}$, $C{=}O_{str}$, $C{=}N_{str}$ and $C{=}C_{str}$ functions in the structures. The ^1H NMR spectra showed peaks in the range of δ 6.09–6.29 for NH_2, δ 8.01–8.71 for N=CH, δ 9.91–10.05 for hydrazinyl NH and δ 10.17–10.25 for pyrimidine NH. The mass spectrum of all the compounds showed a molecular ion peak at M^+, at M + H corresponding to its molecular formula, which confirmed its chemical structure. IR, ^1H NMR, LCMS mass spectra and elemental analysis confirmed the structure of various novel 5-amino-6-(benzo[d] thiazol-2-yl)-2-(2-(substitutedbenzylidene)hydrazinyl)-7-(4-chlorophenyl)pyrido[2,3-*d*]pyrimidin-4(3*H*)-one derivatives (**7a–k**).

3. Pharmacological assay

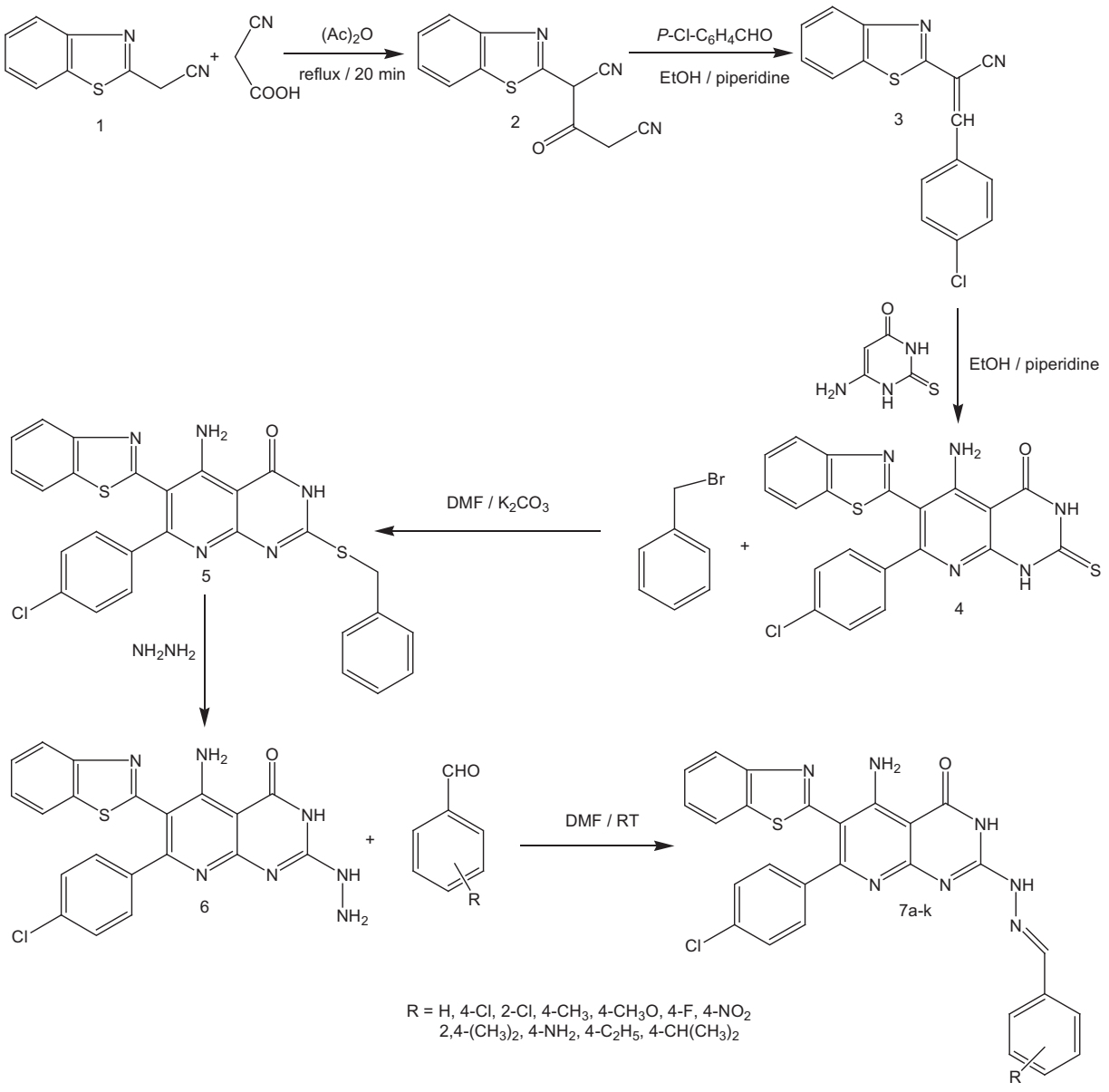
3.1. Antimicrobial activity

All the compounds were screened for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Streptococcus pyogenes* and antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium marneffei* and *Mucor*. Compounds **7b–k** with various substituents in the aromatic ring will give an insight into the steric and electronic effects on the biological activity.

3.2. Evaluation of antibacterial activity

The *in vitro* antibacterial activity of the compounds was tested in nutrient broth for bacteria by the twofold serial dilution method (Vincent and Vincent, 1944). The test compounds were dissolved in dimethyl sulphoxide (DMSO) to obtain 1 mg/ml stock solutions. Seeded broth (broth containing microbial spores) were prepared in NB from 24 h old bacterial cultures on nutrient agar at 37 ± 1 °C. The colony forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^4–10^5 cfu/ml. The antibacterial assay was performed at pH 7.4 ± 0.2, with the final inoculum size of 10^5 cfu/ml. 0.20 ml of the solution of the test compound was added to 1.80 ml of seeded broth to form the first dilution. One millilitre of it was diluted with an equal volume of the seeded broth to give the second dilution. The dilution was continued in one ml increments to obtain six such dilutions. A set of assay tubes containing seeded broth were kept as controls and likewise solvent controls were also run simultaneously. The tubes were incubated in biochemical oxygen demand (BOD) incubators at 37 ± 1 °C for bacteria. The minimum inhibiting concentrations (MICs) were recorded by visual observations after 24 h. Ciprofloxacin was used as a standard for the antibacterial study.

For evaluating the antibacterial activity ciprofloxacin was used as the standard drug. The observed minimum inhibitory concentrations (MICs) are given in Table 1. In general, all the compounds exerted a modest to good antibacterial activity *in vitro* against the tested organisms. Compound **7a** without any substituent in the aryl moiety exhibited antibacterial activity *in vitro* at 100 μg ml^{-1} against *P. aeruginosa,* but its antibacterial activity against the other tested organisms was only at 200 μg ml^{-1}. However **7b**, in which the hydrogen at the para position of the aryl moiety is replaced by chlorine, showed a very good activity against all the tested organisms in the range of 25–50 μg ml^{-1}. Compound **7c**, which had the chlorine in the ortho position, exhibited similar activity as **7b**. Undeniably the compounds **7b–k**, bearing a substituent in the aryl group, are more active than the parent compound, **7a**. Compound **7a** and its isopropyl analogue **7k** have the similar moderate activity against *K. pneumoniae*. However, **7k** showed higher activity than **7a** against all the other tested organisms. Interestingly, **7**f with a nitro substituent, **7i** with an amide and **7g** with fluorine all in the para position, exhibited higher activity relative to Ciprofloxacin.



Scheme 1 Synthesis pathway of benzothiazole pyrimidine derivatives.

Table 1 In vitro antibacterial activity of compounds (**7a–k**).

Compound	Minimum inhibitory concentration (MIC) in μg ml^{-1}				
	S. aureus	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. pyogenes*
7a	200	200	200	100	200
7b	25	25	50	50	25
7c	25	25	50	50	25
7d	50	50	100	100	100
7e	50	50	100	50	50
7f	12.5	25	25	25	12.5
7g	12.5	12.5	12.5	12.5	12.5
7h	100	100	100	100	50
7i	25	12.5	12.5	25	12.5
7j	50	100	50	25	100
7k	100	50	200	100	50
Ciprofloxacin	25	25	50	25	12.5

3.3. Evaluation of antifungal activity

The *in vitro* antifungal activity of the compounds was examined in Sabouraud's dextrose broth for fungi by the two-fold serial dilution method (Vincent and Vincent, 1944). The test compounds were dissolved in dimethyl sulphoxide (DMSO) to obtain 1 mg/ml stock solutions. Seeded broth (broth containing microbial spores) was prepared by suspending 24 h to 7-day old Sabouraud's agar slant cultures in SDB. The colony forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^4–10^5 cfu/ml. For antifungal assay the final inoculum size was 1.1–1.5×10^2 cfu/ml. Testing was carried out by the same procedure as done for antibacterial studies except the temperature which was maintained at 28 ± 1 °C for about 72–96 h of incubation. Clotrimazole was used as a standard for the antifungal study.

For evaluating the antifungal activity clotrimazole was used as the standard drug. The observed minimum inhibitory concentrations (MICs) are given in Table 2. **7a** showed no activity against *A. fumigates* or *C. albicans* even at 200 μg ml^{-1} concentrations. However, it exhibited antifungal activity against all the other tested fungi in the range of 100–200 μg ml^{-1}. Compounds **7b–k** which have substituents in the 4-aryl group were more active than the parent compound, **7a** against all the tested fungi. The *o*-chloro compound, **7c** showed less activity compared to *p*-chloro compound, **7b** against *A. fumigates* and *P. marneffei*. However, against all the tested fungi, both **7b** and **7c** displayed good activity. Against all the tested organisms, compounds **7f**, **7g** and **7i** exhibited higher activity than the standard drug Clotrimazole.

3.4. Influence of aromatic substituent

The results suggest that the antibacterial and antifungal activities are distinctly influenced by the aromatic substituents. Compounds **7b**, **7c**, **7f**, **7g** and **7i** with electron-withdrawing substituents in the aromatic ring, displayed greater antibacterial activity than the other six compounds against all the tested organisms. Furthermore, compounds **7f**, **7g** and **7i** showed greater antifungal activity relating to all the other compounds against all the tested organisms. The aromatic substituents in **7b**, **7c**, **7f**, **7g** and **7i** had positive values[25] for the Hammett substituent constant, σ_p.

4. Experimental

All reagents and solvents were purchased and used without further purification. Melting points determined on a Fisher–Johns melting point apparatus were uncorrected. Crude products were purified by column chromatography on silica gel of 60–120 mesh. IR spectra were obtained on a Perkin Elmer BX series FT-IR 5000 spectrometer using KBr pellet. The ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer for ^{1}H NMR. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker 100 MHz spectrometer. LCMS Mass spectra were recorded on a MASPEC low resolution mass spectrometer operating at 70 eV.

4.1. 2-(Benzo[d]thiazol-2-yl)-3-oxopentanedinitrile (2)

The mixture of cyanoacetic acid (0.01 mol) and acetic anhydride (15 mL) was heated on a water bath for 5 min and 2-(benzo[*d*]thiazol-2-yl)acetonitrile (**1**) (0.01 mol) was added. The reaction mixture was refluxed for 20 min at 85–95 °C left to cool, and the formed solid was filtered off, dried and recrystallized from ethanol to afford 1.69 g (70%) of **2**; mp 275 °C; brown crystals; IR (υ cm^{-1}, KBr): 2185, 2199 (2CN), 1700 (CO). ^{1}H NMR (DMSO-d_6) δ 6.67-7.50 (m, 4H, Ar-H), 5.10 (s, 1H, CH), 4.52 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.2, 162.1, 148.3, 134.2, 123.4, 122.1, 120.3, 120.1, 117.2, 116.5, 48.2, 34.4; LCMS: (*m*/*z*,%): 241 [M^+]. *Anal*. Calcd. for $C_{12}H_7N_3OS$: C, 59.74; H, 2.92; N, 17.42. Found: C, 59.92; H, 2.97; N, 17.53.

4.2. 2-(Benzo[d]thiazol-2-yl)-3-(4-chlorophenyl)-acrylonitrile (3)

To a solution of **2** (0.01 mol) and *p*-chlorobenzaldehyde (0.01 mol) in ethanol (20 mL), a few drops of piperidine were added. The reaction mixture was refluxed for 4 h, then left to cool. The precipitate formed was filtered off, washed, and recrystallized by ethanol to afford 2.11 g (71%) of **3**; mp 148–150 °C; yellow powder; IR (υ cm^{-1}, KBr): 3119 (NH),

Table 2 In vitro antifungal activity of compounds (7a–k).

Compound	Minimum inhibitory concentration (MIC) in μg ml^{-1}				
	A. flavus	*A. fumigatus*	*C. albicans*	*P. marneffei*	*Mucor*
7a	100	–	–	200	200
7b	25	50	50	25	50
7c	25	100	50	50	50
7d	100	200	50	50	100
7e	100	50	50	50	100
7f	12.5	12.5	25	25	12.5
7 g	12.5	12.5	12.5	12.5	12.5
7 h	100	200	100	100	200
7i	25	12.5	25	12.5	25
7j	50	100	50	50	50
7 k	100	50	100	100	100
Clotrimazole	25	25	50	25	50

–, no inhibition even at a higher concentration of 200 μg ml^{-1}.

2188 (CN). ^{1}H NMR (DMSO-d_6) δ 7.32-8.12 (m, arom. + vinylic H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.5, 152.5, 150.6, 148.2, 134.3, 133.2, 132.8, 128.3, 127.6, 124.5, 123.7, 120.9, 117.3, 109.2; LCMS: (*m*/*z*,%): 298 [M + H]. *Anal*. Calcd. for $C_{16}H_9ClN_2S$: C, 64.75; H, 3.06; N, 9.44. Found: C, 64.84; H, 3.17; N, 9.51.

4.3. 5-Amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)-2-thioxo-2,3-dihydropyrido-[2,3-d]-pyrimidin-4(1H)-one (4)

To a mixture of **3** (0.01 mol) and 6-aminothiouracil (0.01 mol) in ethanol (15 mL), a catalytic amount of piperidine (few drops) was added. The reaction mixture was refluxed for 6 h, allowed to cool and poured into ice cold water. The precipitate obtained was filtered off, dried and recrystallized from ethanol. Pale yellow powder; Yield 62%; mp 230–231 °C; IR (υ cm^{-1}, KBr): 3402, 3389 (NH_2), 3141 (NH), 1698 (CO), 1221 (C═S). ^{1}H NMR (DMSO-d_6): δ 13.59 (s, 1H, NH), 13.51 (s, 1H, NH), 7.32-8.49 (m, 8H, Ar-H), 6.22 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.2, 162.0, 156.6, 154.2, 152.6, 150.7, 148.3, 135.0, 133.5, 131.5, 128.3, 128.1, 124.4, 123.6, 120.3, 120.0, 109.2, 92.3; LCMS: (*m*/*z*,%): 438 [M$^+$]. *Anal*. Calcd. for $C_{20}H_{12}ClN_5OS_2$: C, 54.84; H, 2.76; N, 15.99. Found: C, 54.94; H, 2.82; N, 16.04.

4.4. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(benzylthio)-7-(4-chlorophenyl)pyrido[2,3-d] pyrimidin–4(3H)-one (5)

An ice cold solution of cyclic compound of **4** (0.01 mol) in DMF (15 ml), potassium carbonate (0.03 mol) and benzyl bromide (0.02 mol) was taken in a 250 ml round bottomed flask equipped with magnetic stirrer and stirred for 3 h. The residual portion was poured on crushed ice, neutralized with dilute acid and the product obtained (**5**) was collected by filtration.

Pale yellow solid; Yield 69%; mp 196–198 °C; IR (υ cm^{-1}, KBr): 3402-3389 (NH_2), 3141 (NH), 1698 (CO) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.58 (s, 1H, NH), 7.32-8.49 (m, 13H, Ar-H), 6.23 (s, 2H, NH_2), 4.10 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.1, 160.2, 156.8, 154.2, 152.6, 150.9, 150.1, 149.3, 138.2, 133.4, 133.0, 131.4, 128.5, 128.2, 127.7, 127.3, 126.7, 126.2, 124.4, 123.6, 122.1, 109.2, 34.5; LCMS: (*m*/*z*) 528 [M + H]. *Anal*. Calcd. for $C_{27}H_{18}ClN_5OS_2$: C, 61.41; H, 3.44; N, 13.26. Found: C, 61.55; H, 3.53; N, 13.39.

4.5. 5-Amino-6-(benzo[d]thiazol-2-yl)-7-chloro-2-hydrazinylpyrido[2,3-d]pyrimidin-4(3H) one (6)

A mixture of compound **6** (0.01 mol) and hydrazine hydrate 85% (0.03 mol) in 2-propanol (25 ml) was refluxed for 3 h. After cooling, the precipitate was filtered off, recrystallized from alcohol, and dried in vacuum over P_2O_5 to give a brown solid.

Yield: 80%; mp 152–154 °C; IR (υ cm^{-1}, KBr): 3325 (NH–NH_2), 1738 (C═O), 1618 (C═N), 1532 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H, NH–pyrimidine), 8.2–8.3 (d, 2H, NH_2–hydrazinyl), 8–8.2 (t, 1H, NH–hydrazinyl), 7.51–7.98 (m, 4H, Ar–H), 6.11 (s, 2H, NH_2),; ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.2, 162.1, 156.6, 154.3, 153.6, 151.5, 149.9, 133.6, 132.2, 131.7, 128.5, 128.1, 126.3, 126.1, 124.3, 121.2, 120.6, 117.1, 109.5; LCMS: (*m*/*z*) 360 (M + H, 100%). *Anal*. Calcd. for $C_{14}H_{10}ClN_7OS$: C, 46.73; H, 2.80; N, 27.25. Found: C, 46.61; H, 2.87; N, 27.33.c

4.6. 5-amino-6-(benzo[d]thiazol-2-yl)-2-(2-(substitutedbenzylidene)hydrazinyl)-7-(4-chlorophenyl)pyrido[2,3-d]pyrimidin-4(3H)-one derivatives (7a–k)

A mixture of hydrazinyl compound **6** (0.01 mol) and the appropriate aldehyde (0.03 mol) in DMF (20 ml) was stirred at room temperature for 10 h. After the reaction time, the solution was evaporated under reduced pressure and the residue was triturated with water to yield corresponding pure compounds, which were collected by filtration and recrystallized from alcohol.

4.6.1. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-benzylidenehydrazinyl)-7-(4-chlorophenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7a)

Yield: 76%; mp 254–256 °C; IR (υ cm^{-1}, KBr): 3402 (NH_2), 1665 (C═O), 1578 (C═N), 1523 (C═C), cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, NH–pyrimidine), 9.98 (s, 1H, NH–hydrazinyl), 8.15–8.42 (m, 4H, Ar–H), 8.03 (s, 1H, N═CH), 7.45–7.79 (m, 9H, Ar–H), 6.09 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.0, 162.6, 159.2, 155.4, 154.2, 153.1, 151.1, 147.1, 135.3, 134.5, 133.8, 133.1, 131.7, 130.5, 130.2, 129.9, 129.3, 126.2, 125.8, 122.9, 122.2, 119.1, 109.3; LCMS: (*m*/*z*) 524 (M + H, 100%). *Anal*. Calcd. for $C_{27}H_{18}ClN_7OS$: C, 67.19; H, 5.65; N, 11.09. Found: C, 67.24; H, 5.59; N, 11.17.

4.6.2. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(4-chlorobenzylidene)hydrazinyl)-7-(4-chlorophenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7b)

Yield: 85%; mp 201–203 °C; IR (υ cm^{-1}, KBr): 3460 (NH_2), 1609 (C═O), 1594 (C═N), 1547 (C═C), 712 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.20 (s, 1H, NH–pyrimidine), 10.01 (s, 1H, NH–hydrazinyl), 8.11–8.43 (m, 4H, Ar–H), 8.05 (s, 1H, N═CH), 7.48–7.75 (m, 8H, Ar–H), 6.21 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.1, 162.7, 159.3, 155.4, 154.3, 153.2, 151.1, 147.1, 137.3, 135.3, 134.6, 133.1, 132.6, 131.6, 130.5, 129.9, 129.9, 126.2, 125.8, 122.8, 122.2, 119.1, 109.2; LCMS: (*m*/*z*) 558 (M + H, 100%). *Anal*. Calcd. for $C_{27}H_{17}Cl_2N_7OS$: C, 65.65; H, 5.70; N, 10.56. Found: C, 65.73; H, 5.61; N, 10.48.

4.6.3. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(2-chlorobenzylidene)hydrazinyl)-7-(4-chloro phenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7c)

Yield: 69%; mp 191–192 °C; IR (υ cm^{-1}, KBr): 3387 (NH_2), 1648 (C═O), 1575 (C═N), 1538 (C═C), 715 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.21 (s, 1H, NH–pyrimidine), 10.05 (s, 1H, NH–hydrazinyl), 8.10–8.45 (m, 4H, Ar–H), 8.71 (s, 1H, N═CH), 7.39–7.75 (m, 8H, Ar–H), 6.19 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.2, 162.8, 159.3, 155.4, 154.2, 153.1, 151.1, 147.1, 135.3, 134.6, 133.6, 133.1, 132.6, 131.6, 130.3, 129.9, 129.7, 127.9, 126.3, 125.8, 122.8, 122.3, 119.1, 109.3; LCMS: (*m*/*z*) 558 (M + H, 100%). *Anal*. Calcd. for $C_{27}H_{17}Cl_2N_7OS$: C, 68.62; H, 6.31; N, 10.56. Found: C, 68.55; H, 6.25; N, 10.63.

4.6.4. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(4-methylbenzylidene)hydrazinyl)-7-(4-chloro -phenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7d)

Yield: 87%; mp 235 °C; IR (υ cm^{-1}, KBr): 3412 (NH_2), 1660 (C═O), 1569 (C═N), 1528 (C═C), cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.17 (s, 1H, NH–pyrimidine), 9.91 (s, 1H, NH–hydrazinyl), 8.15–8.42 (m, 4H, Ar–H), 8.01 (s, 1H, N═CH), 7.26–7.68 (m, 8H, Ar–H), 6.20 (s, 2H, NH_2), 2.21 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.1, 162.6, 159.3, 155.4, 154.2, 153.1, 151.0, 147.1, 139.9, 135.2, 134.5, 133.1, 131.2, 130.3, 130.2 130.1, 129.8, 126.3, 125.7, 122.8, 122.3, 119.1, 109.2, 23.8; LCMS: (*m*/*z*) 538 (M + H, 100%). *Anal*. Calcd. for $C_{28}H_{20}ClN_7OS$: C, 69.04; H, 6.52; N, 10.06. Found: C, 70.07; H, 6.44; N, 10.16.

4.6.5. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(4-methoxybenzylidene)hydrazinyl)-7-(4-chloro-phenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7e)

Yield: 84%; mp 209–211 °C; IR (υ cm^{-1}, KBr): 3452 (NH_2), 1648 (C═O), 1576 (C═N), 1541 (C═C), 1210 (C–O–C) cm $^{-1}$; ^1H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, NH–pyrimidine), 10.03 (s, 1H, NH–hydrazinyl), 8.12–8.42 (m, 4H, Ar–H), 8.06 (s, 1H, N═CH), 7.01–7.78 (m, 8H, Ar–H), 6.23 (s, 2H, NH_2), 3.78 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.2, 165.41 162.7, 159.3, 155.4, 154.2, 153.1, 151.1, 147.1, 135.3, 134.6, 133.1, 131.4, 130.8, 129.8, 127.2, 126.3, 125.7, 122.9, 122.2, 119.1, 113.7, 109.3, 57.6; LCMS: (*m*/*z*) 553 (M^+, 100%). *Anal*. Calcd. for $C_{28}H_{20}ClN_7O_2S$: C, 65.65; H, 5.70; N, 10.56. Found: C, 65.73; H, 5.79; N, 10.61.

4.6.6. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(4-fluorobenzylidene)hydrazinyl)-7-(4-chloro phenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7f)

Yield: 91%; mp 186–187 °C; IR (υ cm^{-1}, KBr): 3438 (NH_2), 1650 (C═O), 1606 (C═N), 1548 (C═C), 1010 (C–F) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.21 (s, 1H, NH–pyrimidine), 10.01 (s, 1H, NH–hydrazinyl), 8.16–8.38 (m, 4H, Ar–H), 8.06 (s, 1H, N═CH), 7.40–7.77 (m, 8H, Ar–H), 6.24 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.3, 167.2, 162.9, 159.8, 155.4, 154.3, 153.1, 151.1, 147.2, 135.4, 134.7, 133.2, 131.7, 130.5, 130.4, 129.9, 126.3, 125.9, 122.9, 122.3, 119.1, 116.3, 109.4; LCMS: (*m*/*z*) 542 (M + H, 100%). *Anal*. Calcd. for $C_{27}H_{17}ClFN_7OS$: C, 64.28; H, 5.75; N, 9.98. Found: C, 64.39; H, 5.66; N, 10.07.

4.6.7. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(4-nitrobenzylidene)hydrazinyl)-7-(4-chloro phenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7g)

Yield: 79%; mp 224–226 °C; IR (υ cm^{-1}, KBr): 3408 (NH_2), 1669 (C═O), 1574 (C═N), 1520 (C═C), cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.25 (s, 1H, NH–pyrimidine), 10.05 (s, 1H, NH–hydrazinyl), 8.12–8.40 (m, 4H, Ar–H), 8.08 (s, 1H, N═CH), 7.48–7.56 (m, 8H, Ar–H), 6.29 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.3, 163.0, 159.4, 155.5, 154.3, 153.2, 151.2, 149.7, 147.2, 141.1, 135.4, 134.7, 133.2, 131.6, 130.4, 129.9, 126.3, 115.2, 122.2, 122.3, 121.3, 119.1, 109.4; LCMS: (*m*/*z*) 569 (M + H, 100%). *Anal*. Calcd. for $C_{27}H_{17}ClN_8O_3S$: C, 67.11; H, 6.35; N, 9.78. Found: C, 66.98; H, 6.27; N, 9.83.

4.6.8. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(2,4-dimethylbenzylidene)hydrazinyl)-7-(4-chlorophenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7h)

Yield: 85%; mp 168–170 °C; IR (υ cm^{-1}, KBr): 3430 (NH_2), 1665 (C═O), 1580 (C═N), 1532 (C═C), cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, NH–pyrimidine), 9.98 (s, 1H, NH–hydrazinyl), 8.11–8.40 (m, 4H, Ar–H), 8.59 (s, 1H, N═CH), 7.03–7.55 (m, 7H, Ar–H), 6.18 (s, 2H, NH_2), 2.41 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.1, 162.6, 159.3, 155.4, 154.2, 153.1, 151.0, 147.1, 141.2, 138.8, 135.3, 134.6, 133.2, 131.8, 133.4, 129.9, 129.7, 126.9, 126.3, 125.7, 124.4, 122.8, 122.2, 119.1, 109.1, 25.0, 17.4; LCMS: (*m*/*z*) 551 (M^+, 100%). *Anal*. Calcd. for $C_{29}H_{22}ClN_7OS$: C, 67.56; H, 6.53; N, 9.55. Found: C, 67.48; H, 6.59; N, 9.63.

4.6.9. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(4-aminobenzylidene)hydrazinyl)-7-(4-chloro phenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7i)

Yield: 77%; mp 221–223 °C; IR (υ cm^{-1}, KBr): 3450 (NH_2), 1679 (C═O), 1623 (C═N), 1528 (C═C), cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.21 (s, 1H, NH–pyrimidine), 10.02 (s, 1H, NH–hydrazinyl), 8.14–8.40 (m, 4H, Ar–H), 8.04 (s, 1H, N═CH), 6.81–7.52 (m, 8H, Ar–H), 6.27 (s, 2H, NH_2), 5.47 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.3, 162.9, 159.3, 155.4, 154.3, 153.2, 151.0, 151.0, 147.2, 135.3, 134.6, 133.2, 131.4, 130.4, 129.9, 126.3, 125.8, 124.4, 122.9, 122.3, 119.1, 118.3, 109.4; LCMS: (*m*/*z*) 539 (M + H, 100%). *Anal*. Calcd. for $C_{27}H_{19}ClN_8OS$: C, 68.60; H, 6.32; N, 10.32. Found: C, 68.73; H, 6.41; N, 10.25.

4.6.10. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(4-ethylbenzylidene)hydrazinyl)-7-(4-chloro phenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7j)

Yield: 69%; mp 218–219 °C; IR (υ cm^{-1}, KBr): 3432 (NH_2), 1629 (C═O), 1561 (C═N), 1543 (C═C) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.18 (s, 1H, NH–pyrimidine), 9.97 (s, 1H, NH–hydrazinyl), 8.12–8.40 (m, 4H, Ar–H), 8.02 (s, 1H, N═CH), 7.31–7.71 (m, 8H, Ar–H), 6.22 (s, 2H, NH_2), 2.52 (q, 2H, CH_2), 1.27 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.2, 162.7, 159.3, 155.4, 154.2, 153.2, 151.1, 145.1, 142.1, 135.3, 134.6, 133.1, 132.1, 130.3, 129.8, 129.4, 128.3, 126.3, 125.7, 122.6, 122.2, 119.1, 109.2, 31.3, 15.1; LCMS: (*m*/*z*) 552 (M + H, 100%). *Anal*. Calcd. for $C_{29}H_{22}ClN_7OS$: C, 67.11; H, 6.34; N, 9.79. Found: C, 67.23; H, 6.42; N, 9.87.

4.6.11. 5-Amino-6-(benzo[d]thiazol-2-yl)-2-(2-(4-isopropylbenzylidene)hydrazinyl)-7-(4-chloro phenyl)pyrido[2,3-d]pyrimidin-4(3H)-one (7k)

Yield: 78%; mp 206–208 °C; IR (υ cm^{-1}, KBr): 3421 (NH_2), 1650 (C═O), 1574 (C═N), 1523 (C═C), cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.18 (s, 1H, NH–pyrimidine), 9.96 (s, 1H, NH–hydrazinyl), 8.10–8.44 (m, 4H, Ar–H), 8.04 (s, 1H, N═CH), 7.33–7.71 (m, 8H, Ar–H), 6.20 (s, 2H, NH_2), 2.79–2.81 (m, 1H, CH), 1.19 (d, J = 7.6 Hz, 6H, $(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.2, 162.7, 159.3, 155.4, 154.2, 153.1, 151.7, 151.1, 147.1, 135.3, 134.6, 133.1, 131.8, 130.4, 129.5, 129.3, 127.5, 126.3, 125.7, 122.8, 122.3, 119.1, 109.2, 39.3, 21.4; LCMS: (*m*/*z*) 565 ($(M)^+$, 100%). *Anal*. Calcd. for $C_{30}H_{24}ClN_7OS$: C, 69.83; H, 6.89; N, 9.58. Found: C, 69.91; H, 6.78; N, 9.49.

5. Conclusion

In conclusion, we have described a simple and efficient protocol for the synthesis of novel benzothiazole pyrimidine derivatives (**7a–k**) with good yields. The antibacterial and antifungal activities of all the synthesized compounds were investigated. It is evident that newly synthesized compounds, **7b**, **7e**, **7f**, **7g**, **7h** and **7j** have excellent antibacterial and antifungal activities. For the above compounds a significant improvement in their antibacterial and anti fungal activities has been examined over the earlier reported compounds (Maddila et al., 2011). These novel class of new benzothiazole pyrimidine derivatives reported have a probability to emerge as a valuable lead series with great potential to be used as antibacterial and antifungal agents, and as promising candidates for further efficacy evaluation.

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References

Brown, D.J., Evans, R.F., 1985. The Pyrimidines. In: Weissberger, A., Taylor, E.C. (Eds.), The Chemistry of Heterocyclic Compounds. John Wiley & Sons Inc, New Jersey.

Hafez, H.N., El-Gazzar, A.B.A., 2008. Eur. J. Med. Chem. 35, 1043–1052, Bioorg. Med. Chem. Lett. 18, 5222–5227. Kappe, C.O., 2000.

Kappe, C.O., Shishkin, O.V., George, U., Petra, V., 2000. Tetrahedron 56, 1859–1862.

Kapustina, M.V., Omelkin, Yu.O., Kharizomenova, I.A., Shvedov, V.I., Felitis, L.N., 1991. Khim.-farm Zh 25, 38–42.

Maddila, S., Jonnalagadda, S.B., Rao, C.V., 2011a. Orient. J. Chem. 27 (1), 127–133.

Maddila, S., Lavanya, P., Raju, K.N., Jonnalagadda, S.B., Rao, C.V., 2011b. Org. Commun. 4 (2), 33–41.

Maddila, S., Jonnalagadda, S.B., 2012a. Arch. Pharm. Chem. Life Sci. 345, 163–168.

Maddila, S., Jonnalagadda, S.B., 2012b. Bull. Chem. Soc. Ethiopia 26 (1), 121–126.

Maddila, S., Lavanya, P., Rao, C.V., 2016. Arabian. J. Chem. 9 (1), 136–142.

Mayer, T.U., Kapoor, T.M., Haggarty, S.J., King, R.W., Schreiber, S.I., Mitchison, T.J., 1999. Science 286, 971–974.

Okabe, M., Sun, R.C., Zenchoff, G.B., 1991. J. Org. Chem. 56, 4393–4395.

Pasha, M.A., Ramchandra, S.M., Jayashankara, V.P., 2005. Indian J. Chem. 44B, 823–826.

Patil, A.D., Kumar, N.V., Kokke, W.C., Mark, F.B., Alan, J.F., Charles, D.B., 1995. J. Org. Chem. 60, 1182–1188.

Regnier, G., Canevar, L., Le, R.J., Douarec, J.C., Halstop, S., Daussy, J., 1972. J. Med. Chem. 15, 295–298.

Rosowsky, A., Chen, K.N.N., Lin, M., 1973. J. Med. Chem. 16, 191–196.

Saito, K., Kambe, S., Nakano, Y., Sakurai, A., Midorikawa, H., 1983. Synthesis 3, 210–212.

Sasaki, S., Cho, N., Nara, Y., Harada, M., Endo, S., Suzuki, N., Furuya, Sh., Fujino, M., 2003. J. Med. Chem. 46, 113.

Skibo, E.B., Huang, X., Martinez, R., Lemus, R.H., Craigo, W.A., Dorr, R.T., 2002. J. Med. Chem. 45, 5543–5555.

Stefani, H.A., Oliveira, C.B., Almeida, R.B., Pereira, C.M.P., Braga, R.C., Cella, R., Borges, V.C., Savegnago, L., Nogueira, C.W., 2006. Eur. J. Med. Chem. 41, 513–518.

Suguira, K., Schmid, A.F., Schmid, M.M., Brown, F.G., 1973. Cancer Chemother. Rep. Part 23 (1), 231–233.

Suh, M., Kang, M., Yoo, H., Park, S., 2000. Bioorg. Med. Chem. 8, 2079–2083.

Vincent, J.C., Vincent, H.W., 1944. Proc. Soc. Exp. Biol. Med. 55, 162–164.

Williams, R.R., Cline, J.K., 1936. J. Am. Chem. Soc. 58, 1504–1505.

Winter, C.A., Fisley, E.A.R., Nuss, G.W., 1962. Proc. Soc. Exp. Biol. Med. 111, 544–547.