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Research Article

Synthesis of Some New Pyrimidine-Azitidinone Analogues and Their Antioxidant, *In Vitro* Antimicrobial, and Antitubercular Activities

Mallikarjunaswamy Chandrashekaraiah,[1, 2] Mallesha Lingappa,[1] Vathsala Deepu Channe Gowda,[2] and Doddamedur Giddegowda Bhadregowda[2]

[1] *PG Department of Chemistry, JSS College of Arts, Commerce and Science, Ooty Road, Mysore 570025, India*
[2] *Department of Chemistry, Yuvaraja's College, University of Mysore, Mysore 570005, India*

Correspondence should be addressed to Doddamedur Giddegowda Bhadregowda; dgbycm@gmail.com

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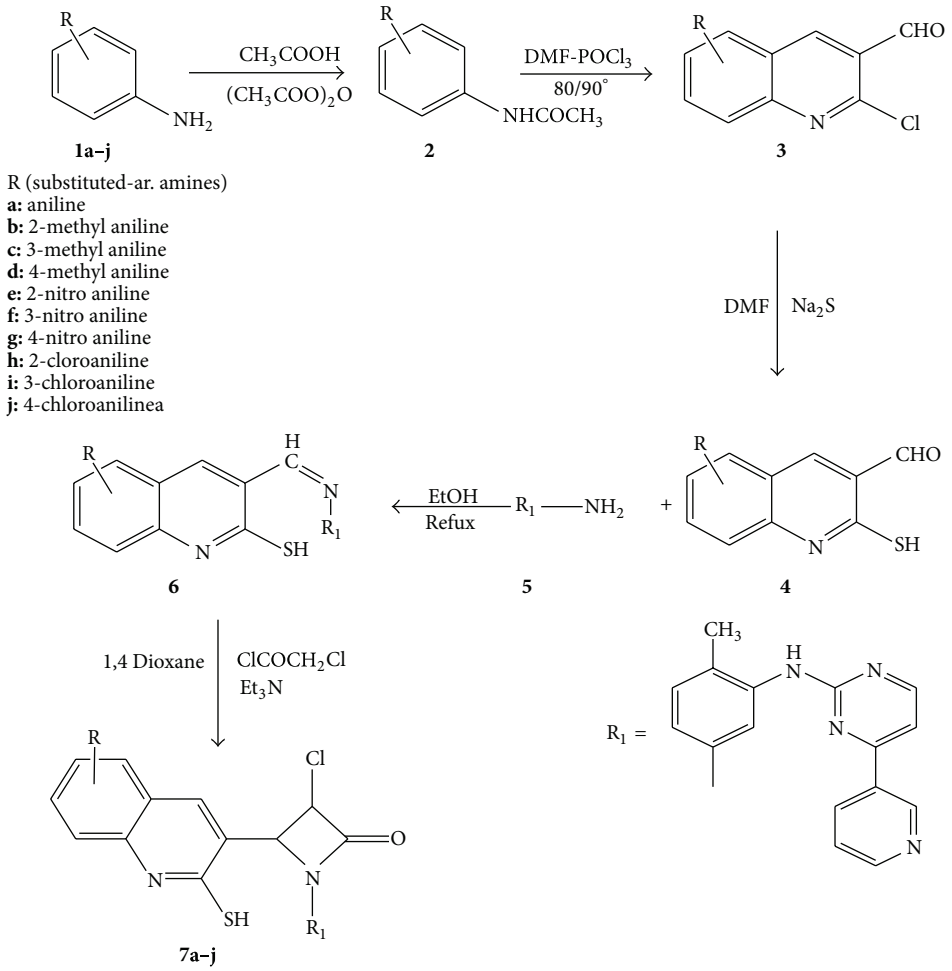
A series of 1-(3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercaptoquinolin-3-yl)azetidin-2-one (**7a-j**) have been synthesized from the condensation of aromatic amines with *N*-phenylacetamide. The thione nucleus formed from 2-chloroquionoline-3-carbadehyde using sodium sulphide in dimethyl formamide (DMF) was followed by the reaction with pyrimidine amine to form the Schiff base intermediates. Attempt has been made to derive final azetidinone analogues from Schiff bases by using chloroacetyl chloride. The newly synthesized analogues were examined for the antimicrobial activity against some bacterial and fungal strains and *in vitro* antituberculosis activity against mycobacterium tuberculosis. These observations provide some predictions to design further antibacterial and antituberculosis active compounds prior to their synthesis according to molecular studies.

1. Introduction

In the family of heterocyclic compounds, nitrogen-containing heterocycles with a sulfur atom is an important class of compounds in medicinal chemistry. There has been considerable interest in the development of preparative methods for the production of pyrimidines. This seems that pyrimidines represent one of the most active classes of compounds, possessing a wide spectrum of biological activities, namely, diuretic [1], antitumor [2], anti-HIV [3], cardiovascular [4], analgesic [5], calcium antagonist [6], anti-inflammatory [7], CNS depressant activity [8], and antimalarialactivity [9].

Over the past few decades, a rapid increase in the opportunistic microbial infections as well as resistance of microbial pathogens against current chemotherapeutics has been observed. To the human civilization, spreading of such dead diseases and epidemics is threatening. The rate of mortality is at more serious stage within the patients having decreased immunity and patients under organ transplantation [10]. Despite the numbers of antimicrobial chemotherapeutics available, the natural occurrence of multidrug resistance in recent years constitutes a substantial need for developing new potentially active antimicrobial entities.

Antimicrobial agents have a chronicle of success in controlling morbidity due to infectious diseases. But, as a consequence of frequent use, bacterial and fungal resistance to known classes of antimicrobial agent has become a global problem in recent years and presents a continuous clinical challenge [11–14]. Pyrimidine nuclei have been a source of great interest to organic, medicinal, and material scientists over many years, which are present in a number of biologically active organic compounds. Furthermore, Schiff base [15, 16], azetidinone [17, 18], and thiazolidinone [19] have also been found to possess promising antimicrobial activity. In view of these observations, we thought that it would be interesting to synthesize the substituted derivatives that may lead to compounds with interesting antimicrobial and antituberculosis profile.



Scheme 1: Synthesis of 1-(3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercaptoquinolin-3-yl)azetidin-2-one.

2. Experimental

All solvents and reagents were purchased from Sigma Aldrich Chemicals. Melting points were determined on an electrically heated VMP-III melting point apparatus. The elemental analyses of the compounds were performed on a Perkin Elmer 2400 Elemental Analyzer. The FT-IR spectra were recorded using KBr discs on FT-IR 4100 Infrared spectrophotometer. The NMR spectra were recorded using Bruker DRX 400 spectrometer at 400 MHz for ^{1}H NMR. Mass spectral data were obtained by LC/MSD Trap XCT. Silica gel for column chromatography was performed using Merck 7734 silica gel and Merck-made TLC plates. Compounds **7a-j** were synthesized by the method summarized in Scheme 1.

*2.1. General Procedure for the Synthesis of 2-Mercapto-quinoline-3-carbaldehyde (**4**).* The substituted 2-chloro-quinoline-3-carbaldehyde (**3**) was prepared according to the literature method [20]. The compound was taken (0.01 mol) in dry DMF (50 mL) and sodium sulphide (0.015 mol) was added and stirred for 1-2 h at room temperature. On completion of the reaction (monitored by TLC), the reaction mixture was poured into crushed ice and made acidic with acetic acid. The product was filtered off, washed well with water, and dried to give desired compound (**4**). The compounds were purified by recrystallisation from DMF. Yield 83%, m.p. 283–285°C. IR (KBr) cm^{-1}: 1687–1693 cm^{-1} (–CHO), 2575–2595 cm^{-1} (–SH).

*2.2. General Procedure for the Synthesis of (Z)-3-((Phenylimino) Methyl) Quinoline-2-thiol(pyrimidine Amine) (**6a–j**).* Substituted 2-mercapto-quinoline-3-carbaldehyde (**4**) (0.01 mol) and pyrimidine amine (**5**) (0.01 mol) were taken in ethanol with catalytic amount of conc. H_2SO_4 (2 mL) and heated to reflux for 6-7 h. After completion of the reaction (TLC), the reaction mixture was poured onto crushed ice; the solid mass separated out thus was filtered, washed with water, and dried to give desired compounds **6a–j**. The compounds were purified by recrystallisation from ethanol.

*2.3. General Procedure for Preparation of New Compounds (**7a–j**).* A mixture of substituted (*Z*)-3-((phenylimino)methyl)quinoline-2-thiol(pyrimidine amine) (**6a–j**, 0.01 mol) and triethylamine (0.02 mol) was dissolved in 1,4-dioxane (50 mL). To this well-stirred cooled solution, chloroacetyl

chloride (0.02 mol) was added dropwise during 30 min. The reaction mixture was refluxed for 10 hours. The triethylamine hydrochloride salt formed was filtered to separate the salt. The filtrate was concentrated to half of its initial volume and then poured into crushed ice. The product obtained was filtered, washed with water, and recrystallized from ethanol.

*2.3.1. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercaptoquinolin-3-yl)azetidin-2-one (**7a**).* FT-IR (KBr, cm^{-1}) ν: 2578 (S–H), 1731 (C=O), 1691 (C=N), 1582 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.24 (s, 3H, Ar–CH_3), 6.55 (s, 1H, CH–N), 6.91–7.83 (m, 14H, Ar–H), 9.52 (s, 1H, NH), 11.05 (s, 1H, SH), 5.64 (d, 1H, CH–Cl). MS (ESI) *m/z*: 524.12. Anal.calcd. for $C_{28}H_{21}ClN_6OS$ (in %): C, 64.05; H, 4.03; N, 16.01. Found C, 64.15; H, 4.13; N, 16.11.

*2.3.2. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercapto-8-methylquinolin-3-yl)azetidin-2-one (**7b**).* FT-IR (KBr, cm^{-1}) ν: 2569 (S–H), 1735 (C=O), 1689 (C=N), 1584 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.31 (s, 6H, 2Ar–CH_3), 5.46 (d, 1H, CH–Cl), 6.52 (s, 1H, CH–N), 7.12–7.82 (m, 13H, Ar–H), 9.53 (s, 1H, NH), 11.03 (s, 1H, SH). MS (ESI) *m/z*: 540.10. Anal.calcd. for $C_{29}H_{23}ClN_6OS$ (in %): C, 64.62; H, 4. 30; N, 15.59. Found C, 64.52; H, 4.40; N, 15.49.

*2.3.3. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercapto-7-methylquinolin-3-yl)azetidin-2-one (**7c**).* FT-IR (KBr, cm^{-1}) ν : 2571 (S–H), 1729 (C=O), 1695 (C=N), 1586 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.34 (s, 6H, Ar–CH_3), 5.34 (d, 1H, CH–Cl), 6.61 (s, 1H, CH–N), 7.22–7.83 (m, 14H, Ar–H), 9.56 (s, 1H, NH), 11.13 (s, 1H, SH). MS (ESI) *m/z*: 540.10. Anal.calcd. for $C_{29}H_{23}ClN_6OS$ (in %): C, 64.62; H, 4. 30; N, 15.59. Found C, 64.49; H, 4.42; N, 15.45.

*2.3.4. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercapto-6-methylquinolin-3-yl)azetidin-2-one (**7d**).* FT-IR (KBr, cm^{-1}) ν: 2568 (S–H), 1726 (C=O), 1693 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.14 (s, 9H, Ar–CH_3), 5.51 (d, 1H, CH–Cl), 6.61 (s, 1H, CH–N), 7.17–7.80 (m, 12H, Ar–H), 9.56 (s, 1H, NH), 11.13 (s, 1H, SH). MS (ESI) *m/z*: 540.05. Anal.calcd. for $C_{29}H_{25}ClN_6OS$ (in %): C, 64.62; H, 4. 30; N, 15.59. Found C, 64.35; H, 4.34; N, 15.36.

*2.3.5. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercapto-8-nitroquinolin-3-yl)azetidin-2-one (**7e**).* FT-IR (KBr, cm^{-1}) ν: 2571 (S–H), 1736 (C=O), 1693 (C=N), 1573 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.41 (s, 3H, Ar–CH_3), 5.49 (d, 1H, CH–Cl), 6.64 (s, 1H, CH–N), 7.19–7.73 (m, 13H, Ar–H), 9.53 (s, 1H, NH), 11.23 (s, 1H, SH). MS (ESI) *m/z*: 571.02. Anal.calcd. for $C_{28}H_{20}ClN_7O_3S$ (in %): C, 59.00; H, 3.54; N, 17.20. Found C, 59.04; H, 3.58; N, 17.26.

*2.3.6. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercapto-7-nitroquinolin-3-yl)azetidin-2-one (**7f**).* FT-IR (KBr, cm^{-1}) ν: 2582 (S–H), 1740 (C=O), 1684 (C=N), 1591 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.38 (s, 3H, Ar–CH_3), 5.61 (d, 1H, CH–Cl), 6.66 (s, 1H, CH–N), 7.17–7.61 (m, 13H, Ar–H), 9.41 (s, 1H, NH), 11.26 (s, 1H, SH). MS (ESI) *m/z*: 571.12. Anal.calcd. for $C_{28}H_{20}ClN_7O_3S$ (in %): C, 59.00; H, 3.54; N, 17.20. Found C, 59.14; H, 3.54; N, 17.21.

*2.3.7. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercapto-6-nitroquinolin-3-yl)azetidin-2-one (**7g**).* FT-IR (KBr, cm^{-1}) ν : 2585 (S–H), 1736 (C=O), 1697 (C=N), 1578 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.39 (s, 3H, Ar–CH_3), 5.52 (d, 1H, CH–Cl), 6.61 (s, 1H, CH–N), 7.18–7.63 (m, 13H, Ar–H), 9.50 (s, 1H, NH), 11.20 (s, 1H, SH). MS (ESI) *m/z*: 571.02. Anal.calcd. for $C_{28}H_{20}ClN_7O_3S$ (in %): C, 59.00; H, 3.54; N, 17.20. Found C, 59.23; H, 3.51; N, 17.28.

*2.3.8. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(8-chloro-2-mercaptoquinolin-3-yl)azetidin-2-one (**7h**).* FT-IR (KBr, cm^{-1}) ν : 2588 (S–H), 1737 (C=O), 1697 (C=N), 1575 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.43 (s, 3H, Ar–CH_3), 5.43 (d, 1H, CH–Cl), 6.69 (s, 1H, CH–N), 7.17–7.66 (m, 13H, Ar–H), 9.53 (s, 1H, NH), 11.22 (s, 1H, SH). MS (ESI) *m/z*: 560.10. Anal.calcd. for $C_{28}H_{20}Cl_2N_6OS$ (in %): C, 60.11; H, 3.60; N, 15.02. Found C, 60.15; H, 3.64; N, 15.12.

*2.3.9. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(7-chloro-2-mercaptoquinolin-3-yl)azetidin-2-one (**7i**).* FT-IR (KBr, cm^{-1}) ν: 2582 (S–H), 1743 (C=O), 1683 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.51 (s, 3H, Ar–CH_3), 5.38 (d, 1H, CH–Cl), 6.65 (s, 1H, CH–N), 7.13–7.71 (m, 13H, Ar–H), 9.51 (s, 1H, NH), 11.23 (s, 1H, SH). MS (ESI) *m/z*: 559.47. Anal.calcd. for $C_{28}H_{20}Cl_2N_6OS$ (in %): C, 60.11; H, 3.60; N, 15.02. Found C, 60.24; H, 3.73; N, 15.19.

*2.3.10. 1-(3-(4-(Pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(6-chloro-2-mercaptoquinolin-3-yl)azetidin-2-one (**7j**).* FT-IR (KBr, cm^{-1}) ν: 2590 (S–H), 1734 (C=O), 1697 (C=N), 1586 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.55 (s, 3H, Ar–CH_3), 5.48 (d, 1H, CH–Cl), 6.67 (s, 1H, CH–N), 7.17–7.73 (m, 13H, Ar–H), 9.54 (s, 1H, NH), 11.27 (s, 1H, SH). MS (ESI) *m/z*: 560.21. Anal.calcd. for $C_{28}H_{20}Cl_2N_6OS$ (in %): C, 60.11; H, 3.60; N, 15.02. Found C, 60.33; H, 3.61; N, 15.22.

2.4. Antioxidant Screening (In Vitro). Compounds **7a–j** are tested for antioxidant property by DPPH, NO, and H_2O_2 methods.

2.5. DPPH Radical Scavenging Activity. The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution

of 1,1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (25, 50, and 75 μg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I%) of free radical production from DPPH was calculated by the following equation:

$$\% \text{ of scavenging} = \left[\frac{\left(A_{\text{control}} - A_{\text{sample}}\right)}{A_{\text{blank}}}\right] \times 100, \quad (1)$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate.

2.6. Nitric Oxide (NO) Scavenging Activity. Nitric oxide scavenging activity was measured by slightly modified methods of Green et al. [21] and Marcocci et al. [22]. The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% N-(1-naphthyl) ethylenediaminedihydrochloride). Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. 1 mL of sodium nitroprusside (10 mmol) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, and 75 μg/mL) of the test compounds and incubated for 150 min at 25°C and 1 mL of the reaction mixture was treated with 1 mL of Griess reagent. The absorbance of the chromatophore was measured at 546 nm. Nitric oxide scavenging activity was calculated using (1).

2.7. Hydrogen Peroxide (H_2O_2) Scavenging Activity. The H_2O_2 scavenging ability of the test compound was determined according to the method of Ruch et al. [23]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 25, 50, and 75 μg/mL concentrations of the test compounds in 3.4 mL phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percent of scavenging of H_2O_2 was calculated using (1).

2.8. Antibacterial Activity. Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria (*Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 7443) and Gram-negative bacteria (*Xanthomonascampestris* MTCC 7908 and *Escherichia coli* MTCC 7410) in DMF by disc diffusion method on nutrient agar medium [24]. The sterile medium (Nutrient Agar Medium, 15 mL) in each petriplate was uniformly smeared with cultures of Gram-positive and Gram-negative bacteria. Sterile discs of 10 mm diameter (Hi-Media) were placed in the petriplates, to which 50 μL (1 mg/mL, i.e., 50 μg/disc) of different synthesized compounds was added. The treatments also included 50 μL of DMF as negative and bacteriomycin and gentamycin as positive control for comparison. For each treatment, three replicates were maintained. The plates were incubated at 37 ± 2°C for 24 h and the zone of inhibition was determined.

2.9. Antifungal Activity. The synthesized compounds were screened for their antifungal activity against Fusariumoxysporum MTCC 2480 in DMF by poisoned food technique [25]. Potato DextroseAgar (PDA) medium was prepared and about 15 mL of PDA was poured into each petriplate and allowed to solidify. 5 mm disc of seven-day-old culture of the test fungi was placed at the center of the petriplate and incubated at 26°C for 7 days. After incubation the percentage inhibition was measured and three replicates were maintained for each treatment. Nystatin was used as standard. All the synthesized compounds were tested (at the dosage of 500 μL of the novel compounds/petriplate, where concentration was 0.1 mg/mL) by poisoned food technique.

2.10. Antituberculosis Activity. The compounds were screened for antituberculosis activity under direction of the U.S. National Institute of Health, NIAID division. All compounds were initially screened against *Mycobacterium tuberculosis* strain H37Rv at single concentration of 6.25 μg/mL in BACTEC 12B medium using a broth microdilution assay. Compounds demonstrating growth inhibition ≥90% in the primary screening were considered active. The active compounds were retested by serial dilution beginning at the concentration of 6.25 μg/mL against *Mycobacterium tuberculosis* H37Rv to determine the actual minimum inhibitory concentration (MIC) in the BACTEC 460 radiometric system and BACTEC 12B medium. The MIC is defined as the lowest concentration reducing fluorescence to 90% of controls. The significance of this value depends on several factors such as compound structure, novelty, toxicity, and potential mechanism of action. INH (MIC = 0.025–0.05 μg/mL) and RMP (0.025–0.125 μg/mL) were used as positive control drugs [26].

3. Results and Discussion

3.1. Chemistry. Various routes have been developed for the synthesis of functionalized quinolones; the Vilsmeier [27] approach is found to be the most efficient. Thus, in this communication, the synthesis of 2-chloroquinoline-3-carbaldehyde **3** from N-aryl acetamides was followed by reaction with Vilsmeier reagent and transformation into different functionalities. The required acetanilide **2** was readily prepared from the reaction of corresponding amines with acetic anhydride in aqueous medium. The Vilsmeier cyclization of acetanilide (**2**) was carried out by adding phosphorus oxychloride to the N-aryl acetamides in DMF at 0–5°C followed by heating at 90°C to afford substituted 2-chloro-3-carbaldehyde (**3**) in good yield. The IR spectra of compound (**3**) showed a strong absorption in the range of 1686–1698 cm^{-1} for the aldehydic group. Thus, the chloro group in few of the substituted 2-chloro-quinoline-3-carbaldehyde

Table 1: Chemical structure and physical data of pyrimidine derivatives **7a–j**.

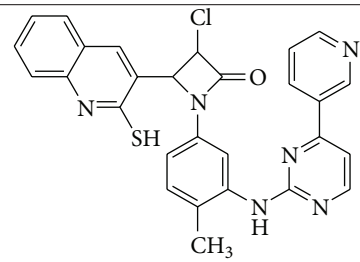
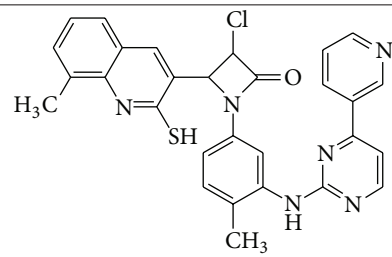
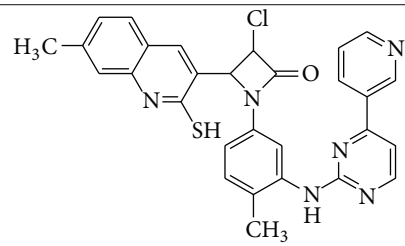
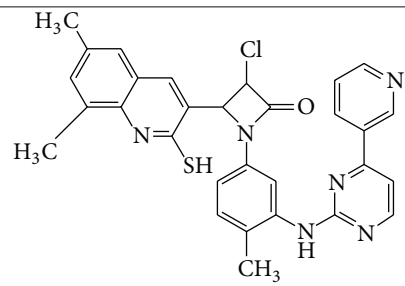
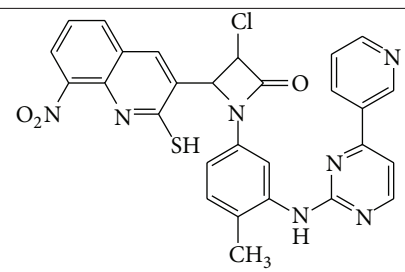
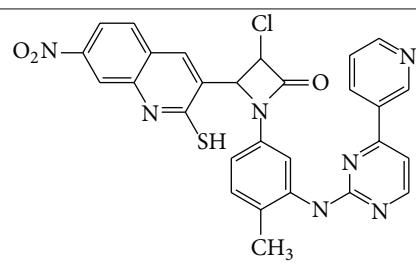
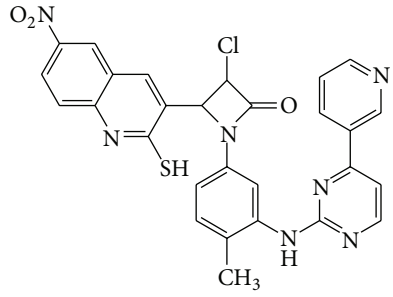
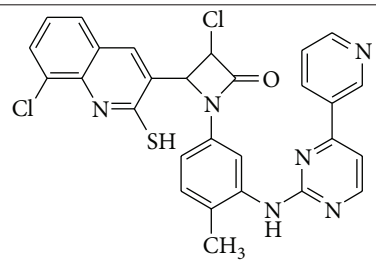
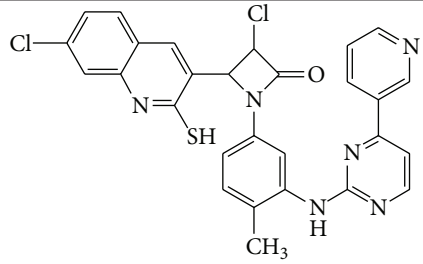
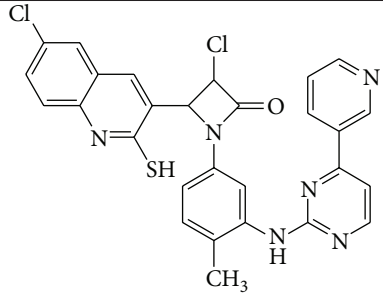
Compound	Structure	Yield (%)	mp (°C)
7a		72	256–258
7b		74	259–251
7c		76	243-244
7d		73	269–261
7e		77	252–254
7f		81	239–231

Table 1: Continued.

Compound	Structure	Yield (%)	mp (°C)
7g		73	234–236
7h		75	245–247
7i		82	232–235
7j		76	245–248

was investigated with various hetero nucleophiles. Due to the replacement of chlorine by sulphur, sodium sulphide in DMF was found to be an efficient reagent affording nucleophilic substitution by sulphur and also providing scope for further reaction and one-pot cyclisation. The substitution was achieved in an hour at room temperature to afford thione (**4**) in quantitative yield. Compound (**4**) showed prominent peak of thione function group (–SH) at 2542–2626 cm^{-1}. Thus, carbaldehyde group in quinoline (**4**) was converted into substituted quinoline Schiff base derivatives **6a–j** in ethanol at refluxed temperature.

Schiff base compounds **6a–j** showed most prominent peak of imine function group (–C=N–) at 1654–1660 cm^{-1}. The substituted Schiff base derivatives 6a–j was also react with chloroacetylchloride in the presence of triethylamine which act as a catalyst in 1,4-dioxane to undergo cyclization to obtain pyrimidine azetidin-2-one derivatives **7a–j**. The IR spectrum of compounds **7a–j** which showed sharp peak near 1723 cm^{-1} indicates the presence of ketone (–C=O) functional group of azetidinone ring. A corresponding peak of C–N–CO was observed at 1555–1575 cm^{-1}. Chlorine functional group exhibited a peak at 763–783 cm^{-1}. Compounds structure and physical data are depicted in the Table 1.

3.2. Antioxidant Activity. Compounds **7a–j** are tested for *in vitro* antioxidant property by 1,1-diphenylpicrylhydrazyl (DPPH) [28, 29], nitric oxide (NO) [21, 22], and hydrogen peroxide (H_2O_2) [23] methods which were summarized in Tables 1–3, respectively. Compounds **7h**, **7i**, and **7j** showed good radical scavenging activity in all three methods due to the presence of mild electron donating groups such as chloro groups attached to the rings when compared with

Table 2: The *in vitro* antioxidant activity of **7a–j** in DPPH method.

Compound	Concentration (μg/mL)			
	25	50	75	IC_{50}
7a	69.30 ± 1.04	72.53 ± 0.85	77.84 ± 1.32	18.01 ± 1.05
7b	64.71 ± 1.45	67.44 ± 1.24	72.84 ± 1.56	19.35 ± 1.15
7c	63.88 ± 0.72	67.91 ± 1.29	71.48 ± 1.29	17.72 ± 0.63
7d	48.60 ± 0.65	52.86 ± 1.24	56.63 ± 0.55	17.21 ± 0.48
7e	52.71 ± 1.71	58.23 ± 1.00	61.37 ± 0.92	19.55 ± 1.21
7f	59.74 ± 1.18	64.42 ± 1.26	68.94 ± 0.88	23.72 ± 1.49
7g	68.43 ± 1.20	72.81 ± 1.45	74.93 ± 1.58	25.65 ± 1.05
7h	70.53 ± 0.20	74.25 ± 0.47	77.85 ± 0.65	20.94 ± 0.80
7i	72.41 ± 0.31	75.86 ± 0.63	78.36 ± 0.70	18.21 ± 1.06
7j	73.80 ± 0.10	77.42 ± 0.45	79.63 ± 0.46	16.92 ± 0.61
Ascorbic acid	82.70 ± 0.20	83.52± 0.38	85.52 ± 0.44	15.15 ± 0.49
Blank	—	—	—	—

(—) showed no scavenging activity. Values were the means of three replicates ± SD.

Table 3: The *in vitro* antioxidant activity of **7a–j** in nitric oxide (NO) method.

Compound	Concentration (μg/mL)			
	25	50	75	IC_{50}
7a	71.23 ± 0.85	74.85 ± 1.08	77.13 ± 1.43	17.55 ± 0.96
7b	68.34 ± 0.95	70.61 ± 1.39	74.18 ± 0.95	18.27 ± 1.05
7c	72.90 ± 0.85	74.06 ± 0.94	78.25 ± 1.06	17.14 ± 0.56
7d	64.37 ± 1.18	69.22 ± 1.57	74.69 ± 1.41	19.45 ± 1.27
7e	53.64 ± 1.39	56.49 ± 1.24	60.03 ± 0.71	23.35 ± 1.14
7f	62.04 ± 1.41	66.83 ± 1.56	70.92 ± 0.78	20.15 ± 0.98
7g	59.95 ± 1.55	60.47 ± 1.21	62.56 ± 1.31	20.49 ± 0.94
7h	73.03 ± 0.23	78.14 ± 0.45	82.44 ± 0.62	17.15 ± 0.95
7i	71.84 ± 0.17	76.29 ± 0.35	79.65 ± 0.54	17.39 ± 1.15
7j	75.23 ± 0.26	79.53 ± 0.37	82.64 ± 0.57	16.65 ± 0.60
Ascorbic acid	84.72 ± 0.18	85.96 ± 0.36	88.32 ± 0.52	14.71 ± 0.54
Blank	—	—	—	—

(—) showed no scavenging activity. Values were the means of three replicates ± SD.

Table 4: The *in vitro* antioxidant activity of **7a–j** in hydrogen peroxide (H_2O_2) method.

Compounds	Concentration (μg/mL)			
	25	50	75	IC_{50}
7a	61.05 ± 0.85	63.84 ± 1.58	68.22 ± 1.07	20.47 ± 1.23
7b	58.25 ± 1.17	62.31 ± 1.17	65.74 ± 1.47	21.22 ± 1.07
7c	60.26 ± 1.06	63.48 ± 1.27	67.84 ± 1.57	20.71 ± 0.54
7d	61.94 ± 1.32	64.51 ± 1.18	68.22 ± 1.07	20.15 ± 0.75
7e	45.02 ± 0.88	47.38 ± 1.17	50.44 ± 1.27	27.75 ± 0.65
7f	53.08 ± 0.89	56.61 ± 1.39	59.95 ± 0.78	23.54 ± 0.42
7g	51.37 ± 1.16	54.03 ± 0.86	58.12 ± 0.97	24.33 ± 1.04
7h	64.12 ± 0.27	67.44 ± 0.64	69.81 ± 0.69	19.49 ± 0.26
7i	66.25 ± 1.15	69.53 ± 1.31	71.68 ± 0.60	18.85 ± 0.60
7j	63.87 ± 0.30	65.49 ± 0.46	68.97 ± 0.65	19.57 ± 1.26
Ascorbic acid	76.41 ± 0.18	78.60 ± 0.33	82.39 ± 0.61	16.31 ± 0.31
Blank	—	—	—	—

(—) showed no scavenging activity. Values were the means of three replicates ± SD.

the standard drug ascorbic acid. Compounds **7a**, **7b**, **7c**, and **7d** showed moderate antioxidant activity, whereas the other compounds **7e**, **7f**, and **7g** displayed mild activity. In general, it was observed that halo substituted **7h**, **7i**, **7j**, and the unsubstituted compounds **7a**, **7b**, **7c**, and **7d** exhibited greater activity when compared with the respective nitro-substituted compounds. The IC_{50} value of the standard ascorbic acid in DPPH method was found to be 15.15 at 25 μg/mL, whereas the IC_{50} values of the compounds **7h**, **7i**, and **7j** were found to be 17.72, 17.21, and 16.92 μg/mL, respectively. Further Tables 2–4 indicate that radical scavenging activity in DPPH, nitric oxide, and hydrogen peroxide methods increases with concentration.

3.3. Antimicrobial Activity. The investigation of antibacterial screening data revealed that all tested compounds showed antibacterial activity against four pathogenic bacterial strains. It is worth to mention that pyrimidine analogues displayed better activity against the mentioned microorganisms. It was observed that the class of newly synthesized analogues with electron withdrawing nitro and electron withdrawing halo (–Cl, –F) substituent demonstrated potential antimicrobial properties. Among the series **7a–j**, compounds **7h**, **7i**, and **7j** showed excellent activity against Gram-positive strain *Staphylococcus aureus*. Final pyrimidine analogues, **7h**, **7i**, and **7j**, displayed strong inhibitory action against Gram-positive *Bacillus subtilis*. Compounds **7e**, **7f**, and **7g** were found to contribute promising activity towards Gram-negative strain *Escherichia coli*. All the remaining final pyrimidine derivatives exerted good to moderate activity profile, whereas some derivatives were found to display weak activily profile. The antifungal bioas say results that revealed those final pyrimidine derivatives **7f**, **7g**, and **7k** displayed antigrowth activity against *Aspergillus niger*. All the remaining final pyrimidine derivatives were found to demonstrate well to moderate activity profile (Table 5).

3.4. Antituberculosis Activity. *In vitro* tuberculosis activities of compounds **7a–j** were assessed against *Mycobacterium tuberculosis* H37Rv. The results indicated that both pyrimidine analogues were active against mycobacteria. Preliminary antituberculosis screening results using BAC TECMGIT method revealed that the final pyrimidine analogues **7i** and **7j** displayed the highest inhibition at a constant concentration level (6.25 μg/mL) against *M. tuberculosis* H37Rv (Table 6).

Table 5: *In vitro* antibacterial and antifungal activities of **7a–j**.

Compound	Zone of inhibition in diameter (mm)			
	B. subtilis	*S. aureus*	*X. campestris*	*E. coli*
7a	22	19	21	24
7b	21	21	20	25
7c	20	22	22	23
7d	22	20	25	27
7e	23	19	30	30
7f	21	22	31	31
7g	22	22	32	33
7h	30	26	20	30
7i	31	25	23	24
7j	33	27	20	
Bacteriocin	—	—	34	—
Gentamicin	35	30	—	35
Nystatin	—	—	—	—

Table 6: Antituberculosis activity of pyrimidine derivatives.

Compound	Inhibition (%)	MIC (lg/mL) *M. tuberculosis* (H37Rv)
7a	58	>6.25
7b	59	>6.25
7c	67	>6.25
7d	58	>6.25
7e	72	>6.25
7f	68	>6.25
7g	71	>6.25
7h	82	>6.25
7i	80	6.25
7j	95	6.25
Rifampicin	—	0.125
Isoniazid	—	0.05

4. Conclusion

In conclusion, a series of new 1-(3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)-4-methylphenyl)-3-chloro-4-(2-mercaptoquinolin-3-yl)azetidin-2-one derivatives, **7a–j**, was synthesized and their antimicrobial and antituberculosis activities have been evaluated. Pyrimidine nucleus is one of the active constituents present in many standard drugs and is known to increase the pharmacological activities of the molecule. The presence of substituted amines is also an instrumental in contributing the net biological activity. In brief, high potency has been observed with the final scaffolds in the form of pyrimidine-azetidinones bearing various amines containing halogen(s) such as chloro or fluoro and nitro functional groups. The overall conclusion placed for synthesized compounds is that most of the compounds showed moderate to promising activity as compared to standard drug against all representative panel of bacterial, fungal, and tuberculosis strains.

Conflict of Interests

The authors report no conflict of interests. They alone are responsible for the content and writing of this paper.

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