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Research Article

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL BROMO-PYRIMIDINE ANALOGS AS ANTICANCER AND ANTIMICROBIAL AGENTS

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ABSTRACT

A novel series of 5-bromo-pyrimidine derivatives (**3,4,5a-l and 6a-h**) were synthesized through multi step reactions starting from 5-bromo-2,4-dichloro pyrimidine. The newly synthesized compounds were characterized using elemental analysis and spectral data (IR, 1H NMR, 13C NMR and LC-MS) analysis. The titled compounds were evaluated for antimicrobial activity by broth dilution method and their in vitro cytotoxic activity. Among the synthesized compounds **5a, 5c, 5e, 6b, 6d,** and **6h** exhibit broad spectrum antimicrobial activity against tested microbial strains. The invitro cancer results ascertain **5c, 5e, 6d, 6g** and **6h** are most potent molecules in comparison to reference standard Dasatinib.

Keywords: 5-Bromo-pyrimidine, Anticancer, Dasatinib derivatives, Cytotoxicity and MTT.

1. INTRODUCTION

Cancer is the worldwide health problem and the most frightening disease of human. Chemotherapy, either alone or as an adjunct to radiotherapy or surgery remains the treatment of choice in most of the cancers.[1-3]

The current anticancer agents are mostly broad acting cytotoxic drugs. They impact structure and function of the rapidly proliferating cancer cells and arrest the cell cycle at a specific phase depending on the mechanism of action of the agents[4-5]. Due to their lack in specificity and adverse effects related to impact on rapidly dividing noncancerous cells, there is an urgent need for identification of novel, potent, selective and less toxic agents, which can overcome cancer resistance to drug treatment that has made many of the currently available chemotherapeutic agents ineffective.[6]

The α, β-unsaturated ketones (chalcones) are considered to be precursors of flavonoids and isoflavonoids, found as naturally- occurring compounds, but it could be considered that their true importance is extended in two branches. The biological activity associated with them, including anti-inflammatory[7-8], antipyretic[9], anti invasive[10], anticancer[11], anti tuberculosis[12] and antifungal activities[13]. And their recognized synthetic utility in the preparation of pharmacologically-interesting heterocyclic systems like pyrazolines, which have been largely studied owing to their pharmacological activities, which includes anti-tumour, anti-inflammatory, anti-parasitary, anti-depressive, anticonvulsant, antimicrobial and inflammatory arthritis[14-17].

Further, in recent times it is reported that the incorporation of fluorine atom into heterocycles provides compounds with enhanced biological properties. The enhanced biological activity of fluorinated heterocycles is due to accumulation of fluorine on carbon and causing increased oxidative and thermal stability. Hence fluorinated drugs due to their inherent characteristics of being metabolically non-degradable and increased lipid solubility are utilized to enhance rate of drug absorption and their in vivo transport[18].

This renewed interest in this class of compounds and in continuation of our research to furnish biologically new active compounds[19-23] has encouraged to synthesize a series of novel bromo-Pyrimidine analogs and evaluate their anticancer and antimicrobial activities.

2. RESULTS AND DISCUSSION

The synthetic scheme utilized for synthesis of titled 5-bromo-pyrimidine derivatives **3, 4, 5a-l and 6a-h** were outlined in Scheme 1[24-26] and their analytical and physical properties were depicted in Table 1.

A series of subsituted pyrimidine-sulfonamide derivatives (**5a-l**) were prepared by treating key intermediates **4** with commercially available subsituted sulfonylchlorides in presence of dichloromethane and triethylamine at 25-30 ^{o}C under an inert nitrogen atmosphere.

The target compounds (**6a-h**) were successfully obtained by amide bond formation through coupling reaction via reaction of intermediates **4** with commercially available subsituted acids in presence of N, N-dimethylformamide as a solvent, N, N-diisopropylethylamine as a base and HATU was used as a coupling reagent, and reaction was carried out under an inert nitrogen atmosphere.

3. Biological activity

The standard strains were procured from the American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The antibacterial activity of the synthesized 5-bromo-pyrimidine derivatives **(4, 5a-l and 6a-h)** was performed by broth dilution method against the following standard bacterial strains *Staphylococcus aureus* (ATCC 11632), *Streptococcus faecalis* (ATCC 14506), *Bacillus subtilis* (ATCC 60511), *Klebsiella pneumoniae* (ATCC 10031), *Escherichia* and *Pseudomonas aeruginosa* (ATCC 10145) and antifungal activity against Yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, CT), mould: *Aspergillus niger* (ATCC 6275).

Subsequently, evaluated for their in vitro anti-cancer activity against tumor cell lines panel consisted of Hela (Human cervixcarcinoma cell line), A549 (Human lung adenocarcinoma cell line), MCF-7 (Human breast adenocarcinoma cell line), A2780 (Human ovarian cancer cell line) and BGC-823 (Human gastric cancer cell line) by using MTT assay Mosmann's method.

The MTT assay is based on the reduction of the soluble 3-(4, 5-methyl -2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT 0.5 mg/mL, 100 µL), into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells.

^{1}H NMR spectrum of intermediate **3** displayed triplet at δ 3.42-3.48 ppm (J 4.72-4.88 Hz) due to piperazine and singlet at δ 8.45 ppm representing CH of Pyrimidine. Absence of tert-butyl signals in ^{1}H NMR spectrum and molecular ion peak in mass spectrum confirm formation of key intermediate **4**. IR spectrum of 5-bromo-4-(4-(substituted sulfonyl) piperazin-1-yl)-2-chloropyrimidine derivatives (**5a-l)** displayed strong absorption band at around 1324 cm-1 (S=O) due to sulfonyl group. In addition, absence of NH peak (δ 7.88 ppm) and appearance of triplet peaks due to CH2 of piperazine at around δ 3.0-3.42 ppm and 3.7-3.82 ppm in 1H NMR spectrum established the formation of compounds **5a-l.**

The IR spectrum of compounds **6a-h** exhibit strong absorption band at around 1654 cm-1 and a resonance peak around δ 161.85 in 13C NMR spectrum due to CO group. Additionally, absence of NH peak (δ 7.88 ppm) of compound **4** in 1H NMR spectrum and results of elemental and mass spectral analysis confirm formation of desired compounds.

4. CONCLUSION

In conclusion, this work demonstrates the synthesis of novel series of 5-bromo-pyrimidine derivatives **(4, 5a-l and 6 a-h)** and in vitro evaluation of their antimicrobial (bacterial and fungal) and anticancer activity against Hela (Human cervix carcinoma cell line), A549 (Human lung adenocarcinoma cell line), MCF-7 (Human breast adenocarcinoma cell line), A2780 (Human ovarian cancer cell line) and BGC-823 (Human gastric cancer cell line) by using MTT assay.

Antimicrobial study revealed that compounds **5a, 5c, 5e, 6b, 6d,** and **6h** demonstrated significant activity against tested Gram-positive and Gram-negative bacteria and fungal species. The invitro anticancer screening of the synthesized series illustrate that all compounds were active, in particular compounds **5c, 5e, 6d, 6g** and **6h** exhibited excellent anticancer activity when compared with reference drug Dasatinib. The promising invitro antimicrobial and anticancer activity of 5-bromo-pyrimidine derivatives make them certainly promising molecules for further lead optimization in the development of novel antimicrobial and anticancer agents.

5. Experimental

5.1 Chemical Protocols

Melting points were determined in open capillary tubes in a Thomas Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT-IR 157, ^{1}H NMR and ^{13}C NMR spectra were recorded (in CDCl3/DMSO-d6) on a Bruker spectrometer at 300/400 MHz using TMS as an internal standard. Mass spectra (EI) on (AMD-604) mass spectrometer operating at 70 eV. Elemental analysis was performed on Thermo Finnigan Flash (EA 1112 CHNS Analyzer).

5.1.1. General procedure for the synthesis of tert-butyl 4-(5-bromo-2-chloropyrimidin-4-yl)piperazine-1-carboxylate (3)

The mixture of 5-bromo-2,4-dichloropyrimidine (70.0 g, 0.3072 mol) and dichloromethane (700 mL) was cooled to 0-5 °C. Further, Triethylamine (123.90 g, 1.2252 mol) and tert-butyl piperazine-1-carboxylate (68.6 g, 0.3682 mol) was slowly added to the above pre cooled reaction mixture at 0-5 °C. The temperature of reaction mixture was increased to 25-30 °C and stirred for 2 h. The reaction completion was monitored by TLC.

Upon completion of reaction, reaction mixture was quenched into ice water and stirred at 25-30 °C for 1 h. The organic layer was separated and concentrated, then triturated using n-heptane, reaction mass was filtered off and dried at 45-50 °C for 5 h. Off white solid obtained (103.24 g, yield 89 %); mp 83-85 °C; IR (KBr) νmax/cm-1 1693 (C=O), 1559 (C=C); 1H NMR (400 MHz, DMSO-d6): *δ* 1.42 (s, 9H, t-butyl), 3.45 (t, *J* 4.72, 4H, 2CH2, piperazine), 3.67 (t, *J* 4.88, 4H, 2CH2, piperazine), 8.45 (s, 1H, CH, Pyrimidine); LC-MS (*m/z*, %): 379 (M+2, 98).

5.1.2. General procedure for the synthesis of 5-bromo-2-chloro-4-(piperazin-1-yl)pyrimidine dihydrochloride (4)

The mixture of *tert*-butyl 4-(5-bromo-2-chloropyrimidin-4-yl)piperazine-1-carboxylate **3** (45 g, 0.1191 mol) and 1,4-dioxane (450 mL) was cooled to 0-5 °C. Further, 4.5M HCl in dioxane (225 mL) was slowly added to the above pre cooled reaction mixture at 0-5 °C and stirred at 25-30 °C for 10 h. Reaction mass was filtered off and dried at 45-50 °C and packed in air tight container. The compound was obtained as off white hygroscopic solid (35.50 g, yield 85 %); mp 174-178 °C; IR (KBr) νmax/cm-1 1557 (C=C); 1H NMR (400 MHz, DMSOd6): *δ* 3.19 (t, *J* 4.60, 4H, 2CH2, piperazine), 3.90(t, *J* 4.78, 4H, 2CH2, piperazine), 7.88 (s,1H, NH), 8.45 (s, 1H, CH, Pyrimidine), 9.74 (s, 2H, HCl); LC-MS (*m/z*, %): 277 (M, 96).

5.1.3. General procedure for the synthesis of 5-bromo-4-(4-(substituted sulfonyl) piperazin-1-yl)-2-chloropyrimidine (5a-l)

The mixture of 5-bromo-2-chloro-4-(piperazin-1-yl)pyrimidine dihydrochloride **4** (0.00285 mol), substituted sulfonyl chloride (0.00391 mol) and dichloromethane (10 V) was cooled to 0-5 °C. Further, Triethylamine (0.00989 mol) was added slowly at 0-5 °C. The reaction mixture was warmed to 25-30 °C and stirred for 16 h. After completion of reaction, water was added to reaction mixture and filtered off. The obtained solid was recrystallized from suitable solvent to obtain target compounds.

5.1.3.1. Synthesis of 5-bromo-2-chloro-4-(4-(4-fluorophenylsulfonyl)piperazin-1-yl) pyrimidine (5a)

Recrystallized from ethanol, off white solid (yield 84 %); mp 148-152 °C; IR (KBr) νmax/cm-1 1560 (C=C), 1324 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 3.06 (t, *J* 4.44, 4H, 2CH2, piperazine), 3.75 (t, *J* 4.52, 4H, 2CH2, piperazine), 7.68 (d, *J* 8.52, 2H, 2CH, 4-fluorophenyl), 8.42 (s, 1H, CH, Pyrimidine), 8.68 (d, *J* 8.48, 2H, 2CH, 4-fluorophenyl); 13C NMR (100 MHz, DMSO-d6): *δ* 45.61, 47.11 (piperazine), 104.40 (pyrimidine-C5), 126.36, 129.57, 132.02, 138.7 (4-fluorophenyl-C2, C6, C3, C5, C1), 157.61, 161.68 (pyrimidine- C2, C6), 163.31 (4-fluorophenyl-C4), 174.74 (pyrimidine- C4); LC-MS (*m/z*, %): 436.0 (M+2, 97).

5.1.3.2. Synthesis of 5-bromo-2-chloro-4-(4-tosylpiperazin-1-yl)pyrimidine (5b)

Recrystallized from ethanol, off white solid (yield 80 %); mp 150-154 °C; IR (KBr) νmax/cm-1 1562 (C=C), 1322 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 2.40 (s, 3H, CH3, methyl benzene), 3.0 (t, *J* 4.36, 4H, 2CH2, piperazine), 3.72 (t, *J* 4.40, 4H, 2CH2, piperazine), 7.45 (d, *J* 8.04, 2H, 2CH, methyl benzene), 7.63 (d, *J* 8.12, 2H, 2CH, methyl benzene), 8.42(s, 1H, CH, Pyrimidine);13C NMR (100 MHz, DMSO-d6): *δ* 21.30 (methyl, methyl benzene), 45.62, 47.10 (piperazine), 104.41 (pyrimidine-C5), 128.32, 129.37, 137.61, 143.31 (methyl benzene–C2, C6, C3, C5, C4, C1), 157.60, 161.68, 174.74 (pyrimidine-C2, C6, C4); LCMS (*m/z*, %): 433 (M+1, 98.2).

5.1.3.3. Synthesis of 5-bromo-2-chloro-4-(4-(4-(trifluoromethyl)phenylsulfonyl) piperazin-1-yl)pyrimidine (5c)

Recrystallized from ethanol, off white solid (yield 83 %); mp 128-132 °C; IR (KBr) νmax/cm-1 1559 (C=C), 1320 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 3.08 (t, *J* 4.41, 4H, 2CH2, piperazine), 3.75 (t, *J* 4.48, 4H, 2CH2, piperazine), 7.69 (m, 2H, 2CH, 4-(trifluoromethyl)phenyl), 7.88 (m, 2H, 2CH, 4-(trifluoromethyl)phenyl), 8.44 (s, 1H, CH, Pyrimidine); 13C NMR (100 MHz, DMSO-d6): *δ* 45.63, 47.12 (piperazine), 104.40 (pyrimidine-C5), 124.11 (trifluoro carbon), 127.1, 128.71, 134.76, 149.60 (4-(trifluoromethyl)phenyl-C3, C5, C2, C6, C4, C1), 157.60, 161.68, 174.73 (pyrimidine-C2, C6, C4); LC-MS (*m/z*,%): 486 (M+2, 98.5).

5.1.3.4. Synthesis of 5-bromo-2-chloro-4-(4-(methylsulfonyl)piperazin-1-yl)pyrimidine (5d)

Recrystallized from ethanol, off white solid (yield 82 %); mp 90-94 °C; IR (KBr) νmax/cm-1 1563 (C=C), 1322 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 3.02 (t, *J* 4.38, 4H, 2CH2, piperazine), 3.32 (s, 3H, methyl), 3.79 (t, *J* 4.42, 4H, 2CH2, piperazine), 8.42 (s, 1H, CH, Pyrimidine); 13C NMR (100 MHz, DMSO-d6): *δ* 40.12 (methyl), 45.60, 47.10 (piperazine), 104.42 (pyrimidine-C5), 157.60, 161.66, 174.70 (pyrimidine-C2, C6, C4); LCMS (*m/z*, %): 356 (M+2, 97.8).

5.1.3.5. Synthesis of 5-bromo-2-chloro-4-(4-(2-nitro-4 (trifluoromethyl)phenylsulfonyl) piperazin-1-yl)pyrimidine (5e)

Recrystallized from ethanol, off white solid (yield 90 %); mp 156-159 °C; IR (KBr) νmax/cm-1 1560 (C=C), 1324 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 3.38(t, *J* 4.80, 4H, 2CH2, piperazine), 3.78 (t, *J* 4.40, 4H, 2CH2, piperazine), 8.21 (s, H, CH, 2-nitro-4-(trifluoromethyl)phenyl), 8.26 (s, 1H, CH, Pyrimidine), 8.45 (s, H, CH, 2-nitro-(trifluoromethyl) phenyl), 8.65 (s, H, CH, 2-nitro-4-(trifluoromethyl)phenyl); 13C NMR (100 MHz, DMSO-d6): *δ* 45.60, 47.12 (piperazine), 104.40 (pyrimidine-C5), 122.44 (2-nitro-4-(trifluoromethyl)phenyl-C3), 124.11 (trifluoro carbon), 129.84, 133.27, 134.48, 137.70, 148.47 (2-nitro-4-(trifluoromethyl)phenyl-C6, C5, C4, C1, C2), 157.65, 161.69, 174.72 (pyrimidine-C2, C6, C4); LC-MS (*m/z*, %): 530 (M+2, 99.2).

5.1.3.6. Synthesis of 5-bromo-2-chloro-4-(4-(naphthalen-2-ylsulfonyl)piperazin-1-yl) pyrimidine (5f)

Recrystallized from ethanol, off white solid (yield 78 %); mp 160-164 °C; IR (KBr) νmax/cm-1 1564 (C=C), 1321 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 3.04 (t, *J* 4.40, 4H, 2CH2, piperazine), 3.78 (t, *J* 4.42, 4H, 2CH2, piperazine), 7.60 (m, 2H, 2CH, naphthalene), 7.77(m, 2H, 2CH, naphthalene), 7.86(m, H, CH, naphthalene), 8.0(m, H, CH, naphthalene), 8.38 (m, H, CH, naphthalene), 8.42 (s, 1H, CH, Pyrimidine); 13C NMR (100 MHz, DMSOd6):*δ* 45.61, 47.13 (piperazine), 104.39 (pyrimidine-C5), 123.41, 126.02, 126.23, 128.14,129.40, 134.11, 136.7, 137.04 (naphthalene), 157.62, 161.68, 173.50 (pyrimidine-C2, C6, C4);LC-MS (*m/z*, %): 468.2 (M+2, 98.5).

5.1.3.7. Synthesis of 4-(4-(3,5-bis(trifluoromethyl)phenylsulfonyl)piperazin-1-yl)-5-bromo-2-chloro pyrimidine (5g)

Recrystallized from ethanol, off white solid (yield 84 %); mp 145-149 °C; IR (KBr) νmax/cm-1 1565 (C=C), 1321 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 3.42 (t, *J* 4.82, 4H, 2CH2, piperazine), 3.80 (t, *J* 4.43, 4H, 2CH2, piperazine), 8.22 (m, 2H, 2CH, 3,5-bis(trifluoromethyl)phenyl), 8.28 (s, 1H, CH, Pyrimidine), 8.48 (m, H, CH, 3,5-bis(trifluoromethyl)phenyl); 13C NMR (100 MHz, DMSO-d6): *δ* 45.61, 47.12 (piperazine), 104.30 (pyrimidine-C5), 124.12 (trifluoro carbon), 126.10, 126.32, 131.61, 143.31(3,5-bis(trifluoromethyl)phenyl-C2, C6, C4, C3, C5, C1), 157.64, 161.68, 174.70 (pyrimidine-C2, C6, C4); LC-MS (*m/z*, %): 554 (M+2, 99.3).

5.1.3.8. Synthesis of 5-bromo-2-chloro-4-(4-(propylsulfonyl)piperazin-1-yl)pyrimidine (5h)

Recrystallized from ethanol, off white solid (yield 77 %); mp 90-94 °C; IR (KBr) νmax/cm-1 1563 (C=C), 1320 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 0.98 (m, 3H, CH3, propyl), 1.73 (m, 2H, CH, propyl), 3.41 (t, *J* 4.40, 4H, 2CH2, piperazine), 3.60 (m, 2H,CH, propyl), 3.82 (t, *J* 4.44, 4H, 2CH2, piperazine), 8.30 (s, 1H, CH, Pyrimidine); 13C NMR (100 MHz, DMSO-d6): *δ* 12.41, 13.31 (CH3, CH2, propyl), 45.61, 47.14 (piperazine), 60.4 (CH2, propyl), 104.40 (pyrimidine-C5), 157.62, 161.65, 171.82 (pyrimidine-C2, C6, C4); LC-MS (*m/z*, %): 384 (M+2, 98.6).

5.1.3.9. Synthesis of 5-bromo-2-chloro-4-(4-(biphenyl-4-ylsulfonyl)piperazin-1-yl)pyrimidine (5i)

Recrystallized from ethanol, off white solid (yield 80 %); mp 148-152 °C; IR (KBr) νmax/cm-1 1558(C=C), 1321 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 3.05 (t, *J* 4.40, 4H, 2CH2, piperazine), 3.79 (t, *J* 4.44, 4H, 2CH2, piperazine), 7.40-7.52 (m, 3H, 3CH, biphenyl), 7.64-7.87 (m, 6H, 6CH, biphenyl), 8.40 (s, 1H, CH, Pyrimidine); 13C NMR (100 MHz, DMSO-d6): *δ* 45.09, 47.12 (piperazine), 104.40 (pyrimidine-C5), 127.11, 127.60, 127.81, 127.92, 129.21, 138.61, 140.82 (biphenyl), 157.61, 161.69, 172.30 (pyrimidine-C2, C6, C4); LC-MS (*m/z*, %): 494 (M+2, 98.8).

5.1.3.10. Synthesis of 5-bromo-2-chloro-4-(4-(3,5-dimethylisoxazol-4-ylsulfonyl) piperazin-1-yl) pyrimidine (5j)

Recrystallized from ethanol, yellowish to off white solid (yield 83 %); mp 138-142 °C; IR (KBr) νmax/cm-1 1561 (C=C), 1322 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 2.34 (s,3H, CH3, 3,5-dimethylisoxazol), 2.62 (s, 3H, CH3, 3,5-dimethylisoxazol), 3.20 (t, *J* 4.80, 4H,2CH2, piperazine), 3.81 (t, *J* 4.72, 4H, 2CH2,

piperazine), 8.46 (s, 1H, CH, Pyrimidine); 13CNMR (100 MHz, DMSO-d6): *δ* 11.44, 13.19 (methyl, 3,5-dimethylisoxazol), 45.18, 46.78 (piperazine), 104.22 (pyrimidine-C5), 112.74 (3,5-dimethylisoxazol-C1), 150.03, 157.64 (3,5-dimethylisoxazol-C2, C3), 158.07, 161.55, 174.73 (pyrimidine-C2, C6, C4); LC-MS (*m/z*, %):437 (M+2, 98.4).

5.1.3.11. Synthesis of 5-bromo-4-(4-(4-tert-butylphenylsulfonyl)piperazin-1-yl)-2-chloro pyrimidine (5k)

Recrystallized from ethanol, off white solid (yield 81 %); mp 135-139 °C; IR (KBr) νmax/cm-1 1562 (C=C), 1323 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 1.14 (s, 9H, 3CH3, tbutyl), 3.03 (t, *J* 4.74, 4H, 2CH2, piperazine), 3.70 (t, *J* 4.44, 4H, 2CH2, piperazine), 7.48 (m, 2H, 2CH, 4-tert-butylphenyl), 7.68 (m, 2H, 2CH, 4-tert-butylphenyl), 8.42 (s, 1H, CH, Pyrimidine); 13C NMR (100 MHz, DMSO-d6): *δ* 31.3 (CH3, tert-butyl), 34.2 (tert-butyl), 45.60, 47.11 (piperazine), 104.23 (pyrimidine-C5), 125.31, 128.05, 143.21, 154.60 (4-tert-butylphenyl-C3, C5, C2, C6, C1, C4), 157.64, 161.66, 174.20 (pyrimidine-C2, C6, C4); LC-MS(*m/z,* %): 475 (M+2, 99.4).

5.1.3.12. Synthesis of 5-bromo-2-chloro-4-(4-(cyclopropylsulfonyl)piperazin-1-yl) pyrimidine (5l)

Recrystallized from ethanol, off white solid (yield 78 %); mp 148-152 °C; IR (KBr) νmax/cm-1 1559 (C=C), 1324 (S=O); 1H NMR (400 MHz, DMSO-d6): *δ* 0.94 (t, *J* 2.36, 2H, 2CH, cyclopropyl), 0.98 (t, *J* 3.68, 2H, 2CH, cyclopropyl), 2.63 (m, 1H, CH, cyclopropyl), 3.30 (t, *J* 4.81, 4H, 2CH2, piperazine), 3.78 (t, *J* 4.43, 4H, 2CH2, piperazine), 8.49 (s, 1H, CH,Pyrimidine); 13C NMR (100 MHz, DMSO-d6): *δ* 4.0, 37.5 (cyclopropyl), 46.20, 47.14 (piperazine), 104.42 (pyrimidine-C5), 157.62, 161.68, 174.70 (pyrimidine-C2, C6, C4); LCMS (*m/z*, %): 382 (M+2, 98.7).

5.1.4. General procedure for the synthesis of (4-(5-bromo-2-chloropyrimidin-4-yl) piperazin-1-yl)(Susbsituted acid)methanone (6a-h)

The mixture of 5-bromo-2-chloro-4-(piperazin-1-yl)pyrimidine dihydrochloride **4** (0.00285mol), substituted acid (0.00371 mol) and N, N, dimethylformamide (10 V) was cooled to 0-5 °C. Further, N, N-Diisopropylethylamine (0.0142 mol), HATU (0.00371 mol) was added slowly at 0-5 °C and mixture was stirred for 20 min. The reaction mass warmed to 25-30 °C and stirred for 16 h. After completion of reaction, the reaction mass was diluted with ethyl acetate (25 V), organic layer was washed using 1M citric acid solution, 1M lithium hydroxide solution and followed by water. Organic layer was concentrated and triturated using n-heptane. Reaction mass was filtered off and obtained solid was recrystallized with
suitable solvent to obtain titled compounds.

5.1.4.1. Synthesis of methyl 1-(4-(5-bromo-2-chloropyrimidin-4-yl)piperazin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (6a)

Recrystallized from ethanol, off white solid (yield 86 %); mp 96-99 °C; IR (KBr) νmax/cm-1 1654 (C=O), 1571 (C=C); 1H NMR (300 MHz, DMSO-d6): *δ* 0.86 (s, 6H, 2CH3, isopropyl), 1.94 (m, 1H, CH, isopropyl), 3.53 (s, 3H, CH3, -O-CH3), 3.68 (m, 8H, 4CH2, piperazine), 4.26 (s, 1H, CH, methane), 7.34 (d, *J* 7.88, 1H, NH, sec. amide), 8.46 (s, 1H, CH, Pyrimidine); 13C NMR (75 MHz, DMSO-d6): *δ* 18.55, 19.69, 30.25 (isopropyl), 44.99, 47.35 (piperazine), 51.93 (methoxy), 55.99 (-CH-NH-), 104.08 (pyrimidine-C5), 157.20 (NHCO-O-), 157.68, 161.74 (pyrimidine-C2, C6), 161.85 (CO), 170.87 (pyrimidine-C4); LC-MS(*m/z*, %): 435 (M+2, 98.9).

5.1.4.2. Synthesis of (4-(5-bromo-2-chloropyrimidin-4-yl) piperazin-1-yl)(4-fluorophenyl) methanone (6b)

Recrystallized from ethanol, off white solid (yield 79 %); mp 126-129 °C; IR (KBr) νmax/cm-1 1643 (C=O), 1563 (C=C); 1H NMR (300 MHz, DMSO-d6): *δ* 3.38 (t, *J* 4.42, 4H, 2CH2, piperazine), 3.76 (t, *J* 4.01, 4H, 2CH2, piperazine), 7.37 (m, 2H, 2CH, 4-fluorophenyl), 8.43 (s, 1H, CH, Pyrimidine), 8.67 (m, 2H, 2CH, 4-fluorophenyl); 13C NMR (75 MHz, DMSO-d6): *δ* 46.26, 47.35 (piperazine), 104.20 (pyrimidine-C5), 115.30, 128.83, 130.32, 131.28 (4-fluorophenyl-C3, C5, C2, C6, C1), 157.89, 161.76 (pyrimidine-C2, C6), 161.82(CO), 163.31 (4-fluorophenyl-C4), 170.93 (pyrimidine- C4); LC-MS (*m/z*, %): 401 (M+2, 99.6).

5.1.4.3. Synthesis of (4-(5-bromo-2-chloropyrimidin-4-yl)piperazin-1-yl)(2-chloro-5-iodophenyl)methanone (6c)

Recrystallized from ethanol, yellowish to off white solid (yield 85 %); mp 135-139 °C; IR (KBr) νmax/ cm-1 1646 (C=O), 1562 (C=C); 1H NMR (300 MHz, DMSO-d6): *δ* 3.39 (t,*J* 4.42, 4H, 2CH2, piperazine), 3.75 (t, *J* 4.0, 4H, 2CH2, piperazine), 7.38 (d, *J* 6.54, 1H, CH,2-chloro-5-iodophenyl), 7.98 (d, *J* 7.04, 1H, CH, 2-chloro-5-iodophenyl), 8.42 (s, 1H, CH,Pyrimidine), 8.69 (s, 1H, CH, 2-chloro-5-iodophenyl); 13C NMR (75 MHz, DMSO-d6): *δ*46.26, 47.34 (piperazine), 98.42 (2-chloro-5-iodophenyl-C5), 104.25 (pyrimidine-C5), 130.30,133.24, 134.86, 136.21, 140.02 (2-chloro-5-iodophenyl-C2, C3, C1, C6,

C4), 157.93, 161.81(pyrimidine-C2, C6), 162.20(CO), 170.92 (pyrimidine-C4); LC-MS (*m/z*, %): 542 (M+2,98.4).

5.1.4.4. Synthesis of (4-(5-bromo-2-chloropyrimidin-4-yl)piperazin-1-yl)(2,6-difluorophenyl) methanone (6d)

Recrystallized from ethanol, off white solid (yield 81 %); mp 130-134 °C; IR (KBr) νmax/cm-1 1643 (C=O), 1563 (C=C); 1H NMR (300 MHz, DMSO-d6): *δ* 3.39(t, *J* 4.44, 4H, 2CH2, piperazine), 3.77 (t, *J* 3.72, 4H, 2CH2, piperazine), 7.19 (t, *J* 7.92, 2H, 2CH, 2,6-difluorophenyl), 7.51 (m, *J* 6.78, 1H, CH, 2,6-difluorophenyl) 8.45 (s, 1H, CH, Pyrimidine); 13C NMR (75 MHz, CDCl3): *δ* 46.25, 47.37 (piperazine), 104.17 (pyrimidine-C5), 111.70, 113.30, 131.28 (2,6-difluorophenyl-C3, C5, C1, C4), 157.14 (CO), 158.49 (pyrimidine-C2), 160.27 (2,6-difluorophenyl-C2, C6), 161.45, 170.98 (pyrimidine-C6, C4); LC-MS (*m/z*, %): 418 (M+2, 99.3).

5.1.4.5. Synthesis of (4-(5-bromo-2-chloropyrimidin-4-yl)piperazin-1-yl)(4,5,6-trichloropyridin -2-yl)methanone (6e)

Recrystallized from ethanol, off white solid (yield 82 %); mp 158-162 °C; IR (KBr) νmax/ cm-1 1640 (C=O), 1561 (C=C); 1H NMR (300 MHz, DMSO-d6): *δ* 3.39 (t, *J* 4.41, 4H,2CH2, piperazine), 3.75 (t, *J* 4.13, 4H, 2CH2, piperazine), 8.45 (s, 1H, CH, Pyrimidine), 9.21(s, 1H, CH, 4,5,6-trichloropyridin); 13C NMR (75 MHz, DMSO-d6): *δ* 46.24, 47.32 (piperazine), 104.30 (pyrimidine-C5), 122.32, 132.82, 145.67, 149.23, 152.10 (4,5,6-trichloropyridin-C2, C4, C3, C5, C1), 157.84, 161.80 (pyrimidine-C2, C6), 162.34 (CO), 170.76(pyrimidine-C4); LC-MS (*m/z*, %): 487.8 (M+3, 99.5).

5.1.4.6. Synthesis of (4-(5-bromo-2-chloropyrimidin-4-yl)piperazin-1-yl)(4-chloro-2-nitrophenyl)methanone (6f)

Recrystallized from ethanol, off white solid (yield 87 %); mp 152-156 °C; IR (KBr) νmax/cm-1 1647 (C=O), 1561 (C=C); 1H NMR (300 MHz, DMSO-d6): *δ* 3.38 (t, *J* 4.40, 4H, 2CH2, piperazine), 3.76 (t, *J* 3.98, 4H, 2CH2, piperazine), 8.45 (s, 1H, CH, Pyrimidine), 8.56 (d, *J* 6.84, 1H, CH, 4-chloro-2-nitrophenyl), 8.88 (d, *J* 6.23, 1H, CH, 4-chloro-2-nitrophenyl), 9.21 (s, 1H, CH, 4-chloro-2-nitrophenyl); 13C NMR (75 MHz, DMSO-d6): *δ* 46.30, 47.42 (piperazine), 104.30 (pyrimidine-C5), 124.12, 129.56, 131.23, 134.61, 134.74, 147.84 (4-chloro-2-nitrophenyl-C3, C6, C1, C4, C5, C2), 157.88, 161.90 (pyrimidine-C2, C6), 162.42(CO), 170.77 (pyrimidine-C4); LC-MS (*m/z*, %): 462 (M+2, 98.9).

5.1.4.7. Synthesis of (4-(5-bromo-2-chloropyrimidin-4-yl)piperazin-1-yl)(4-(trifluoromethyl)phenyl)methanone (6g)

Recrystallized from ethanol, off white solid (yield 89 %); mp 132-136 °C; IR (KBr) νmax/cm-1 1644 (C=O), 1564 (C=C); 1H NMR (300 MHz, DMSO-d6): *δ* 3.40 (t, *J* 4.52, 4H, 2CH2, piperazine), 3.79 (t, *J* 4.10, 4H, 2CH2, piperazine), 7.70 (m, *J* 6.80, 2H, 2CH, 4-(trifluoromethyl)phenyl), 7.94 (m, *J* 6.77, 2H, 2CH, 4-(trifluoromethyl)phenyl), 8.44 (s, 1H, CH, Pyrimidine); 13C NMR (75 MHz, DMSO-d6): 46.24, 47.32 (piperazine), 104.30 (pyrimidine-C5), 124.11 (trifluoro carbon), 126.9, 128.61, 134.66, 148.50 (4-(trifluoromethyl)phenyl-C3, C5, C2, C6, C4, C1), 157.90, 161.12 (pyrimidine-C2, C6), 162.54 (CO), 170.78(pyrimidine-C4); LC-MS (*m/z*, %): 450.0 (M+2, 98.5).

5.1.4.8. Synthesis of (4-(5-bromo-2-chloropyrimidin-4-yl) piperazin-1-yl) (2, 5-dichlorophenyl)methanone (6h)

Recrystallized from ethanol, off white solid (yield 78 %); mp 148-152 °C; IR (KBr) νmax/cm-1 1645 (C=O), 1562 (C=C); 1H NMR (300 MHz, DMSO-d6): *δ* 3.40 (t, *J* 4.19, 4H, 2CH2, piperazine), 3.76 (t, *J* 4.14, 4H, 2CH2, piperazine), 7.56 (d, *J* 6.73, 1H, CH, 2,5-dichlorophenyl), 7.72 (d, *J* 7.24, 1H, CH, 2,5-dichlorophenyl), 7.98 (s, 1H, CH, 2,5-dichlorophenyl), 8.44 (s, 1H, CH, Pyrimidine); 13C NMR (75 MHz, DMSO-d6): *δ* 46.20, 47.31 (piperazine), 104.30 (pyrimidine-C5), 126.62, 128.72, 132.21, 132.32, 132.42, 134.73 (2,5-dichlorophenyl-C4, C6, C5, C3, C2, C1), 157.84, 161.80 (pyrimidine-C2, C6), 162.24 (CO),170.86 (pyrimidine-C4); LC-MS (*m/z*, %): 451 (M+2, 97.9).

5.1.5. General procedure for the synthesis of tert-butyl 4-(5-bromo-2-morpholinopyrimidin-4-yl) piperazine-1-carboxylate (7)

The mixture of *tert*-butyl 4-(5-bromo-2-chloropyrimidin-4-yl)piperazine-1-carboxylate **3** (50 g, 0.1324 mol), ethanol (500 mL) and triethylamine (26.79 g, 0.2647 mol) was cooled to 20-25 °C. Further, morpholine (23.06 g, 0.2647 mol) was added slowly to the above reaction mixture at 20-25 °C and refluxed for 12 h. The resulting solution was concentrated, quenched into ice water and stirred at room temperature for 1 h. The solid formed was filtered and recrystallized with ethanol to obtain off white solid (51 g, yield 90 %); mp 86-89 °C; IR (KBr) νmax/ cm-1 1695 (C=O), 1565 (C=C); 1H NMR (400 MHz, DMSO-d6): *δ* 1.41 (s, 9H,tbutyl), 3.42 (t, *J* 4.23, 4H, 2CH2, piperazine), 3.48 (t, *J* 3.44, 4H, 2CH2, piperazine), 3.62 (m,

8H, 4CH2, morpholine), 8.13 (s,1H, CH, Pyrimidine); LC-MS (*m/z*, %): 428.4 (M+2, 98).

5.1.6. General procedure for the synthesis of 5-bromo-2-morpholino-4-(piperazin-1-yl) pyrimidine dihydrochloride (8)

The mixture of tert-butyl 4-(5-bromo-2-morpholinopyrimidin-4-yl)piperazine-1-carboxylate **7** (46 g, 0.1074 mol) in 1,4-Dioxane (460 mL) was cooled to 0-5 °C. Further, 4.5M HCl in dioxane (230 mL) slowly was added to above pre cooled reaction mixture at 0-5 °C and stirred at 25-30 °C for 10 h. Solids were filtered, dried at 45-50 °C for 7 h and packed in air tight container. The compound was obtained as off white hygroscopic solid (37.0 g, yield 86 %); mp 168-172 °C; IR (KBr) νmax/cm-1 1567 (C=C); 1H NMR (400 MHz, DMSO-d6): δ 3.17 (t, *J* 4.41, 4H, 2CH2, piperazine), 3.66 (m, 8H, 4CH2, morpholine), 3.84(t, *J* 4.03, 4H, 2CH2, piperazine), 8.12 (s, 1H, NH), 8.20 (s,1H, CH, Pyrimidine), 9.64 (s, 2H, HCl); LC-MS (*m/z*, %): 330 (M, 98.2).

5.2 Biological protocol

5.2.1 Antimicrobial Activity

The antimicrobial susceptibility testing was performed in vitro by broth micro dilution method (Hassan E et al., 1993); (Khalil MA1993). The MIC determination of the synthesized 6-di fluoro Chalcone derivatives **(3, 4, 5, 6, 7a-h, 8, 9a-g and 10a-g)** was carried out in side-by-side comparison with ciprofloxacin and norfloxacin against Gram-positive (*S.aureus*, *S.faecalis, B.subtilis*) and Gram-negative (*K.penumoniae, E.coli. P.aeruginosa*) bacteria. The antifungal activity was assayed against yeasts (*C.tropicalis, S.cerevisiae*) and moulds (*A.niger*). The minimal inhibitory concentrations (MIC, µg/mL) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. Test compounds (10mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL) then diluted in culture medium (Mueller-Hinton Broth for bacteria and Sabouraud Liquid Medium for fungi), further progressive dilutions to obtain final concentrations of 1, 2, 4, 8, 16, 31.25, 62.5, 125, 250 and 500 µg/mL. DMSO never exceeded 1% v/v. The tubes were inoculated with 105 cfu/ mL (colony forming unit/mL) and incubated at 37°C for 24h. The growth control consisting of media (positive control) and media with DMSO (negative control) at the same dilutions as used in the experiments were employed.

5.2.2Anticancer activity

The synthesized ,6-di fluoro Chalcone derivatives **(3, 4, 5a-l and 6a-h)** were tested in vitro for their cytotoxic properties against tumor cell lines panel consisted of Hela (Human cervix carcinoma cell line), A549 (Human lung adenocarcinoma cell line), MCF-7 (Human breast adenocarcinoma cell line), A2780 (Human ovarian cancer cell line) and BGC-823 (Human gastric cancer cell line) by using MTT assay Mosmann's method. The MTT assay is based on the reduction of the soluble 3-(4,5-methyl -2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT 0.5 mg/mL, 100 µL), into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells (Mosmann T 1983). The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin and streptomycin at 37^{o}C and humidified at 5% CO2. Briefly cells were placed on 96-well plates at 100 µL total volume with density of 1-2.5 X 104 cells/mL and were allowed to adhere for 24 h before treatment with tested drugs in DMSO solution (10-5, 10-6, 10-7 mol/L final concentration). Triplicate wells were treated with media and agents. Cell viability was assayed after 96 h of continuous drug exposure with a tetrazolium compound. The supernant medium was removed, and 150 µL of DMSO solution was added to each well. The plates were gently agitated using mechanical plate mixer until the color reaction was uniform and the OD570 was determined using microplate reader. The 50% inhibitory concentration (IC50) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of vehicle in the MTT assay. Assays were performed in triplicate on three independent experiments. The results had good reproducibility between replicate wells with standard errors below 10%.

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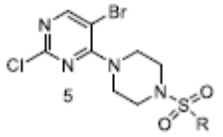
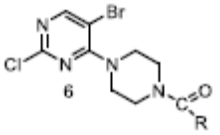
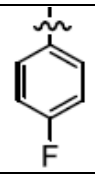
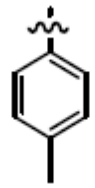
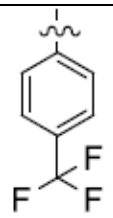
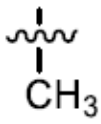
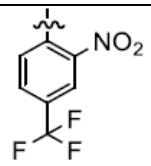
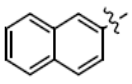
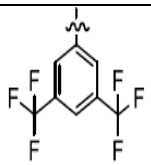
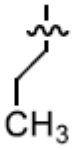
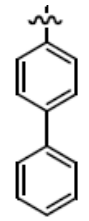
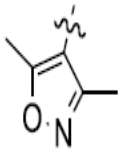
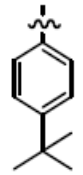
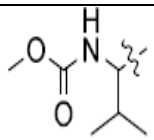
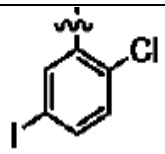
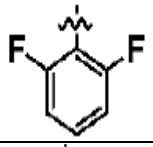
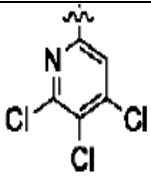
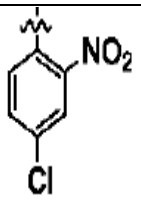
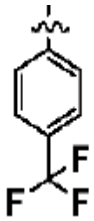
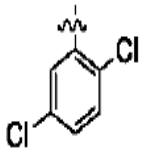
Table 1: Analytical and Physico-chemical data of synthesized compounds (3, 4, 5a-l, and 6a-h)





Compound	R	Molecular Formula	M.W[a]	M.p ([o]C)[b]/crystallization solvent	Yield (%)	% Analysis of C,H,N found (Calc.)[c]		
						C	H	N
3	-	C13H18BrClN4O2	377.66	(83-85) Ethanol	91	NA	NA	NA
4	-	C8H12BrCl2N3	350.47	(175-178) Ethanol	86	NA	NA	NA
5a		C14H13BrClFN4O2S	435.7	(145-148) Ethanol	87	73.76 (73.72)	4.13 (4.11)	16.24 (16.22)
5b		C15H16BrClN4O2S	431.74	(150-152) Ethanol	78	60.55 (60.53)	4.13 (4.11)	13.24 (13.22)
5c		C15H13BrClF3N4O2S	485.71	(128-130) Ethanol	76	61.81 (61.82)	4.48 (4.44)	9.83 (9.87)
5d		C9H12BrClN4O2S	355.64	(90-94) Ethanol	87	58.10 (58.12)	4.14 (4.13)	16.94 (16.92)
5e		C15H12BrClF3N5O4S	530.7	(156-158) Ethanol	82	60.33 (60.35)	3.56 (3.57)	13.03 (13.04)
5f		C18H16BrClN4O2S	467.77	(160-164) Ethanol	85	55.87 (55.85)	3.47 (3.49)	12.07 (12.02)
5g		C16H12BrClF6N4O2S	553.7	(145-148) Ethanol	88	60.90 (60.92)	4.60 (4.55)	11.84 (11.82)
5h		C11H16BrClN4O2S	383.69	(90-94) Ethanol	69	63.95 (63.94)	4.81 (4.83)	15.43 (15.44)

5i		C20H18BrClN4O2S	493.8	(148-152) Ethanol	87	63.26 (63.23)	4.36 (4.30)	13.17 (13.14)
5j		C13H15BrClN5O3S	436.71	(138-142) Ethanol	87	65.23 (65.20)	4.50 (4.53)	13.58 (13.55)
5k		C18H22BrClN4O2S	473.81	(135-138) Ethanol	69.5	60.49 (60.48)	3.67 (3.69)	11.76 (11.74)
5l		C11H14BrClN4O2S	381.68	(238-241) Ethanol	84.1	58.24 (58.22)	3.19 (3.11)	8.86 (8.85)
6a		C15H21BrClN5O3	434.72	(245-247) Ethanol	81.9	68.13 (68.12)	4.23 (4.21)	10.36 (10.35)
6b		C15H13BrClFN4O	542	(212-216) Ethanol	74.3	62.80 (62.82)	3.67 (3.66)	9.55 (9.57)
6c		C15H12BrCl2IN4O	485.98	(253-255) Ethanol	78.0	61.33 (61.32)	3.57 (3.58)	12.44 (12.47)
6d		C15H12BrClF2N4O	461.1	(231-233) Ethanol	73.4	68.72 (68.71)	4.57 (4.49)	10.02 (10.07)
6e		C14H10BrCl4N5O	449.65	(247-249) Ethanol	83.2	65.54 (65.55)	4.07 (4.09)	9.97 (9.92)

6f		C15H12BrCl2N5O3	450.54	(132-134) Ethanol	84.3	66.19 (66.15)	4.40 (4.41)	9.65 (9.66)
6g		C16H13BrClF3N4O	428.32	(148-149) Ethanol	80.3	69.05 (69.08)	4.10 (4.12)	10.07 (10.05)
6h		C15H12BrCl3N4O	401.13	(86-89) Ethanol	68.2	66.01 (66.03)	3.85 (3.82)	13.39 (13.37)

[a] Molecular weight of the compound
[b] Melting point of the compound
[c] Elelmental analysis of C, H, and N were with in ±0.4% of theoretical value

Table 2: Antimicrobial activity expressed as MIC (µg/mL)

Compounds	Gram-positive organisms[a]			Gram-negative organisms[a]			Fungi[a]		
	Sa	Sf	Bs	Kp	Ec	Pa	Sc	Ct	An
3	31.25	125	125	125	125	8	62.5	62.5	62.5
4	31.25	31.25	8	62.5	62.5	31.25	125	31.25	62.5
5a	**8**	**4**	**4**	16	8	4	125	31.25	62.5
5b	16	31.25	31.25	31.25	31.25	31.25	62.5	31.25	62.5
5c	31.25	62.5	62.5	**4**	**4**	**4**	31.25	8	31.25
5d	16	16	62.5	62.5	16	16	62.5	4	16
5e	**4**	**4**	**4**	62.5	62.5	62.5	62.5	31.25	125
5f	31.25	125	31.25	16	16	31.25	31.25	31.25	16
5g	31.25	31.25	62.5	31.25	62.5	62.5	31.25	62.5	16
5h	250	125	16	125	16	125	250	125	125
5i	125	16	16	125	125	16	8	8	16
5a	8	16	16	16	62.5	62.5	62.5	31.25	125
5b	125	125	16	16	125	31.25	8	16	16
5c	16	16	31.25	16	62.5	16	31.25	31.25	31.25
5d	125	125	31.25	31.25	16	125	16	62.5	8
5e	16	8	31.25	16	16	62.5	62.5	31.25	125
5f	31.25	125	31.25	16	8	62.5	62.5	8	8
5g	31.25	16	62.5	31.25	31.25	8	8	125	125
5h	16	8	16	62.5	16	62.5	62.5	125	62.5
5i	16	31.25	31.25	16	16	62.5	16	16	31.25
5j	16	16	62.5	62.5	16	62.5	16	16	16
5k	31.25	16	16	62.5	31.25	62.5	62.5	125	125
5l	31.25	31.25	31.25	31.25	16	16	31.25	16	16
6a	62.5	8	31.25	125	16	31.25	125	62.5	62.5
6b	**4**	**8**	**4**	**4**	**8**	**8**	62.5	125	62.5
6c	16	125	31.25	16	62.5	62.5	31.25	125	16
6d	**4**	**8**	**4**	16	125	31.25	125	125	16
6e	16	16	8	16	31.25	31.25	62.5	62.5	62.5
6f	62.5	16	31.25	62.5	8	31.25	62.5	125	62.5
6g	62.5	16	31.25	62.5	8	31.25	62.5	125	62.5
6h	**4**	**8**	**4**	**4**	**8**	**8**	62.5	125	62.5
Ciprofloxacin	≤5	≤5	≤1	≤1	≤1	>5	-	-	-
Norfloxacin	<5	<5	≤1	≤1	≤1	>5	-	-	-
Flucanozole	-	-	-	-	-	-	≤1	≤1	≤1

[a]The screening organisms. Gram-positive bacteria: *Staphylococcus aureus* (ATCC 11632, Sa), *Streptococus faecalis* (ATCC 14506, Sf), and *Bacillus subtilis* (ATCC 60511, Bs).

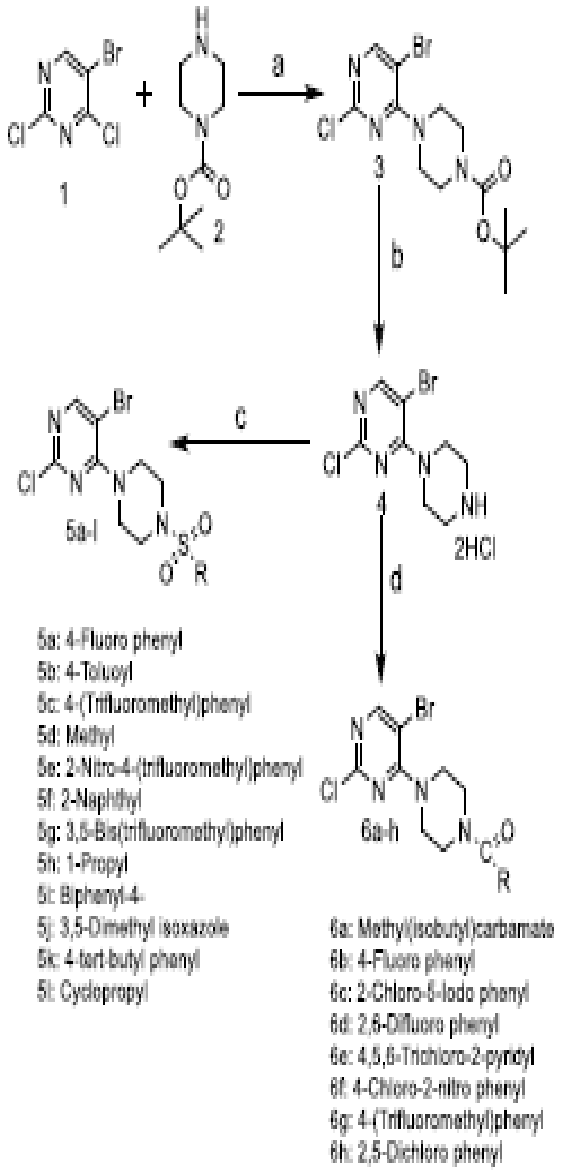
[a]The screening organisms. Gram-negative bacteria: *Klebsiella penumoniae* (ATCC 10031, Kp), *Escherichia coli* (ATCC 10536, Ec), and *Pseudomonas aeruginosa* (ATCC 10145, Pa).

[a]The screening organisms. Yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, Ct), mould: *Aspergillus niger* (ATCC 6275, An).

Table 3: Cytotoxicity of synthesized compounds (3, 4, 5, 6, 7a-h, 8, 9a-g and 10a-g) against human tumor cells (IC50 ±SD, µM)

Compounds	Human tumor cells				
	Hela	A549	MCF-7	A2780	BGC-823
3	6.25±1.24	2.55±0.15	2.15±2.10	3.62±1.29	1.84±0.72
4	6.31±3.29	4.18±0.24	2.11±0.54	3.91±1.54	3.85±0.38
5a	**7.23±0.39**	**0.96±0.28**	**1.20±0.92**	2.54±0.48	1.32±0.53
5b	6.88±1.44	1.84±0.32	2.02±0.41	3.88±1.69	1.95±0.71
5c	**3.98±0.50**	**1.14±0.37**	**0.85±0.47**	3.89±0.45	1.56±0.65
5d	7.04±1.13	2.30±0.43	192±0.50	4.30±0.42	1.09±0.56
5e	**2.87±0.08**	**1.87±0.52**	1.85±0.34	3.44±0.70	1.76±0.71
5f	6.30±0.32	1.84±0.48	1.74±0.43	3.57±0.46	1.54±0.39
5g	5.91±0.81	1.50±0.72	1.65±0.53	2.62±0.44	1.45±0.28
5h	7.23±0.47	4.48±0.84	2.12±0.49	2.78±0.56	1.22±0.37
5i	6.09±0.33	1.89±0.42	2.60±0.48	3.93±0.57	1.28±0.30
5a	6.45±1.84	4.58±0.11	1.94±0.21	5.32±1.25	2.35±0.16
5b	6.13±0.35	3.25±0.87	1.89±0.35	4.51±2.32	3.31±0.54
5c	5.71±0.24	2.11±0.64	1.72±1.98	4.33±1.59	1.97±0.91
5d	4.94±0.91	1.56±0.15	1.69±1.14	2.72±0.41	1.41±0.29
5e	6.62±0.56	3.26±0.95	3.22±2.05	4.35±0.36	6.11±0.73
5f	6.59±0.75	4.21±0.51	3.23±0.85	5.14±0.87	3.22±0.24
5g	6.23±0.41	3.44±0.23	4.32±0.11	3.14±0.23	1.04±0.34
5h	6.49±0.19	2.53±0.56	2.04±0.43	3.26±0.87	1.23±0.89
5i	5.91±0.47	3.01±0.50	2.23±0.50	3.09±1.34	2.01±0.04
5j	5.98±0.24	1.43±0.35	2.45±0.52	3.18±1.52	1.11±1.11
5k	6.24±0.48	2.11±0.41	2.76±0.47	3.56±0.34	1.43±1.22
5l	**4.86±0.49**	**1.21±0.92**	2.50±0.42	3.03±1.54	1.20±0.56
6a	7.09±0.53	1.57±0.08	3.04±0.58	2.45±1.02	2.11±0.43
6b	1.76±0.60	1.93±0.59	2.09±0.44	2.26±0.64	2.04±0.30
6c	6.12±0.77	1.43±0.52	2.14±0.58	2.45±0.70	1.36±0.59
6d	6.76±0.60	1.93±0.59	**2.09±0.44**	**2.26±0.64**	**1.07±0.30**
6e	6.16±0.32	1.72±1.98	4.33±1.59	4.33±1.59	1.65±0.10
6f	5.46±0.43	1.69±1.14	2.72±0.41	2.72±0.41	1.44±0.20
6g	**4.36±0.26**	**0.93±0.59**	3.22±2.05	4.35±0.36	1.53±0.30
6h	**3.77±0.76**	**0.88±0.59**	2.09±0.44	2.26±0.64	1.43±0.30
Dasatinib (Control)	5.71±0.57	1.33±0.55	1.62±0.44	2.32±0.39	0.92±0.19

[a] Mean value ±SD (standard deviation from three experiments).
[b] Boldface: IC50 ≤ the control



Scheme 1. Convergent synthesis pathway of clubbed 5-bromo-pyrimidine derivatives

a) Dichloromethane, Triethylamine, 2h at 25-30°C. b) 1,4-Dioxane, 4.5M HCl in dioxane, 10h at 25-30°C. c) Dichloromethane, Triethylamine, subsituted sulphonyl chlorides stirred for 16h at 25-30°C. d) N,N-dimethylformamide, N,N-Diisopropylethylamine, subsituted acids, 1-Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate(HATU),16h at 25-30°C.

Scheme. 1:

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