A Convenient Synthesis of New Annelated Pyrimidines and Their Biological Importance

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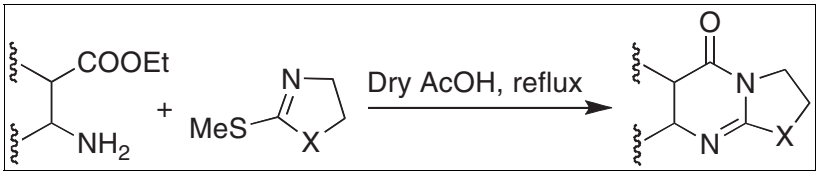

Several bicyclic/tricyclic-fused pyrimidines were synthesized from the reactions of amino esters and bifunctional nucleophiles such as 2-methylthio-thiazoline and 2-methylthio-imidazoline. The synthesized compounds were tested for their *in vitro* antimicrobial activities that revealed mild to moderate growth inhibitory potentials.

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INTRODUCTION

Fused heterocycles represent an important class of organic compounds because of their structural diversity and potential applications in medicinal chemistry [1–3]. Such heterocycles frequently form part(s) of pharmacophores or fragments of naturally occurring bioactive molecules. Perhaps the most important of them are heterocyclic systems containing pyrimidine skeleton. Fused pyrimidines have drawn considerable interest over the last few years as reflected by the recent number of articles describing their synthetic routes and broad-spectrum activities. Compounds of this class have been reported to block platelet aggregation [4–6], demonstrate promising antihypertensive [7,8], antimalarial/antiparasitic [9–11], anti-inflammatory [12], and anticancer [13,14] activities. However, the macromolecular target-based inhibition studies on fused pyrimidines have lagged behind these biological activity data. Substituted pyrazolopyrimidines were found to inhibit signaling kinases such as glycogen synthase kinase 3 and cyclin-dependent kinase 5 [15]. Whereas glycogen synthase kinase 3 has been implicated in a number of diseases, including type II diabetes, Alzheimer's disease, inflammation, cancer, and bipolar disorder, cyclin-dependent kinase 5 is involved in the processes of neuronal maturation. Such a widespread use of fused pyrimidines in the pharmaceutical, agrochemical, and veterinary industries prompted an immense interest to develop new facile routes for efficient and cost-effective syntheses.

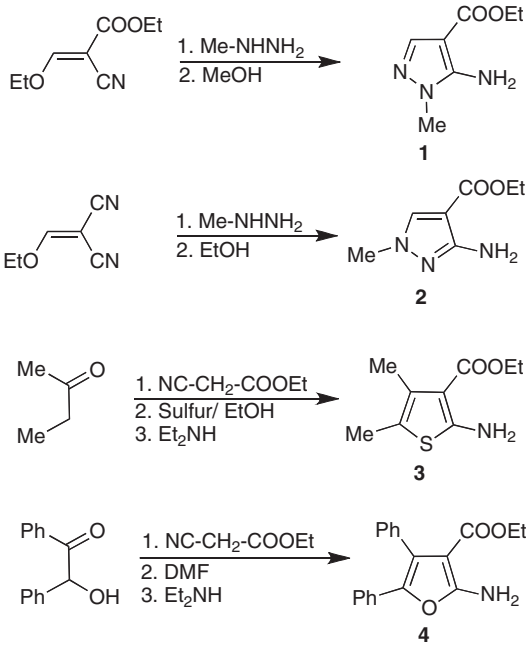
Over the years, various methods for the synthesis of fused pyrimidines have been developed and used in heterocyclic chemistry [16–20]. However, some of these methods suffer from undesired complexities such as longer reaction time, use of expensive or hazardous reagents, and often inconvenient work-up. As part of our ongoing interest in medicinal chemistry, we attempted to develop a general and facile method for the syntheses of a novel series of linear/angular-fused pyrimidines. The current work describes the syntheses of fused pyrimidines (**9–15**) on the basis of S_N—S_N tandem reactions of amino esters and annelating reagents. The ease and generality of the reactions coupled with the simple work-up may merit their use in pharmaceutical industries for the syntheses of molecules with similar scaffolds.

RESULTS AND DISCUSSION

Chemistry. Several fused pyrimidines were synthesized in the current study from annelated substrates and reagents. The reaction progress was in general monitored by TLC employing suitable solvent mixtures. The final products were primarily characterized on the basis of their spectral data interpretation and wherever appropriate, confirmed by comparison with reported values.

The annelated substrates, ethyl esters of 5-amino-1-methyl-1*H*-pyrazole-4-carboxylate (**1**), 3-amino-1-methyl-1*H*-pyrazole-4-carboxylate (**2**), 2-amino-4,5-dimethyl-thiophene-3-carboxylate (**3**), and 2-amino-4,5-diphenyl-furan-3-carboxylate (**4**) were prepared from ethyl (ethoxymethylene) cyanoacetate, ethoxymethylene malononitrile, butan-2-one, and benzoin, respectively by Gewald procedure [21,22] in 57–84% yields (Scheme 1). The compounds were identified by careful observation of their spectral data. NMR and IR spectral characteristics for functional groups including —COOEt and —NH_2 were conserved in all annelated substrates (**1–4**). **1** and **2** are isomeric, and hence their spectral properties were similar but not identical. Apart from their very different melting points (90 and 222°C for **1** and **2**), certain features distinguished them. Notably, the UV absorption patterns were different for the two compounds; **1**

Scheme 1



absorbed at λ 254, 226, and 206 nm, whereas **2** showed bathochromic shift to λ 268 and 210 nm. ^{1}H-NMR spectra for the two compounds were similar except for the broad 2H singlet for the *ortho*-amino group centered around δ 5.28 and 4.38 ppm in **1** and **2**, respectively.

The annelating reagents, 2-methylthio-thiazoline (**6**) and 2-methylthio-imidazoline (**8**) were prepared starting from ethanolamine and ethylene diamine, respectively by Jensen method (Scheme 2) [23,24]. The final methylation step was carried out in methanol with equimolar amounts of methyl iodide. The compounds were readily identified by their spectral characteristics and by comparison with literature values. Chemically, these annelating agents are interesting because they contain a good leaving group (—SMe) and show both nucleophilic and electrophilic characters. In particular, 2-methylthio-thiazoline (**6**) is widely used as a carbon—nitrogen double bond fragment for the synthesis of heterocyclic compounds having a thiazole ring.

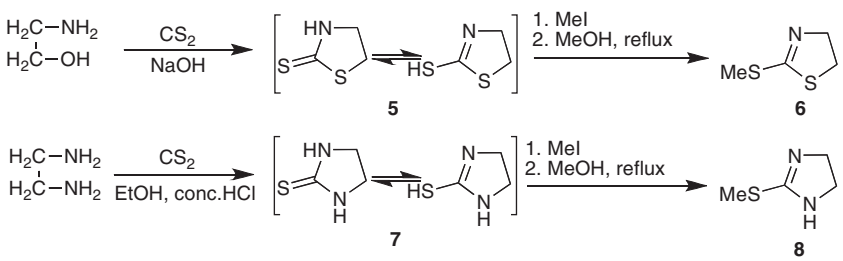
Fused pyrimidine derivatives (**9**) and (**10**) were synthesized from annelated substrate, amino ester (**1**), whereas **11–13** from **2** to **4**, respectively (Scheme 3). In brief, the reactions were carried out in mixtures containing an amino ester (**1–4**) and an annelating reagent (**6** or **8**) in dry acetic acid at 100/160°C under reflux for 1–8 h. The formed precipitate was filtered off, occasionally washed with water, and recrystallized from ethanol to furnish products in reasonably good yields (51–73%). The reactions likely proceed via a conserved mechanism involving an initial nucleophilic attack of the amino group of esters to the electron-deficient alkenic carbon (C-2) of reagents (**6** or **8**). The intermediate (s) thus formed then eliminates the leaving group (—SMe) followed by ring closure in the next step by a second nucleophilic attack of the heterocyclic nitrogen of the reagents to the sp^2-hybridized carbon of the ester (substrate). This results in an elimination of ethanol to yield the final products via intermolecular cyclization (Scheme 4).

In addition, the annelating substrate (**1**) was also used in the synthesis of 1-methyl-5-(4-methyl-benzoyl)-1,5-dihydro-pyrazolo[3,4-*d*]pyrimidin-4-one (**15**) in a two-step route as shown in Scheme 5. The target compound (**15**) was synthesized from 1-methyl-1,5-dihydro-pyrazolo[3,4-*d*] pyrimidin-4-one (**14**) and *p*-toluoyl chloride in dry ether in approximately 65% yield.

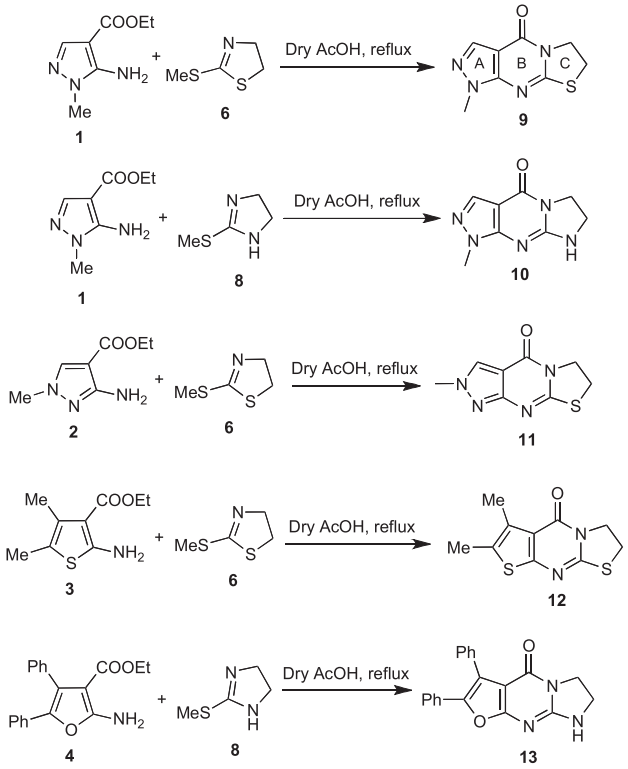
In the present study, characterization of the reagents and products employed various spectral techniques including UV, IR, and high-field NMR analysis. Three of the synthesized compounds, **9–11** contain thiazolo/imidazo pyrimidine moiety (ring BC) fused with a pyrazole ring (A) that has an *N*-methyl substituent. These three compounds had relatively higher melting points (>250°C) compared with the rest and exhibited some common ^{1}H-NMR features such as a downfield 1H singlet at δ 7.76 ppm for an unsubstituted pyrazole ring proton, two 2H triplets around δ 4.15 (t, 2H, J = 7.2–7.8 Hz) and 3.26 (t, 2H, J = 7.2–7.8 Hz) for two methylenes on ring C, and a sharp 3H singlet (δ 2.5–3.0) for the *N*-methyl group. The other compounds (**12** and **13**) had additional spectral characteristics for two methyl (**12**) or two phenyl (**13**) groups.

The ^{13}C-NMR spectra for the compounds were particularly helpful in assigning the double bonded carbons and were in good agreement with the structures. In addition, DEPT ^{13}C-NMR revealed different hydrocarbon status (CH, CH_2, or tertiary) that was also consistent with the structures. The IR spectra were in general useful for

Scheme 2



Scheme 3

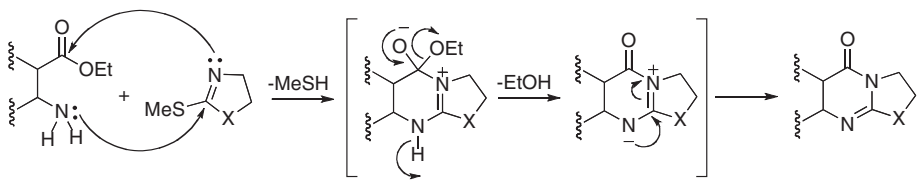


identifying —NH_2 and —COO^- groups in the amino esters (starting material) and the carbonyl group in the final product. Complete analysis of the UV, IR, ^{1}H-NMR, and ^{13}C-NMR spectra were supported by the molecular formula derived from the elemental analysis and allowed unambiguous determination of the structures presented here.

Biological. Antimicrobial activities of fused primidine derivatives were evaluated by a standard *in vitro* bioassay [25]. The synthesized compounds demonstrated mild growth inhibition (mean zone of inhibition, 6.5–12.0 mm) at 200-μg dose against antibiotic-susceptible standard strains of gram-positive and gram-negative bacteria as well as human fungal pathogens (Table 1). The data show that the annelated substrates (**1, 2,** and **4**) and the reagents (**6** and **8**) possess some degree of growth inhibitory activity especially against the fungus such as *Candida albicans*. Compound **3** appears to be mildly active against gram-positive *Bacillus* and gram-negative *Escherichia coli* and the fungus *Aspergillus niger*. In contrast, such selectivity towards fungi was significantly altered with the bigger tricyclic final products, as they seemed to inhibit the growth (to variable degrees) of an increased number of pathogens (Table 1).

When using living cells, restricted growth phenotypes often depend on cell permeability of the small molecule inhibitors. Log of calculated partition coefficient (CLogP) for *n*-octanol/water is a calculated (by CSChemDraw Ultra v8.03) lipophilicity index that often correlates with biological activity and lipophilicity (and therefore permeability) of compounds [25]. On the other hand, polar functional groups are important for interacting with biological macromolecules such as proteins and nucleic acids. Protecting such functionality with acyl or alkyl groups often allows a small molecule to cross the cell membrane which then becomes active via metabolic deprotection in cells. The standard antibiotic used in the current study, kanamycin, is highly functionalized with low ClogP value (−5.17), whereas **13** has the highest CLogP value (3.76) amongst the tested compounds because of the presence of aromatic rings. Although lipophilicity index suggests that the compound **13** should be cell permeable, but when tested for growth inhibition, it did not show significant antimicrobial activity presumably because of poor functionalization.

Scheme 4



Scheme 5

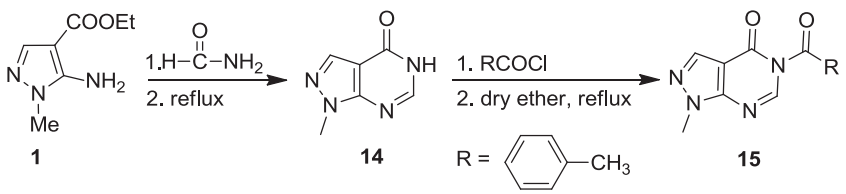


Table 1

Spectrum of antimicrobial activity of fused pyrimidines and reagents against gram-positive and gram-negative bacteria and fungi.

	Diameters of mean zone of inhibition (MZI)												
	1	2	3	4	6	8	9	10	11	12	13	14	Kan
Gram positive													
Bacillus cereus QL 29	-	-	8.1	-	-	-	7.4	7.2	7.6	7.6	8.5	7.2	31.9
Bacillus megaterium QL 38	-	-	7.5	-	-	-	9.4	8.6	10.1	10.1	9.8	8.5	34.2
Bacillus subtilis QL 40	-	-	-	6.5	-	-	-	-	8.6	8.6	8.6	8.1	30.1
Gram negative													
Esherichia coli ATCC 25922	-	-	8.2	-	-	-	7.2	7.1	6.5	6.5	7.9	8.2	34.2
Pseudomonas aeruginosa ATCC 27853	-	-	-	-	-	-	-	-	-	-	-	8.9	34.8
Salmonella paratyphii A AM 16590	-	-	-	-	-	-	-	-	-	-	-	6.5	26.9
Shigella dysenteriae ATCC 26131	-	-	-	-	-	-	7.4	7.4	7.8	9.1	8.2	7.0	36.9
Fungi													
Aspergillus niger	-	-	9.5	-	-	-	-	-	-	8.1	9.8	-	35.0
Candida albicans ATCC 10231	10.0	9.8	-	9.5	12.0	9.4	-	9.8	-	10.5	-	11.5	32.5
Rhizopus oryzae ATCC 20344	-	-	-	-	-	-	-	-	-	-	-	-	27.6
Saccharomyces cerevisiae AB 972	-	-	7.8	-	-	7.8	8.8	8.6	9.5	-	8.1	8.6	32.1

The word Kan means kanamycin, and "-" indicates no sensitivity or MZI lower than 6 mm.
Doses for kanamycin and the newly tested compounds were 30 and 200 micrograms per disk, respectively.
Compound **15** at 200 μg/disk was fully resistant to all the tested organisms and is therefore not included in the table.

CONCLUSION

Overall, we described a convenient, general, and facile method for the synthesis of fused heterocyclic compounds via heteroannelations of amino ester substrates with annelating agents. The most important features of the syntheses are that readily available, inexpensive starting materials were used at relatively milder reaction conditions that furnished products in reasonably good yields. In addition, no hazardous compounds/effluents were generated via the procedure. The method appears to be versatile in that it can be used to introduce a variety of fused heterocycles at variable positions for drug discovery. Although the compounds in this class showed mild growth inhibitory activities in our bioassay, they might be potent inhibitors of some of the biological targets such as tyrosine kinase SRC, human heat shock protein 90, and platelet-derived growth factor receptor kinase as suggested by a structural search using Ligand Activity by Surface Similarity Order descriptors in the ChemSpider server [26].

EXPERIMENTAL

General. Reagents were procured from Fluka, Merck, and Sigma-Aldrich Company Ltd. (Dorset, UK) Melting points were recorded on a Gallenkamp melting point apparatus (Loughborough, UK) and paraffin oil bath and are uncorrected. UV and IR spectra were recorded on Shimadzu UV–VIS and Shimadzu FTIR spectrophotometers, (Kyoto, Japan), respectively. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were acquired in $CDCl_3$/DMSO-d_6 on an Ultra Shield Bruker DPX 400 spectrometer (Analytische Messtechnik, Karlsruhe, Germany); the chemical shifts are reported in parts per million relative to the residual nondeuterated solvent signals. The number of attached protons for ^{13}C signals was determined using the DEPT 135 pulse sequence. The reaction progress and product homogeneity were monitored by TLC on Kieselgel gel 60 F_{254} precoated sheets (E. Merck, Darmstadt, Germany), and the spots were detected under UV exposure at 254 nm. Column chromatography was carried out on silica gel (60–120 mesh ASTM). The CLogP values were determined by using the CS ChemDraw Ultra version 8.03, by Cambridge-Soft.com.

Ethyl-5-amino-1-methyl-1*H*-pyrazole-4-carboxylate (1). To a solution of ethyl (ethoxymethylene)cyanoacetate (10.00 g, 59 mmol) in methanol (35 mL) was added methyl hydrazine (equivalent amount) dropwise whilst maintaining the temperature below 60°C. The mixture was then heated under reflux for 1 h, filtered off, dried, and recrystallized from ethanol that afforded **1**. White crystals (yield: 8.39 g, 84%), mp 90–91°C; UV (EtOH) λ: 254, 226 nm; IR (KBr) ν: 3401, 3284, 3115, 2986, 1679, 1562, 1385 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.59 (s, 1H, CH), 5.35–5.20 (br.s, 2H, NH_2), 4.25 (q, 2H, J = 7.1 Hz, CH_2), 3.61 (s, 3H, NCH_3), 1.33 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 164.31, 149.24, 138.86, 95.76, 59.29, 33.86, 14.25; *Anal*. Calcd for $C_7H_{11}N_3O_2$: C, 49.52; H, 6.41; N, 24.65. Found: C, 49.48; H, 6.38; N, 24.70%.

Ethyl-3-amino-1-methyl-1*H*-pyrazole-4-carboxylate (2) [15,27]. Compound **2** was synthesized from a mixture of ethoxymethylene malononitrile (10.85 g, 89 mmol) in ethanol (35 mL) and methyl hydrazine (3.60 g, 78 mmol) by a similar procedure described for **1**. Yellowish crystals (yield: 7.61 g, 58%), mp 222–223°C; UV (EtOH) λ: 268 nm; IR (KBr) ν: 3433, 3285, 3198, 1692, 1576, 1261, 1105 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.52 (s, 1H, CH), 4.38 (br.s, 2H, NH_2), 4.25 (q, 2H, J = 7.1 Hz, CH_2), 3.66 (s, 3H, NCH_3), 1.28 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 164.09, 156.38, 133.24, 99.10, 59.72, 38.90, 14.46.

Ethyl-2-amino-4,5-dimethyl-thiophene-3-carboxylate (3) [28]. A solution of butanone (8.9 mL, 100 mmol), ethylcyanoacetate (11.31 g, 100 mmol), and sulfur (3.20 g, 100 mmol) in 95% ethanol (30 mL) was treated with diethylamine (4 mL) whilst maintaining the temperature below 60°C using an ice bath. The reaction mixture was stirred for 2 h at RT before being poured into ice water. This yielded precipitate that was filtered,

washed thoroughly with water, and recrystallized from ethanol to give **3**. Yellowish crystals (yield: 15.16 g, 76%), mp 89–91°C; UV (EtOH) λ: 240 nm; IR (KBr) ν: 3433, 3285, 3197, 1648, 1502, 1452, 1314, 1261, 1026 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 5.27 (s, 2H, NH_2), 4.29 (q, 2H, J = 7.2 Hz, CH_2), 3.60 (s, 6H, 2 × CH_3), 1.39 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 164.31, 138.86, 113.96, 95.76, 59.29, 46.69, 33.87, 25.04, 14.26.

Ethyl-2-amino-4,5-diphenyl-furan-3-carboxylate (4) [19]. A solution of ethylcyanoacetate (2.13 g, 19 mmol) and benzoin (5.25 g, 25 mmol) in DMF (7.5 ml) was treated with diethylamine (4 mL). After 12 h of stirring at RT, the mixture was poured into water (60 mL), and the solid material was filtered and recrystallized from ethanol. Yellowish crystals (yield: 3.51 g, 61%), mp 163–165°C; UV (EtOH) λ: 253 nm; IR (KBr) ν: 3384, 3060, 2934, 1677, 1595, 1389, 1206, 977, 929, 755 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 9.26 (br.s, 2H, NH_2), 7.62–7.64 (m, 2H, Ar—H), 7.45–7.49 (m, 1H, Ar—H), 7.45 (d, 2H, Ar—H), 7.36–7.40 (m, 5H, Ar—H), 4.27 (q, 2H, J = 7.2 Hz, CH_2), 1.04 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 164.87, 161.47, 136.05, 132.89, 132.56, 130.88, 128.97, 128.60, 128.50, 128.28, 117.32, 113.40, 100.73, 60.29, 42.24, 29.68, 11.13; DEPT ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 132.89, 130.89, 128.97, 128.60, 128.50, 128.28, 42.25, 11.14.

2-Methylthio-thiazoline (6). To a mixture of ethanolamine (3.66 g, 60 mmol) and sodium hydroxide (9.60 g) in water (26 mL), carbon disulfide (158 mmol) was added at 30°C with continuous stirring. The mixture was heated under reflux for 7 h, and the resulting solution was left to cool, which afforded solid deposits. An addition of 200 mL of concentrated HCl to the reaction mixture led to some more precipitates. The solid material was finally filtered off, washed with water, dried, and recrystallized from water to give 2-mercaptothiazoline (**5**). Yellowish crystals (yield: 6.01 g, 84%), mp 102–104°C; UV (EtOH) λ: 240 nm; IR (KBr) ν: 3133, 1507, 1296, 1050 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 8.16 (s, 1H, SH), 3.98 (t, 2H, J = 7.9 Hz, CH_2), 3.55 (t, 2H, J = 7.9 Hz, CH_2,). Methylation of **5** (6.01 g, 50 mmol) was carried out by standard protocol using equimolar amount of methyl iodide in methanol (30.3 mL). The reaction mixture was heated under reflux for an hour before being cooled and diluted with ether. This afforded white crystalline hydroiodide salts that were filtered. The product salts were then dissolved in water and decomposed with 15% NaOH to yield **6** that was finally extracted with $CHCl_3$ and was evaporated to dryness under reduced pressure in a vacuum evaporator. Yellow oil (yield: 4.35 g, 65%), bp 70°C; UV (EtOH) λ: 253 nm; IR (KBr) ν: 2932, 2853, 1564, 1304, 1255, 1001, 920, 727 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 4.2 (t, 2H, J = 7.9 Hz, CH_2), 3.39 (t, 3H, J = 7.9 Hz, CH_2) 2.50 (s, 3H, SCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 165.47, 63.74, 44.31, 35.26, 14.83.

2-Methylthio-imidazoline (8). To a mixture of ethylene diamine (5.00 g, 83 mmol), rectified spirit (100 mL), and water (100 mL) was added dropwise carbon disulfide (equimolar) with occasional stirring for 2 h. The reaction mixture was heated with reflux for an hour before adding concentrated HCl (15 mol) and continued for another 9 h. The resulting solid on cooling was filtered, washed with cold acetone (80 mL), and recrystallized from ethanol to give **7**. Yellowish crystals (yield: 3.98 g, 47%), mp 155–156°C; UV (EtOH) λ: 245.6 nm; IR (KBr) ν: 3249, 2880, 2571, 1499, 1492, 1275, 1196, 920 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 6.0 (s, 2H, NH, NH/SH), 3.75–3.77 (m, 4H, 2 × CH_2). Methylation of **7** was carried out by adding methyl iodide (1.85 mL, 29 mmol) to a solution of **7** in methanol (18 mL). The reaction mixture was heated under reflux for 2 h with stirring. Methanol was then removed under reduced pressure, and the solid white hydroiodide salt was neutralized with 15% NaOH (2.4 mL). The final product (**8**) was extracted with $CHCl_3$ (30 mL × 4) and dried over sodium sulfate, and the solvent was evaporated to dryness under reduced pressure. White crystals (yield: 1.51 g, 44%), mp 119–121°C; UV (EtOH) λ: 251 nm; IR (KBr) ν: 3393, 3150, 1604, 1582, 1107 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.29 (s, 1H, NH), 4.20 (d, J = 7.9 Hz, 2H, CH_2), 3.39 (d, J = 7.9 Hz, 2H, CH_2), 2.48 (s, 3H, SCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 164.85, 50.13, 13.25.

1-Methyl-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidin-4-one (9) [12]. A solution of amino ester (**1**) (0.68 g, 4 mmol) and 2-methylthio-thiazoline (**6**) (0.52 g, 4 mmol) in dry acetic acid (6 mL) was heated under reflux for 4 h. The reaction progress was monitored by TLC (chloroform–methanol 13:1, R_f = 0.86) that indicated conversion of the starting materials into product (**9**). The reaction mixture was cooled at RT and stirred for an hour before adding crushed ice (35 g) into it. Reddish crystals (yield: 0.45 g, 55%), mp >250°C; UV (EtOH) λ: 268 nm; IR (KBr) ν: 3097, 3040, 2899, 1677, 1599, 1396, 927 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.76 (s, 1H, CH), 4.14 (t, 2H, J = 7.4 Hz, CH_2), 3.27 (t, 2H, J = 7.4 Hz, CH_2), 2.47 (s, 3H, NCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 164.40, 140.8, 136.8, 115.2, 72.1, 44.0, 42.9, 35.84.

1-Methyl-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]imidazo[1,2-*a*] pyrimidin-4(8*H*)-one (10) [29]. A solution of amino ester (**1**) (0.68 g, 4 mmol) in dry acetic acid was treated with 2-methylthio-imidazoline (**8**) (0.52 g, 4 mmol) at 160°C under nitrogen atmosphere for an hour. The reaction progress was monitored by TLC (*n*-hexane–ethyl acetate, 9:1, R_f = 0.54), which indicated formation of the product. After cooling at RT, crushed ice (35 g) was added, and the mixture was stirred for another hour. The formed precipitate was collected and recrystallized from methanol to give (**10**). Reddish crystals (yield: 0.56 g, 73%), mp >250°C; UV (EtOH) λ: 275 nm; IR (KBr) ν: 3433, 3285, 3197, 2986, 1692, 1502, 1105 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.76 (s, 1H, CH), 6.98 (s, 1H, NH), 4.15 (t, 2H, J = 7.8 Hz, CH_2), 3.25 (t, 2H, J = 7.8 Hz, CH_2), 3.03 (s, 3H, NCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 165.40, 137.32, 125.12, 110.25, 72.05, 39.32, 34.37.

2-Methyl-6,7-dihydro-2*H*-pyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidin-4-one (11) [30,31]. A solution of amino ester (**2**) (1.00 g, 6 mmol) and 2-methylthio-thiazoline (**6**) (0.79 g, 6 mmol) in dry acetic acid (6 mL) was heated under reflux in a 100-mL round-bottomed flask. The reaction was monitored by TLC (chloroform–methanol, 11:1, R_f = 0.7). After completion, the reaction mix was cooled at RT followed by adding crushed ice (25 g) and stirring for an hour. The formed precipitate was filtered, washed, and crystallized from methanol to give (**11**). Reddish crystals (yield: 0.63 g, 51%), mp >250°C; UV (EtOH) λ: 280 nm; IR (KBr) ν: 3148, 3097, 3017, 2897, 2861, 1675, 1599, 1396, 928, 785 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.76 (s, 1H, CH), 4.15 (t, 2H, J = 7.2 Hz, CH_2), 3.26 (t, 2H, J = 7.2 Hz, CH_2), 2.47 (s, 3H, NCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 164.48, 149.23, 139.01, 96.13, 59.14, 46.78, 33.99, 14.43; DEPT ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 139.01, 59.14, 33.99, 14.43.

6,7-Dimethyl-2,3-dihydro-thiazolo[3,2-*a*]thieno[2,3-*d*] pyrimidin-5-one (12) [32,33]. The solution of ethyl-2-amino-4,5-dimethyl-thiophene-3-carboxylate (**3**) (1.00 g, 5 mmol) and 2-methylthio-thiazoline (**6**) (0.67 g, 5 mmol) in dry acetic acid (6 mL) was heated under reflux for 8 h. The reaction progress was monitored by TLC (*n*-hexane–ethyl acetate, 9:1, R_f = 0.51) that showed formation of **12**. The mixture was then poured into ice water, and the resulting precipitate was filtered off, washed with water, and recrystallized from ethanol that afforded the final product (**12**). Reddish crystals (yield: 0.66 g, 55%), mp 171–

172°C; UV (EtOH) λ: 265 nm; IR (KBr) ν: 3097, 3018, 2970, 1675, 1534, 1396, 928 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 4.22 (t, 2H, J = 7.5 Hz, CH_2), 3.41 (t, 2H, J = 7.5 Hz, CH_2), 2.53 (s, 6H, 2 × CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 178.33, 170.48, 138.87, 128.06, 61.49, 60.43, 46.91, 37.75, 25.39, 14.06.

2,3-Diphenyl-5,6-dihydro-imidazo[3,2-*a*]furo[2,3-*d*]pyrimidin-4(8*H*)-one (13). A solution of amino ester (**4**) (0.78 g, 3 mmol) and 2-methylthio-imidazoline (**8**) (0.52 g, 4 mmol) in dry acetic acid (6 mL) was heated under reflux for 6 h. The progress of the reaction was monitored by TLC (chloroform–methanol, 13:1, R_f = 0.45) that showed the conversion of the starting material into product. After cooling the reaction mixture to RT, crushed ice was added, and the mixture was stirred for an hour. The precipitate was collected and crystallized from methanol to give (**13**). Light yellow crystals (yield: 0.50 g, 60%), mp 201–203°C; UV (EtOH) λ: 288 nm; IR (KBr) ν: 3393, 3335, 3149, 2207, 1667, 1580, 1452, 1199, 972 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 8.76 (s, 1H, NH), 7.78–7.88 (m, 4H, Ar—H), 7.49 (t, 2H, J = 8.1 Hz, Ar—H), 7.26 (t, 2H, J = 8.0 Hz, Ar—H), 7.11 (t, 2H, J = 7.6 Hz, Ar—H), 3.77 (t, 2H; J = 7.8 Hz, CH_2), 2.21 (t, 2H, J = 7.8 Hz, CH_2); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 172.47, 171.38, 142.74, 137.88, 131.00, 129.50, 128.26, 124.10, 123.93, 122.32, 118.68, 61.37, 60.62, 59.33, 37 .95; DEPT ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 130.81, 128.69, 128.06, 123.90, 122.12, 118.49, 60.42, 37.75.

1-Methyl-5-(4-methyl-benzoyl)-1,5-dihydro-pyrazolo[3,4-*d*] pyrimidin-4-one (15). A solution of amino ester (**1**) (1.00 g, 6 mmol) in formamide (4 mL) was refluxed at 180°C for 4 h. The reaction progress was checked by TLC (*n*-hexane–ethylacetate, 1:2, R_f = 0.35). The resulting mixture was poured into ice water and stirred for an hour. The formed precipitate was filtered and recrystallized from ethanol to give (**14**). White crystals (yield: 0.68 g, 77%), mp >250°C; UV (EtOH) λ: 253 nm; IR (KBr) ν: 3148, 3097, 2897, 1675, 1396 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 8.05 (s, 1H, CH), 8.00 (s, 1H, NH), 3.88 (s, 3H, NCH_3), 2.58 (s, 1H, CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 166.13, 157.18, 144.8, 132.02, 114.17, 36.23, 33.09. To a solution of **14** (0.25 g, 2 mmol), *p*-toluoyl chloride (0.32 g, 2 mmol), and diethyl ether (6 mL) were added. Then, the mixture was heated under reflux for about 6 h with continuous stirring. The reaction was monitored by TLC (*n*-hexane–ethyl acetate, 1:3, R_f = 0.61). The mixture was evaporated to dryness under reduced pressure in vacuum evaporator. The resulting solid was recrystallized from ethanol to give **15**. White crystal (yield: 0.29 g, 65%); mp 220–222°C; UV (EtOH): 263.0, 220.0 nm; IR (KBr) ν: 2979, 1758, 1734, 1539, 1347, 1188 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.97 (d, 2H, J = 8.0 Hz, Ar—H), 7.92 (d, 2H, J = 8.0 Hz, Ar—H), 7.58 (s, 1H, CH), 5.47 (s, 1H, CH), 3.60 (s, 3H, NCH_3), 2.49 (s, 3H, Ar—CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 173.61, 164.11, 151.53, 149.25, 136.63, 127.79, 127.60, 127.11, 126.71, 95.32, 59.12, 57.08, 42.93, 28.89.

Antimicrobial activity. Antimicrobial susceptibility testing was performed by the standardized disk diffusion of the National Committee for Clinical Laboratory Standards [25]. Inhibitory zone diameters were measured on nutrient agar for bacteria and potato dextrose agar (Difco Lab.) for fungi, with conventional metrical filter paper disks (BBL, Cocksville, USA, 6 mm in diameter) containing 200 μg of compounds. All experiments were conducted twice and repeated if the results differed significantly. The inhibitory zone diameters were read with a caliper. For each tested strain, the growth conditions and the sterility of the medium were checked in negative controls. The results obtained were compared with standard antibiotic kanamycin (30 μg/disk) disks bought from Mast Diagnostics, UK.

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