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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and in vitro antimicrobial evaluation of new 5-chloro-8-bromo-3-aryl-1,2,4-triazolo[4,3-*c*]pyrimidines

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Abstract A series of new 5-chloro-8-bromo-3-aryl-1,2,4-triazolo[4,3-*c*]pyrimidines (**4a–j**) have been accomplished in excellent yields by the oxidative cyclization of pyrimidinylhydrazines (**3a–j**) of various aryl aldehydes with one equivalents of iodobenzene diacetate in methanol. The chemical structures of the synthesized compounds were confirmed by elemental analyses, FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectral studies. Ten new compounds (**4a–j**) were tested in vitro for their antimicrobial activity against clinically isolated strains. Variable and modest activities were observed against the investigated strains of bacteria and fungi. Compounds **4f**, **4i**, and **4j** demonstrated good antimicrobial activity against all the tested microbial strains.

Keywords 8-Bromo-2,4-dichloropyrimidine · Triazolopyrimidines · Iodobenzene diacetate · Antimicrobial activity · Oxidative cyclization

Introduction

In recent years, organohypervalent iodine reagents (Moriarty, 2005; Tohma and Kita, 2004) have emerged as reagent of choice for various synthetically useful transformations due to their low toxicity, ready availability, and ease of handling. The hypervalent iodine reagents are mostly carried out under the mild reaction conditions and these developments have offered superior alternative to the reported traditional methods (Zhdankin, 2009). Organoiodine (III) reagent such as iodobenzene diacetate (IBD) is excellent reagent for the oxidation of phenolic compounds (Kumar *et al.*, 2004) α-functionalization of carbonyl compounds and synthesis of wide variety of heterocyclic compounds. Triazolo pyrimidines are useful building blocks in the synthesis of herbicidal drugs. 1,2,4-Triazolo [1,5-*a*]pyrimidine derivatives are thermodynamically more stable.

Pyrimidine scaffold being an integral part of DNA and RNA plays a vital role in several biological processes and have considerable chemical and pharmacological importance. Antimicrobial studies of some pyrimidine derivatives have been reported (Bukhari *et al.*, 2011; Giles *et al.*, 2012; Cieplik *et al.*, 2011; Bansal *et al.*, 2013). Pyrimidines and their derivatives are considered to be important for drugs and agricultural chemicals. A large number of pyrimidine derivatives are reported to exhibit antimycobacterial (Kumar *et al.*, 2002), antitumor (Baraldi *et al.*, 2002), antiviral (Nasr and Gineinah, 2002), anticancer (Sondhi *et al.*, 2001), anti-inflammatory (Gangjee *et al.*, 2001), and antimicrobial (Kumar *et al.*, 2001) activities. In view of these reports, this paper reports the synthesis of 5-chloro-8-bromo-3-aryl-1,2,4-triazolo[4,3-*c*]pyrimidines (**4a–j**) and characterized by different spectral studies. Antibacterial and antifungal activities of (**4a–j**) were reported.

Experimental

Chemistry

All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd. Melting range was determined

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by Veego Melting Point VMP III apparatus. Elemental analyses were recorded on VarioMICRO superuser V1.3.2 Elementar. The FT-IR spectra were recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-500 spectrometer at 400 MHz using DMSO-d_6 as solvent and TMS as an internal standard. Mass spectral electrospray ionization (ESI) measurements were obtained by LC/MSD Trap XCT. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck-made TLC plates. All the synthesized molecules (**4a**–**j**) were structurally characterized by ESI, ^{1}H NMR, ^{13}C NMR and FT-IR spectral studies.

General procedure for the synthesis of 1-(5-bromo-2-chloropyrimidine-yl)hydrazine (**2**)

The starting material 5-bromo-2,4-dichloropyrimidine (**1**) (1 mmol) was dissolved in methanol (25 ml). To this mixture was added hydrazine hydrate (3 mmol) drop wise under ice bath. The reaction mixture was stirred at room temperature for 1 h, and then made cool in the ice bath. The yellow solid was precipitated was collected through filtration and washed the chilled water and dried to afford compound **2** (Yield 87 %). m.p.: 148–150 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.17 (s, 1H, Py–H), 6.2 (s, 1H, NH–NH$_2$). ^{13}C NMR (DMSO-d_6, 400 MHz): 98.6, 148.6, 160.0, 163.5.

General procedure for synthesis of 2-aryl-1-(5-bromo-2-chloropyrimidine-4-yl)hydrazine (**3a**–**j**)

1-(5-Bromo-2-chloropyrimidine-4-yl)hydrazine (**2**) was dissolved in ethanol and aryl aldehydes were added to it. The contents were refluxed on a water bath for 2 h and allowed to stand at room temperature. The crystalline solid, thus obtained was filtered, washed with ethanol and dried to afford hydrazones (**3a**–**j**).

*2-Benzylidene-1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**3a**)*

The product obtained from (**2**) (0.01 mol) and benzaldehyde (0.012 mol). Yield 67 %. m.p.: 157–160 °C. FT-IR (KBr, cm^{-1}): 3357 (N–H), 2936 (C–H), 1577 (HC=N), 1461 (C=C), 1376 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.46 (s, 1H, NH), 8.43 (s, 1H, Py–H), 8.11 (s, 1H, CH), 7.69–7.66 (d, 2H, Ar–H, $J = 9.0$ Hz), 7.45–7.37 (m, 3H, Ar–H). ^{13}C NMR (DMSO-d_6, 400 MHz): 99.7, 124.2, 126.2, 128.6, 143.2, 147.6, 149.2, 156.2, 159.2, 161.6.

*2-(4-Bromobenzylidene)-1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**3b**)*

The product obtained from (**2**) (0.01 mol) and 4-bromobenzaldehyde (0.012 mol). Yield 68 %. m.p.: 122–125 °C. FT-IR (KBr, cm^{-1}): 3372 (N–H), 2957 (C–H), 1577 (HC=N), 1468 (C=C), 1347 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.53 (s, 1H, NH), 8.40 (s, 1H, Py–H), 8.11 (s, 1H, CH), 7.61 (s, 4H, Ar–H). ^{13}C NMR (DMSO-d_6, 400 MHz): 101.0, 123.3, 128.8, 131.8, 133.2, 133.2, 145.2, 146.1, 150.2, 153.6.

*2-(4-Chlorobenzylidene)-1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**3c**)*

The product obtained from (**2**) (0.01 mol) and 4-chlorobenzaldehyde (0.012 mol). Yield 77 %. m.p.: 137–139 °C. FT-IR (KBr, cm^{-1}): 3382 (N–H), 2940 (C–H), 1532 (HC=N), 1465 (C=C), 1360 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.53 (s, 1H, NH), 8.41 (s, 1H, Py–H), 8.12 (s, 1H, CH), 7.70 (d, 2H, Ar–H, $J = 8.6$ Hz), 7.50 (d, 2H, Ar–H, $J = 4.2$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): 101.8, 131.1, 132.2, 132.4, 145.3, 146.1, 150.2, 150.8, 154.2.

*2-(3-Chlorobenzylidene)-1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**3d**)*

The product obtained from (**2**) (0.01 mol) and 3-chlorobenzaldehyde (0.012 mol). Yield 73 %. m.p.: 132–124 °C. FT-IR (KBr, cm^{-1}): 3349 (N–H), 2956 (C–H), 1562 (HC=N), 1451 (C=C), 1352 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.52 (s, 1H, NH), 8.41 (s, 1H, Py–H), 8.11 (s, 1H, CH), 7.70 (d, 2H, Ar–H, $J = 8.6$ Hz), 7.49 (d, 2H, Ar–H, $J = 4.3$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): 100.9, 113.1, 123.5, 128.8, 132.4, 144.6, 150.1, 156.4, 158.2, 160.4.

*2-(2-Chlorobenzylidene)-1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**3e**)*

The product obtained from (**2**) (0.01 mol) and 2-chlorobenzaldehyde (0.012 mol). Yield 75 %. m.p.: 144–145 °C. FT-IR (KBr, cm^{-1}): 3374 (N–H), 2982 (C–H), 1592 (HC=N), 1473 (C=C), 1366 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.82 (s, 1H, NH), 8.86 (s, 1H, Py–H), 8.13 (s, 1H, CH), 8.02 (d, 1H, Ar–H, $J = 8.1$ Hz), 7.52 (d, 1H, Ar–H, $J = 7.8$ Hz), 7.40–7.37 (m, 2H, Ar–H). ^{13}C NMR (DMSO-d_6, 400 MHz): 101.4, 123.1, 128.1, 129.3, 130.1, 130.4, 132.1, 134.8, 155.5, 155.9, 161.0.

*2-(4-Chloro-2-fluorobenzylidene)-1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**3f**)*

The product obtained from (**2**) (0.01 mol) and 4-chloro-2-fluorobenzaldehyde (0.012 mol). Yield 73 %. m.p.: 168–170 °C. FT-IR (KBr, cm^{-1}): 3358 (N–H), 2959 (C–H), 1560 (HC=N), 1481 (C=C), 1367 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.68 (s, 1H, NH), 8.40 (s, 1H, Py–H), 8.13 (s, 1H, CH), 7.67 (d, 2H, Ar–H, $J = 8.7$ Hz), 7.54 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d_6, 400 MHz): 101.4, 119.6, 127.2, 128.9, 131.5, 138.2, 146.2, 155.6, 159.2.

*2-(2-Methylbenzylidene)-1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**3g**)*

The product obtained from (**2**) (0.01 mol) and 2-methylbenzaldehyde (0.012 mol). Yield 71 %. m.p.: 197–198 °C. FT-IR (KBr, cm^{-1}): 3351 (N–H), 2982 (C–H), 1558 (HC=N), 1481 (C=C), 1366 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.44 (s, 1H, NH), 8.72 (s, 1H, Py–H), 8.10 (s, 1H, CH), 7.78 (d, 1H, Ar–H, $J = 4.2$ Hz), 7.25 (d, 1H, Ar–H, $J = 8.7$ Hz), 7.23–7.21 (t, 2H, Ar–H, $J = 5.1$ Hz), 2.32 (s, 3H CH_3). ^{13}C NMR (DMSO-d_6, 400 MHz): 20.2, 99.1, 122.6, 128.3, 128.7, 131.9, 132.8, 136.0, 144.4, 155.7, 158.2, 160.2.

*2-(4-Propylbenzylidene)-1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**3h**)*

The product obtained from (**2**) (0.01 mol) and 4-propylbenzaldehyde (0.012 mol). Yield 68 %. m.p.: 166–168 °C. FT-IR (KBr, cm^{-1}): 3372 (N–H), 2934 (C–H), 1577 (HC=N), 1468 (C=C), 1384 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.39 (s, 1H, NH), 8.40 (s, 1H, Py–H), 8.09 (s, 1H, CH), 7.59-7.56 (d, 2H, Ar–H, $J = 8.2$ Hz), 7.25-7.23 (d, 2H, Ar–H, $J = 6.2$ Hz), 2.58-2.48 (t, 2H, CH_2, $J = 7.6$ Hz), 1.62–1.54 (m, 2H, CH_2), 0.90 (t, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 400 MHz): 14.9, 24.5, 39.1, 101.6, 128.7, 130.6, 133.7, 136.2, 137.0, 140.1, 147.4, 157.6.

*2-(2-Fluoro-3-methoxybenzylidene)-1-(5-bromo-2-chloropyrimidin-4-yl)hydrazine (**3i**)*

The product obtained from (**2**) (0.01 mol) and 2-fluoro-3-methoxybenzaldehyde (0.012 mol). Yield 79 %. m.p.: 147–148 °C. FT-IR (KBr, cm^{-1}): 3374 (N–H), 2998 (C–H), 1556 (HC=N), 1452 (C=C), 1370 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.67 (s, 1H, NH), 8.69 (s, 1H, Py–H), 8.12 (s, 1H, CH), 7.48 (t, 1H, Ar–H, $J = 7.7$ Hz), 7.17–7.15 (d, 2H, Ar–H, $J = 9.0$ Hz), 3.84 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 400 MHz): 56.1, 100.4, 116.6, 124.5, 127.2 142.6, 145.2, 146.2, 148.7, 148.8, 152.8, 157.6.

*2-(2-Fluoro-5-methoxybenzylidene)-1-(5-bromo-2-morpholinopyrimidin-4-yl)hydrazine (**3j**)*

The product obtained from (**2**) (0.01 mol) and 2-fluoro-5-methoxybenzaldehyde (0.012 mol). Yield 75 %. m.p.: 168–169 °C. FT-IR (KBr, cm^{-1}): 3380 (N–H), 2968 (C–H), 1560 (HC=N), 1471 (C=C), 1370 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.76 (s, 1H, NH), 8.62 (s, 1H, Py–H), 8.13 (s, 1H, CH), 7.41 (d, 1H, Ar–H, $J = 5.6$ Hz), 7.19 (s, 1H, Ar–H), 7.00 (d, 1H, Ar–H, $J = 4.2$ Hz), 3.75 (s, 3H, CH_3), 3.67-3.62 (m, 8H, $4CH_3$). ^{13}C NMR (DMSO-d_6, 400 MHz): 100.1, 117.6, 124.5, 127.2 142.6, 145.2, 145.2, 148.7, 148.8, 154.8, 156.6.

General procedure for synthesis of 5-chloro-8-bromo-3-aryl-1,2,4-triazolo[4,3-*c*]pyrimidines (**4a–j**)

Hydrazones (**3a–j**) (0.01 mol) was dissolved in methanol (10 vol.) and IBD (0.0105 mol) were added to it. The reaction mixture was stirred at room temperature for 1 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, reaction mass cooled to 5–10 °C in ice bath and stirred for 3 h. The solid thus obtained was filtered and dried to afford the target molecule (**4a–j**).

*8-Bromo-5-chloro-3-phenyl-[1,2,4]triazolo[4,3-c]pyrimidine (**4a**)*

The general experimental procedure described above afforded **4a**, and the product obtained from hydrazine (**3a**) (2.74 g, 0.01 mol) and benzaldehyde (1.06 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2937 (C–H), 1637 (C=N), 1462 (C=C), 1376 (C–N), 522 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.43 (s, 1H, Py–H), 7.69–7.66 (d, 2H, Ar–H, $J = 9.0$ Hz), 7.45–7.37 (m, 3H, Ar–H). ^{13}C NMR (DMSO-d_6, 400 MHz): 99.6, 124.2, 125.8, 127.6, 128.6, 144.5, 146.3, 158.1, 161.0. MS (ESI) *m/z*: 312.0. Anal. Calcd. for $C_{11}H_6BrClN_4$ (in %): C, 42.68; H, 1.95; N, 18.10. Found: C, 42.70; H, 1.89; N, 18.07.

*8-Bromo-3-(4-bromo-phenyl)-5-chloro-[1,2,4]triazolo[4,3-c]pyrimidine (**4b**)*

The general experimental procedure described above afforded **4b**, and the product obtained from hydrazine (**3b**) (2.74 g, 0.01 mol) and 4-bromobenzaldehyde (1.85 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2936 (C–H), 1635 (C=N), 1461 (C=C), 1376 (C–N), 521 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.40 (s, 1H, Py–H), 7.69–7.66 (d, 2H,

Ar–H, $J = 9.0$ Hz), 7.45–7.37 (d, 2H, Ar–H, $J = 4.5$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): 101.6, 124.5, 125.9, 129.0, 131.8, 133.2, 145.2, 146.1, 150.2, 153.6. MS (ESI) *m/z*: 390.1. Anal. Calcd. for $C_{11}H_5Br_2ClN_4$ (in %): C, 34.01; H, 1.30; N, 14.42. Found: C, 34.09; H, 1.26; N, 14.40.

*8-Bromo-5-chloro-3-(4-chloro-phenyl)-[1,2,4] triazolo[4,3-c]pyrimidine (**4c**)*

The general experimental procedure described above afforded **4c**, and the product obtained from hydrazine (**3c**) (2.74 g, 0.01 mol) and 4-chlorobenzaldehyde (1.40 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2937 (C–H), 1635 (C=N), 1463 (C=C), 1375 (C–N), 722 (C–Cl), 522 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.42 (s, 1H, Py–H), 7.65–7.61 (d, 2H, Ar–H, $J = 8.7$ Hz), 7.40–7.32 (d, 2H, Ar–H, $J = 4.2$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): 101.4, 131.6, 131.4, 133.0, 145.4, 146.1, 152.2, 152.4, 155.5. MS (ESI) *m/z*: 344.0. Anal. Calcd. for $C_{11}H_5BrCl_2N_4$ (in %): C, 38.41; H, 1.47; N, 16.29. Found: C, 38.39; H, 1.42; N, 16.35.

*8-Bromo-5-chloro-3-(3-chloro-phenyl)-[1,2,4] triazolo[4,3-c]pyrimidine (**4d**)*

The general experimental procedure described above afforded **4d**, and the product obtained from hydrazine (**3d**) (2.74 g, 0.01 mol) and 3-chlorobenzaldehyde (1.40 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2935 (C–H), 1635 (C=N), 1464 (C=C), 1376 (C–N), 721 (C–Cl), 522 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.42 (s, 1H, Py–H), 7.66-7.60 (d, 2H, Ar–H, $J = 8.7$ Hz), 7.40–7.32 (d, 2H, Ar–H, $J = 4.2$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): 100.4, 113.7, 124.8, 129.2, 134.4, 145.1, 150.5, 156.1, 157.1, 161.0. MS (ESI) *m/z*: 344.1. Anal. Calcd. for $C_{11}H_5BrCl_2N_4$ (in %): C, 38.41; H, 1.47; N, 16.29. Found: C, 38.47; H, 1.41; N, 16.34.

*8-Bromo-5-chloro-3-(2-chloro-phenyl)-[1,2,4] triazolo[4,3-c]pyrimidine (**4e**)*

The general experimental procedure described above afforded **4e**, and the product obtained from hydrazine (**3e**) (2.74 g, 0.01 mol) and 2-chlorobenzaldehyde (1.40 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2936 (C–H), 1634 (C=N), 1465 (C=C), 1376 (C–N), 722 (C–Cl), 521 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.41 (s, 1H, Py–H), 7.65–7.60 (d, 2H, Ar–H, $J = 8.7$ Hz), 7.41–7.33 (d, 2H, Ar–H, $J = 4.2$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): 101.7, 124.6, 131.2, 131.5, 131.7, 132.7, 134.6, 136.1, 137.3, 148.8, 155.6. MS (ESI) *m/z*: 344.2. Anal. Calcd. for $C_{11}H_5BrCl_2N_4$ (in %): C, 38.41; H, 1.47; N, 16.29. Found: C, 38.40; H, 1.50; N, 16.28.

*8-Bromo-5-chloro-3-(4-chloro-2-fluoro-phenyl)-[1,2,4]triazolo[4,3-c]pyrimidine (**4f**)*

The general experimental procedure described above afforded **4f**, and the product obtained from hydrazine (**3f**) (2.74 g, 0.01 mol) and 4-chloro-2-fluorobenzaldehyde (1.58 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2936 (C–H), 1635 (C=N), 1466 (C=C), 1376 (C–N), 1270 (C–F), 722 (C–Cl), 522 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.40 (s, 1H, Py–H), 7.68 (s, 1H, Ar–H), 7.40–7.35 (d, 2H, Ar–H, $J = 4.4$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): 99.9, 117.2, 124.5, 129.6, 132.6, 136.6, 137.4, 148.0, 157.2, 161.0. MS (ESI) *m/z*: 364.1. Anal. Calcd. for $C_{11}H_4BrCl_2FN_4$ (in %): C, 36.50; H, 1.11; N, 15.48. Found: C, 36.48; H, 1.06; N, 15.40.

*8-Bromo-5-chloro-3-o-tolyl-[1,2,4]triazolo[4,3-c]pyrimidine (**4g**)*

The general experimental procedure described above afforded **4g**, and the product obtained from hydrazine (**3g**) (2.74 g, 0.01 mol) and 2-methylbenzaldehyde (1.20 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2935 (C–H), 1636 (C=N), 1470 (C=C), 1376 (C–N), 722 (C–Cl), 521 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.42 (s, 1H, Py–H), 7.68 (d, 1H, Ar–H, $J = 4.3$ Hz), 7.50 (d, 1H, Ar–H, $J = 4.6$ Hz), 7.25-7.10 (m, 2H, Ar–H), 3.42 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 400 MHz): 20.7, 99.7, 124.7, 128.5, 128.6, 130.1, 131.0, 134.2, 144.1, 153.6, 157.2, 158.5. MS (ESI) *m/z*: 326.0. Anal. Calcd. for $C_{12}H_8BrClN_4$ (in %): C, 44.54; H, 2.49; N, 17.31. Found: C, 44.48; H, 2.56; N, 17.27.

*8-Bromo-5-chloro-3-(4-propyl-phenyl)-[1,2,4] triazolo[4,3-c]pyrimidine (**4h**)*

The general experimental procedure described above afforded **4h**, and the product obtained from hydrazine (**3h**) (2.74 g, 0.01 mol) and 4-propylbenzaldehyde (1.48 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2936 (C–H), 1640 (C=N), 1472 (C=C), 1376 (C–N), 721 (C–Cl), 521 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.60 (s, 1H, Py–H), 7.43 (d, 2H, Ar–H, $J = 4.6$ Hz), 7.20 (d, 2H, Ar–H, $J = 5.1$ Hz), 2.54-2.48 (t, 2H, CH_2, $J = 7.6$ Hz), 2.00–1.85 (m, 2H, CH_2), 1.05 (t, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 400 MHz): 14.6, 24.2, 38.4, 101.7, 125.9, 133.0, 133.4, 134.4, 140.8, 152.6, 158.1. MS (ESI) *m/z*: 354.1. Anal. Calcd. for $C_{14}H_{12}BrClN_4$ (in %): C, 47.82; H, 3.44; N, 15.93. Found: C, 47.78; H, 3.46; N, 15.97.

*8-Bromo-5-chloro-3-(2-fluoro-3-methoxy-phenyl)-[1,2,4]triazolo[4,3-c]pyrimidine (**4i**)*

The general experimental procedure described above afforded **4i**, and the product obtained from hydrazine (**3i**) (2.74 g, 0.01 mol) and 2-fluoro-3-methoxybenzaldehyde (1.54 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2935 (C–H), 1641 (C=N), 1473 (C=C), 1376 (C–N), 1290 (C–F), 722 (C–Cl), 522 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.64 (s, 1H, Py–H), 7.05 (d, 1H, Ar–H, $J = 4.1$ Hz), 6.90 (t, 1H, Ar–H, $J = 3.1$ Hz), 6.74 (d, 1H, $J = 4.2$ Hz), 3.75 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz): 56.35, 99.8, 117.6, 124.6, 129.2, 137.3, 138.4, 145.1, 146.4, 148.9, 150.5, 155.7. MS (ESI) *m/z*: 358.0. Anal. Calcd. for $C_{12}H_7BrClFN_4O$ (in %): C, 40.31; H, 1.97; N, 15.67. Found: C, 40.48; H, 1.76; N, 15.70.

*8-Bromo-5-chloro-3-(2-fluoro-5-methoxy-phenyl)-[1,2,4]triazolo[4,3-c]pyrimidine (**4j**)*

The general experimental procedure described above afforded **4j**, and the product obtained from hydrazine (**3j**) (2.74 g, 0.01 mol) and 2-fluoro-5-methoxybenzaldehyde (1.54 g, 0.01 mol). FT-IR (KBr, cm^{-1}): 2936 (C–H), 1642 (C=N), 1474 (C=C), 1376 (C–N), 1299 (C–F), 722 (C–Cl), 522 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.65 (s, 1H, Py–H), 7.01 (s, 1H, Ar–H), 6.94 (d, 1H, Ar–H, $J = 3.7$ Hz), 6.71 (d, 1H, $J = 4.3$ Hz), 3.74 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz): 56.25, 99.6, 117.6, 124.1, 128.2, 138.3, 138.4, 145.1, 146.4, 147.9, 151.5, 156.7. MS (ESI) *m/z*: 358.0. Anal. Calcd. for $C_{12}H_7BrClFN_4O$ (in %): C, 40.31; H, 1.97; N, 15.67. Found: C, 40.32; H, 1.77; N, 15.71.

Biology

Antibacterial activity

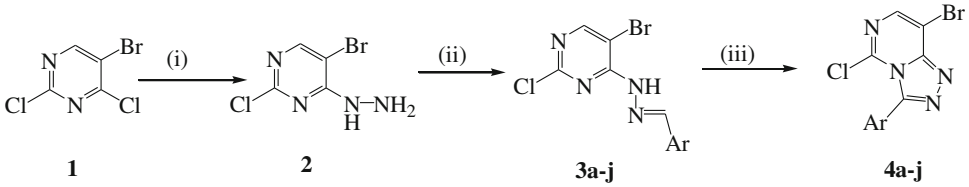
Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria (*Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 7443) and Gram-negative bacteria (*Xanthomonas campestris* MTCC 7908 and *Escherichia coli* MTCC 7410) in DMF by disc diffusion method on nutrient agar medium (Andrews, 2001). The sterile medium (nutrient agar medium, 15 ml) in each Petri plate was uniformly smeared with cultures of Gram-positive and -negative bacteria. Sterile discs of 10 mm diameter (Hi-Media) was placed in the Petri plates, to which 50 µl (1 mg/ml, i.e. 50 µg/disc) of the different synthesized compounds were added. The treatments also included 50 µl of DMF as negative, 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.

Antifungal activity

The synthesized compounds were screened for their antifungal activity against *Fusarium oxysporum* MTCC 2480 in DMF by poisoned food technique (Satish *et al.*, 2007). Potato dextrose agar (PDA) media was prepared and about 15 ml of PDA was poured into each Petri plate and allowed to solidify. 5 mm disc of 7 days old culture of the test fungi was placed at the center of the Petri plate 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 new compounds/Petri plate, where concentration was 0.1 mg/ml) by poisoned food technique.

Results and discussion

The new 5-chloro-8-bromo-3-aryl-1,2,4-triazolo[4,3-*c*]pyrimidines (**4a–j**) were synthesized according to Scheme 1. Reaction of 5-bromo-2,4-dichloropyrimidine (**1**) with hydrazine hydrate at 5–10 °C resulted in the formation of 1-(5-bromo-2-chloropyrimidine-4-yl)hydrazine (**2**). Further condensation with different aldehydes in ethanol at a reflux temperature gave the corresponding hydrazones (**3a–j**) with high yield. Then the reactions of hydrazones (**3a–j**) were carried out with 1.05 equivalent of IBD in methanol by stirring for 1 h to give the corresponding 5-chloro-8-bromo-3-aryl-1,2,4-triazolo[4,3-*c*] pyrimidines (**4a–j**). The structures of the newly synthesized compounds (**4a–j**) were confirmed by FT-IR, ^{1}H NMR, and mass spectral data. The chemical structures and physical data of all the synthesized compounds are tabulated in Table 1.



Scheme 1 Reagents and conditions: (*i*) $NH_2NH{\cdot}H_2O$, MeOH, TEA, 5–10 °C. (*ii*) ArCHO, ethanol, reflux, 2 h. (*iii*) IBD, MeOH, 15–20 °C, 2 h

Table 1 Chemical structures and physical data of pyrimidine compounds (**4a–j**)

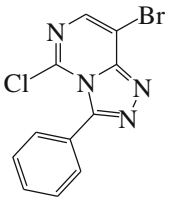
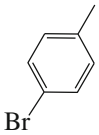
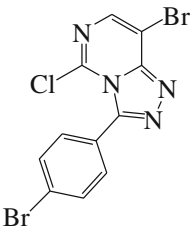
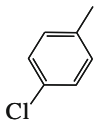
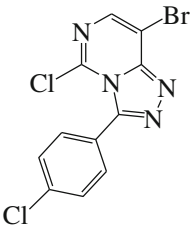
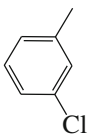
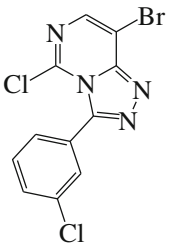
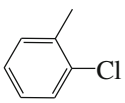
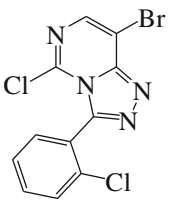
Compounds	Ar	Structure	Yield (%)	m.p. (°C)
4a			76	124–126
4b			74	160–163
4c			81	170–172
4d			80	179–181
4e			79	200–202

Table 1 continued

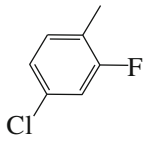
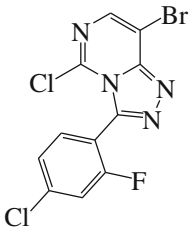
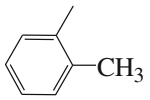
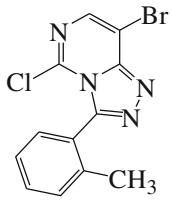
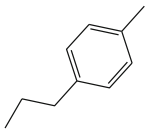
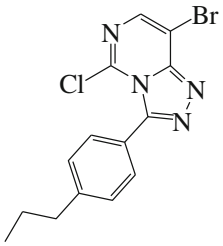
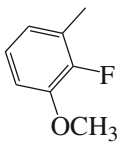
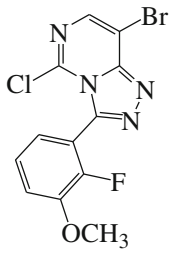
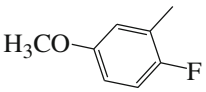
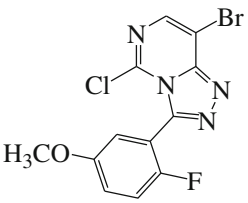
Compounds	Ar	Structure	Yield (%)	m.p. (°C)
4f			81	164–166
4g			80	137–140
4h			74	139–141
4i			84	188–190
4j			75	182–184

Table 2 In vitro antibacterial and antifungal activities of the synthesized compounds

Compounds	Zone of inhibition in diameter (mm)				% Inhibition
	B. subtilis	*S. aureus*	*X. campestris*	*E. coli*	*F. oxysporum*
4a	15	16	14	14	52.6
4b	24	23	21	23	64.5
4c	22	20	21	20	62.4
4d	22	21	22	21	63.0
4e	23	22	21	22	63.4
4f	28	27	29	28	86.6
4g	16	18	16	15	55.2
4h	17	19	18	19	57.9
4i	27	25	25	27	74.0
4j	26	24	24	26	67.8
Bacteriomycin	–	–	34	–	–
Gentamycin	35	30	–	35	–
Nystatin	–	–	–	–	100

The characterization of products (**4a–j**) was based upon a careful comparison of ^{1}H NMR spectra with those of **3a–j**. An important characteristic feature in the ^{1}H NMR spectra of **4a–j** was the disappearance of the singlet due to N–CH around 8.10–8.30 and NH proton around 8.50–8.8 singlet which was presence in the spectra **3a–j**. The FT-IR spectra of **4a–j** were recorded using KBr pellets in the range of 4,000–400 cm^{-1}. The absorption bands around 2,925 cm^{-1} are assigned to the C–H stretch. The strong bands around 1,375 cm^{-1} are assigned to the C–N stretch. New bands appeared at 1,270 cm^{-1} (**4f**); 1,290 cm^{-1} (**4i**) and 1,299 cm^{-1} (**4j**) corresponding to C–F stretching frequency. The strong bands at 720–723 cm^{-1} are assigned to the C–Cl stretch in **4c**, **4d**, **4e**, and **4f**. The ^{1}H NMR spectra of **4f** and **4i** showed singlet in the region of δ 8.40 and 8.64, respectively. The mass spectra of **4f** showed molecular ion peak at *m*/*z* 363.8 which is in agreement with the molecular formula $C_{11}H_4BrCl_2FN_4$. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ±0.4 %.

Compounds (**4a–j**) were tested in vitro for their antibacterial activity against two Gram-positive and two Gram-negative bacterial strains. Commercial antibiotics such as bacteriomycin and gentamycin were used as standard drugs. The results were compared with standard drugs and depicted in Table 2. Compound **4f** was found to be more potent against Gram-positive and -negative bacterial strains with the zone of inhibition, respectively, 27–29 mm. Compounds **4i** and **4j** exhibited good antibacterial activity against all the bacterial strains. Compounds **4b–4e** were showed moderate antibacterial activity and compound **4b** was found to be slightly active than **4c–4e**. Compounds **4g** and **4h** were found to be moderate antibacterial activity and compound **4a** was weakly active against tested bacterial strains.

The in vitro antifungal activity of the new pyrimidine derivatives (**4a–j**) was studied against the fungal strain, *Fusarium oxysporum*. Nystatin was used as a standard drug and the results are given in Table 2. Compounds **4f**, **4i**, and **4j** showed good inhibition against *F. oxysporum.* Compounds **4b**, **4c**, **4d**, and **4e** exhibited moderate antifungal activity against tested fungal strain. Compounds **4g** and **4h** exhibited moderate antifungal activity against *F. oxysporum*. On the other hand, the lowest antifungal effect was detected for compound **4a** against tested fungal strain. Among the compounds (**4a–j**) the antimicrobial inhibitory activity follows the order **4f** > **4i** > **4j** > **4b** > **4e** > **4d** > **4c** > **4h** > **4g** > **4a** against tested microbial strains.

Initial structure–activity relationship (SAR) can be drawn for the compounds (**4a–j**). Antibacterial activity of 1,2,4-triazolo[4,3-*a*]pyrimidines (Khera *et al.*, 2011) and SAR studies of pyrimido[4,5-*d*]pyrimidine-2,5-dione derivatives (Sondhi *et al.*, 2001) have been reported. In this study, different electron withdrawing and electron donating groups attached to phenyl ring as substituent linkage to 1,2,4-triazole ring. The close survey of antimicrobial efficacy indicated that the inhibition values of all the compounds exhibited a varied range of 14–29 mm and 52.6–86.6 % of antibacterial and antifungal activities, respectively, against the entire tested microbial strains. The electron withdrawing halogen groups in **4f** produces enhanced antimicrobial activity against the tested organisms. The electron-donating methoxy group in **4i** produces enhanced activity probably by *m*-position compared to the *o*-position in **4j**. This indicates the positional requirement of methoxy group on phenyl ring for enhanced activity. **4b** showed better antimicrobial activity compared to **4h** and

4g probably due to the presence of electron withdrawing group in **4b**. The chlorine atom in **4c**, **4d**, and **4e** showed moderate antimicrobial activity whereas the presence of phenyl ring alone in **4a** produces weak antimicrobial activity against tested microbial strains. The biological results for compounds (**4a**–**j**) showed that the substitution pattern on phenyl ring appears to be vital for broad spectrum activity. The above SAR studies reveal that, the nature of the functional groups is crucial for biological activity.

Conclusion

In conclusion, a series of new 5-chloro-8-bromo-3-aryl-1,2,4-triazolo[4,3-*c*]pyrimidines (**4a**–**j**) were synthesized in good yield, characterized by different spectral studies and their antimicrobial activities have been evaluated. Compounds **4f**, **4i**, and **4j** demonstrated good inhibition against microbial strains tested. The SAR studies reveal that the substituent on phenyl ring is responsible for the antimicrobial activity of these classes of agents. On the basis of their activity, these derivatives were identified as viable leads for further studies.

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