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Synthesis of novel piperazine and morpholine linked substituted pyrimidine derivatives as antimicrobial agents

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Abstract

Several 6-(substituted)-5-cyano-2-thiouracils **1a-d**, were prepared by the condensation of different aldehydes with ethylcyanoacetate and thiourea through a single step reaction by conventional and microwave irradiation technique. The compounds 6-(substituted)-5-cyano-2-thiouracils **1a-d** were further treated with methyl iodide in presence of potassium carbonate and N,N-dimethyl formamide, as solvent to get 1-methyl-2-methylthio-4-(substituted)-5-cyano-1,6-dihydropyrimidine-6-ones **2a-d**. Methylated compounds were further treated with heterocyclic secondary amines like N-methylpiperazine, piperazine and morpholine to get **3a-d, 4a-d** and **5a-d.** All the synthesized compounds were screened for their antimicrobial activity and were characterized by elemental analyses, IR, ^{1}H NMR and mass spectral data.

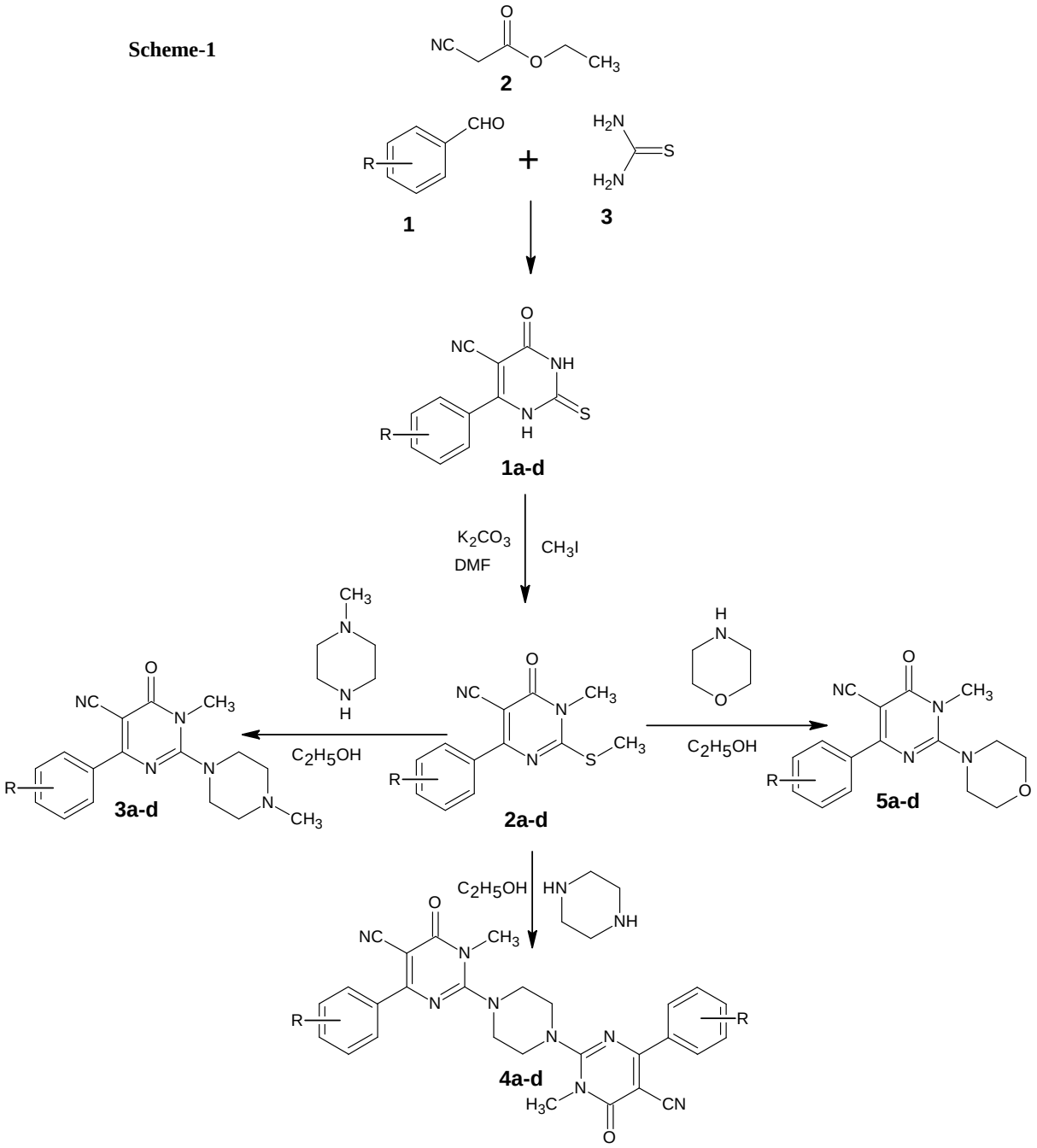
Key words: Antimicrobial activity, morpholines, microwave methods, pyrimidines, piperazines,

Introduction

The medicinal value of pyrimidine derivatives is significant among various heterocycles, as they are found to possess antineoplastic,[1-3] antiviral,[4-6] antibiotic[7] and anti-inflammatory[8] including other biological activities. Further drugs containing piperazine and morpholine moiety have exhibited remarkable biological properties, viz Diethylcarbamazine as potent filaricide, Phendimetrazine as CNS stimulant, Cyclizine as antihistamine and Prazocin as antihypertensive. Some analogs of these class like 2,6-diketopiperazine derivative Razoxane is more effective in the soft tissue sarcoma and leukemia.[9] It has been observed that piperazine linked with pyrimidine lead, Ritanserin as 5HT2 antagonist with anxiolytic activity,[10] Buspirone is a well known drug with pyrimidine moiety linked with piperazine analog indicated in the management of anxiety disorder encompassed with or without depression.[11-12] The several phenothiazines, tricyclic antipsychotic drugs are substituted with piperazine heterocycle by replacing long chain of aliphatic amine, these substitutions produced the most potent phenothiazine antipsychotic compounds. Terry et al, have evaluated pyrimidine derivatives linked to morpholine group for its VEGF-R2 inhibitor activity and showed to be effective in a mouse model of corneal neovascularization.[13] The novel 4-benzylamino-2-[(4-morpholin-4-ylphenyl)amino] pyrimidine-5-carboxamide derivatives (YM-341619, AS1617612) potently inhibited STAT6 activation.[14] All these observations point out that pyrimidines, piperazine and morpholine occupies distant and remarkable place in medicinal chemistry. Keeping in view of these above facts, it was thought of synthesizing some pyrimidine derivatives incorporated with piperazine and morpholine groups to explore their biological profile.

The starting material 6-(Substituted)-5-cyano-2-thiouracils **1a-d,** obtained by reported procedure,[15] were stirred with methyl iodide in presence of N,N-Dimethylformide and K_2CO_3 to get 1-methyl-2-methylthio-4-(substituted)-5-cyano-1,6-dihydropyrimidine-6-ones **2a-d**. The formation of the **2b** was confirmed by recording its ^{1}H NMR which exhibited three singlets at δ 2.2, 3.2 and 3.9 for N-CH_3 S-CH_3 and O-CH_3 respectively and two doublets at δ 7.1 to 7.4 for aromatic protons, it was further confirmed by appearance of molecular ion peak at m/z 287 in its mass spectra. These 1-methyl-2-methylthio-4-(substituted)-5-cyano-1,6-dihydropyrimidine-6-ones **2a-d** were then treated with N-methyl piperazine, piperazine and morpholine to obtain 1-

Scheme-1



3a;4a;5a	R = C_6H_5	3c;4c;5c	R = 4-CH_3O -C_6H_4
3b;4b;5b	R = 4-Cl-C_6H_4	3d;4d;5d	R = 3-NO_2-C_6H_4

SCHEME-1:General synthetic procedure for synthesis of 6-(substituted)-5-cyano-2-thiouracils **1a-d,** 1-methy-2-methythio-4-substituted-5-cyano-1,6-dihydropyrimidine-6-ones **2a-d,** 1-methyl-2-(*p*-methylpiperazin-1-yl)- 4-(substituted)-5-cyano-1,6-dihydropyrimidine-6-ones **3a-d,** 2,2'-(piperazine-1,4-diyl)bis(1-methyl-4-(substituted)-5-cyano-1,6-dihydropyrimidine-6-ones **4a-d** and 1-methyl-2-morpholino-4-(substituted)-5-cyano-1,6-dihydropyrimidine-6-ones **5a-d.**

methyl-2-(*p*-methylpiperazin-1-yl)-4-(substituted)-5-cyano-1,6-dihydropyrimidine-6-ones **3a-d,** 2,2'-(piperazine-1,4-diyl)bis(1-methyl-4-(substituted)-5-cyano-1,6-dihydro pyrimidine-6-ones **4a-d** and 1-methyl-2-morpholino-4-(substituted)-5-cyano-1,6-dihydropyrimidine-6-ones **5a-d** respectively**.** Structures of all the synthetized compounds were characterized by elemental analysis, spectroscopic data, and have been screened for antimicrobial activity.

Materials and methods

Experimental Section

General Procedures. Melting points were determined in open capillaries and are uncorrected. IR spectra (KBr pellet technique) were recorded using a Perkin-Elmer 237 spectrophotometer. ^{1}H NMR spectra were recorded on Bruker AM 400 instrument (at 400 MHz) using tetramethylsilane (TMS) as an internal standard and DMSO-*d6* as a solvent. Chemical shifts are given in parts per million (ppm). Splitting patterns are designated as follows: s- singlet, d-doublet, t- triplet, q- quartet and m-multiplet. Mass spectra (MS) were recorded on Schimadzu MS operating at 70eV. Elemental analysis (C, H, N, and S) were performed on Perkin Elmer 240 analyzer. All the synthesized compounds were purified by recrystallization and Column chromatography on Merck silicagel (60-120 mesh) using suitable solvents. The reactions were followed up and the purity of compounds was monitored on pre-coated TLC plates and visualizing the spots in ultraviolet light.

Synthesis of 6-phenyl-**5-cyano-2-thiouracil. (1a).**

Conventional method. A mixture of ethylcyanoacetate (5.7g. 50 mmol) thiourea (3.8g, 50 mmol), benzaldehyde (2.12g, 50 mmol) and potassium carbonate (6.9g, 50 mmol) in absolute ethanol (50mL) was refluxed for 12hrs. Then, it was neutralized with glacial acetic acid. The product was filtered and recrystallized from aqueous ethanol.

Microwave method, All above said reactants were taken in conical flask and subjected for microwave irradiation for three intermittent cycles of duration 2 minutes at 160 watts. Then, it was neutralized with glacial acetic acid. The obtained product was filtered and crystallized by aqueous ethanol. The compounds **1b-d** were also prepared by same procedure as described above and their characterization data were recorded.

6-Phenyl -5-cyano-2-thiouracil. (1a). Yield 65%**,** light yellow; mp 210 °C, IR (KBr) ν (cm^{-1}): 2225 (CN),1685 (C=O), 1230 (C=S), 1545 (C=N), 1500 (C=C); ^{1}H NMR DMSO δ: 13.0 (s, 1H, NH), 7.5 (m, 5H, Ar-H), 1.2 (s, 1H, NH); *Analysis*. Calcd. For $C_{11}H_7N_3OS$ C, 57.63%; H, 3.08%; N, 18.33%. found C, 57.59%; H, 3.04%; N, 18.32%;

6-(p-Anisyl)-5-cyano-2-thiouracil. (1b). Yield 65%**,** yellow; mp 256 °C, IR (KBr) ν (cm^{-1}): 2220 (CN),1680 (C=O), 1240 (C=S), 1500 (C=C), 1520─1545 (C=N), 1215 (C-O-C); ^{1}H NMR DMSO δ: 13.1 (s, 1H, NH), 7.5 (d, 2H, Ar-H), 7.1 (d, 2H, Ar-H), 3.8 (s, 3H, O-CH_3); *Analysis*. Calcd. For $C_{12}H_9N_3O_2S$ C, 55.59%; H, 3.50%; N, 16.21%. found C, 55.53%; H, 3.45%; N, 16.17%;

6-(*p*-Chlorophenyl)-5-cyano-2-thiouracil. (1c). Yield 65%**,** dark yellow; mp 221 °C, IR (KBr) ν (cm^{-1}): 2222 (CN), 1700 (C=O), 1220 (C=S), 1545 (C=N), 1500 (C=C), 735 (C-Cl); ^{1}H NMR (DMSO) δ: 13.2 (s, 1H, NH), 7.4 (d, 2H, Ar-H), 7.1 (d, 2H, Ar-H), 1.3 (s, 1H, NH); *Analysis*. Calcd. For $C_{11}H_6ClN_3OS$ C, 50.10%; H, 2.29%; N, 15.93%. found C, 50.08%; H, 2.26%; N, 15.90%;

6-(*m*-Nitro-phenyl)-5-cyano-2-thiouracil. (4d). Yield 65%**,** deep yellow; mp 230 °C, IR (KBr) ν (cm^{-1}): 2225 (CN), 1695 (C=O), 1222 (C=S), 1540 (C=N), 1505 (C=C), 1250 (C-NO_2); ^{1}H NMR (DMSO) δ: 13.0 (s, 1H, NH), 8.4 (d, 1H, Ar-H), 8.2 (d, 1H, Ar-H), 7.8 (t, 1H, Ar-H), 7.2 (s, 1H, Ar-H), 1.4 (s, 1H, NH);

Analysis. Calcd. For $C_{11}H_6N_4O_3S$ C, 48.17%; H, 2.21%; N, 20.43%. found C, 48.15%; H, 2.20%; N, 20.41%;

Synthesis of 1-methyl-2-methylthio-4-phenyl-5-cyano-1,6-dihydropyrimidine-6-one. (2a).

To a solution of 6-(p-anisyl)-5-cyano-2-thiouracil (2.29 g, 10 mmol) in DMF (20ml), potassium carbonate (2.76 g, 20 mmol) and methyl iodide (2.84 g, 20 mmol) were added and stirred for 3 hrs. Then the reaction mixture was diluted with cold water and neutralized by glacial acetic acid. The product was filtered off and crystallized from suitable solvents. The compounds **2b-d** were prepared by same procedure as given above and their characterization data are recorded as below.

1-Methyl-2-methylthio-4-phenyl-5-cyano-1,6-dihydropyrimidine-6-one.

(2a). Yield 75%; mp 185 °C; IR (KBr) ν (cm^{-1}): 2230 (CN), 1665 (C=O), 1544 (C=C), 1525 (C=N); ^{1}H NMR DMSO δ: 7.2 (m, 5H, Ar-H), 3.0 (s, 3H, S-CH_3), 2.2 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{13}H_{11}N_3OS$ C, 60.68%; H, 4.31%; N, 16.33%; Found; C, 60.65%; H, 4.26%; N, 16.29%.

1-Methyl-2-methylthio-4-(anisyl)-5-cyano-1,6-dihydropyrimidine-6-one.

(2b). Yield 70%; mp 163 °C; IR (KBr) ν (cm^{-1}): 2250 (CN), 1617 (C=O), 1542 (C=C), 1520 (C=N), 1215 (C-O-C); ^{1}H NMR DMSO δ: 8.1(d, 2H, Ar-H), 7.1 (d, 2H, Ar-H), 3.8 (s, 3H, O-CH_3), 3.5 (s, 3H, S-CH_3), 2.7 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{14}H_{13}N_3O_2S$ C, 58.52%; H, 4.56%; N, 14.62%; Found; C, 58.45%; H, 4.50%; N, 14.58%.

1-Methyl-2-methylthio-4-(*p*-chlorophenyl)-5-cyano-1,6-dihydropyrimidine-6-one. (2c). Yield 65%; mp 265 °C; IR (KBr) ν (cm^{-1}): 2245 (CN), 1690 (C=O), 1610 (C=C), 1545 (C=N), 750 (C-Cl); ^{1}H NMR δ 13.3 (s, 1H, NH), 7.4 (d, 2H, Ar-H), 7.0 (d, 2H, Ar-H), 3.4 (s, 3H, S-CH_3), 2.8 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{13}H_{10}ClN_3OS$ C, 53.52%; H, 3.45%; N, 14.40%; Found; C, 53.51%; H, 3.42%; N, 14.35%.

1-Methyl-2-methylthio-4-(*m*-nitrophenyl)-5-cyano-1,6-dihydropyrimidine-6-one. (2d). Yield 60%; mp 210 °C; IR (KBr) ν (cm^{-1}): 2210 (CN), 1710 (C=O), 1600 (C=C), 1560–1515 (C=N), ^{1}H NMR δ : 7.8 (m, 4H, Ar-H), 3.0 (s, 3H, S-CH_3), 2.3 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{13}H_{10}N_4O_3S$ C, 51.65%; H, 3.33%; N, 18.53%; Found; C, 51.61%; H, 3.28%; N, 18.45%.

Synthesis of 1-methyl-2-(*p*-methylpiperazin-1-yl)-4-phenyl-5-cyano-1,6-dihydropyrimidine-6-one.

(3a). To the solution of 1-methyl-2-methylthio-4-phenyl-5-cyano-1,6-dihydropyrimidine-6-one (0.257 g, 1 mmol) in dry ethanol with N-methylpiperazine (0.1 g, 1 mmol) was refluxed for 12 hrs. Then the reaction mixture was subjected for filtration, and filtrate was added to crushed ice. Further it was neutralized with glacial acetic acid. The product was isolated and crystallized from suitable solvents. The compounds **3b-d** were prepared by same procedure as given above and their characterization data are recorded as below.

1-Methyl-2-(*p*-methylpiperazin-1-yl)-4-phenyl-5-cyano-1,6-dihydropyrimidine-5-one. (3a). Yield 45%; mp 175 °C; IR (KBr) ν (cm^{-1}): 2215 (CN), 1670 (C=O), 1600 (C=C), 1530 (C=N), 1015 (N-CH_3), ^{1}H NMR δ: 7.8 (m, 5H, Ar-H), 3.1 (t, 4H, $(CH_2)_2$), 2.8 (t, 4H, $(CH_2)_2$), 2.3 (s, 3H, N-CH_3) 2.2 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{17}H_{19}N_5O$ C, 66.00%; H, 6.19%; N, 22.64%; Found; C, 65.89%; H, 6.15%; N, 22.60%;

1-Methyl-2-(*p*-methylpiperazin-1-yl)-4-anisyl-5-cyano-1,6-dihydropyrimidine-5-one. (3b). Yield 55%; mp 215 °C; IR (KBr) ν (cm^{-1}): 2209 (CN), 1673 (C=O), 1608 (C=C), 1534 (C=N), 1005 (N-CH_3), ^{1}H NMR δ: 7.9 (d, 2H, Ar-H), 6.9 (d, 2H, Ar-H), 3.8 (s, 3H, OCH_3), 3.2 (t, 4H, $(CH_2)_2$), 2.7 (t, 4H, $(CH_2)_2$), 2.4 (s, 3H, N-CH_3), 2.3 (s, 3H, N-CH_3); *Analysis*. Calcd.

For $C_{18}H_{21}N_5O_2$ C, 63.70%; H, 6.24%; N, 20.64%; Found; C, 62.68%; H, 6.22%; N, 20.61%;

1-Methyl-2-(*p*-methylpiperazin-1'-yl)-4-(*p*-chlorophenyl)-5-cyano-1,6-dihydro pyrimidine-5-one. (3c). Yield 50%; mp 185 °C; IR (KBr) ν (cm^{-1}): 2202 (CN), 1653 (C=O), 1581 (C=C), 1523 (C=N), 1010 (N-CH_3), ^{1}H NMR δ : 8.0 (d, 2H, Ar-H), 7.8 (d, 2H, Ar-H), 3.0 (t, 4H, $(CH_2)_2$), 2.8 (t, 4H, $(CH_2)_2$), 2.4 (s, 3H, N-CH_3), 2.3 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{17}H_{18}ClN_5O$ C, 59.39%; H, 5.28%; N, 20.37%; Found; C, 59.35%; H, 5.26%; N, 20.31%;

1-Methyl-2-(*p*-methylpiperazin-1'-yl)-4-(*m*-nitrophenyl)-5-cyano-1,6-dihydro pyrimidine-5-one. (3d).Yield 65%; mp 215 °C; IR (KBr) ν (cm^{-1}): 2208 (CN), 1705 (C=O), 1610 (C=C), 1522 (C=N), 1009 (N-CH_3) , ^{1}H NMR δ : 8.4 (d, 1H, Ar-H), 8.1 (d, 1H, Ar-H), 7.9 (s, 1H, Ar-H), 7.6 (t, 1H, Ar-H), 3.2 (t, 4H, $(CH_2)_2$), 3.0 (t, 4H, $(CH_2)_2$), 2.6 (s, 3H, N-CH_3), 2.5 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{17}H_{18}ClN_5O$ C, 57.62%; H, 5.12%; N, 23.72%; Found; C, 57.56%; H, 5.10%; N, 23.71%;

Synthesis of 2,2'-(piperazine-1,4-diyl)bis(1-methyl-4-phenyl-5-cyano-1,6-dihydropyrimidine-6-one). (4a). To the solution of 1-methyl-2-(methylthio)-4-phenyl-5-cyano-1,6-dihydropyrimidine-6-one (0.257 g, 1 mmol) in dry ethanol with piperazine (0.086 g., 1 mmol) was refluxed for 12 hrs. Then the reaction mixture was subjected for filtration, the filterate was added into crushed ice, the resulting mixture was neutralized with glacial acetic acid thus obtained precipitate was filtered, dried and recrystallized from suitable solvents. The compounds **4b-d** were prepared by same procedure as given above and their characterization data are recorded as below.

2,2'-(piperazine-1,4-diyl)bis(1-methyl-4-phenyl-5-cyano-1,6-dihydropyrimidine-6-one). (4a). Yield 70%; mp 163 °C; IR (KBr) ν (cm^{-1}): 2210 (CN), 1710 (C=O), 1615 (C=C), 1510 (C=N), 1430 (C=C); ^{1}H NMR DMSO δ: 7.6(m,5H,Ar-H), 7.1(m,5H,Ar-H), 2.9 (t, 4H, $(CH_2)_2$), 2.7 (t, 4H, $(CH_2)_2$)), 2.3 (s, 3H, N-CH_3), 2.1 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{28}H_{24}N_8O_2$ C, 66.65%; H, 4.79%; N, 22.21%; Found; C, 66.65%; H, 4.79%; N, 22.21%.

2,2'-(Piperazine-1,4-diyl)bis(1-methyl-4-anisyl-5-cyano-1,6-dihydropyrimidine-6-one). (4b). Yield 70%; mp 163 °C; IR (KBr) ν (cm^{-1}): 2200 (CN), 1728 (C=O), 1600 (C=C), 1519 (C=N), 1250 (C-O-C); ^{1}H NMR DMSO δ: 8.6 (d,2H,Ar-H), 8.4 (d,2H,Ar-H), 8.2 (d,2H,Ar-H), 7.9 (d,2H,Ar-H), 2.8 (t, 4H, $(CH_2)_2$), 2.6 (t, 4H, $(CH_2)_2$), 2.2 (s, 3H, N-CH_3), 2.1 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{30}H_{28}N_8O_4$ C, 63.82%; H, 5.00%; N, 19.85%; Found; C, 63.82%; H, 5.00%; N, 19.85%;

2,2'-(Piperazine-1,4-diyl)bis(1-methyl-4-(*p*-chlorophenyl)-5-cyano-1,6-dihydro pyrimidine-6-one. (4c).Yield 70%; mp 163 °C; IR (KBr) ν (cm^{-1}): 2202 (CN), 1653 (C=O), 1581 (C=C), 1523 (C=N), 795 (C-Cl); ^{1}H NMR DMSO δ: 7.99 (d, 2H, Ar-H), 7.90 (d, 2H, Ar-H), 7.5 (d,2H,Ar-H), 7.4 (d,2H,Ar-H), 3.2 (t, 4H, $(CH_2)_2$), 3.4 (t, 4H, $(CH_2)_2$), 2.5 (s, 3H, N-CH_3), 2.1 (s,3H,N-CH_3); *Analysis*. Calcd. For $C_{28}H_{22}Cl_2N_8O_2$ C, 58.65%; H, 3.87%; N, 19.54%; Found; C, 58.62%; H, 3.85%; N, 19.52%.

2,2'-(Piperazine-1,4-diyl)bis(1-methyl-4-(*m*-nitrophenyl)-5-cyano-1,6-dihydro pyrimidine-6-one. (4d).Yield 70%; mp 169°C; IR (KBr) ν (cm^{-1}): 2204 (CN), 1645 (C=O), 1529 (C=C), 1529 (C=N), 1349 (C-NO_2); *Analysis*. Calcd. For $C_{28}H_{22}N_{10}O_6$ C, 56.56%; H, 3.73%; N, 23.56%; Found; C, 56.51%; H, 3.70%; N, 23.51%; m/z 594, 410,386,374, 327 and 299;

Synthesis of 1-methyl-2-morpholino-4-phenyl-5-cyano-1,6-dihydropyrimidine-6-one. (5a).

To the solution of 1-methyl-2-methylthio-4-phenyl-5-cyano-1,6-dihydropyrimidine-5-one (0.257g, 1 mmol) in dry ethanol with morpholine (0.087g, 1 mmol) was

refluxed for 14 hrs. Then the reaction mixture was filtered, then filterate was added into crushed ice. Further it was neutralized with glacial acetic acid thus obtained precipitate was filtered, dried and crystallized from suitable solvents. **5b-d** were prepared by same procedure as given above and their characterization data are recorded as below.

1-Methyl-2-morpholino-4-phenyl-5-cyano-1,6-dihydropyrimidine-6-one. (5a).

Yield 60%; mp 205 °C; IR (KBr) ν (cm^{-1}): 2216 (CN), 1661 (C=O),1585 (C=C), 1519 (C=N), 2176 (C-O-C) ^{1}H NMR δ: 7.4 to 7.5 (m, 5H, Ar-H), 3.8 (t, 4H, $(CH_2)_2$), 3.7 (t, 4H, $(CH_2)_2$), 3.4 (s, 3H, N-CH_3); Analysis. Calcd. For $C_{16}H_{16}N_4O_2$ C, 64.85%; H, 5.44%; N, 18.91%; Found; C, 64.82%; H, 5.41%; N, 18.85%.

1-Methyl-2-morpholino-4-anisyl-5-cyano-1,6-dihydropyrimidine-6-one. (5b).

Yield 65%; mp 215 °C; IR (KBr) ν (cm^{-1}): 2210 (CN), 1675 (C=O), 1560 (C=C), 1510 (C=N), 2170 (C-O-C); ^{1}H NMR δ 7.4 (d, 2H, Ar-H), 7.2 (d, 2H, Ar-H), 3.8 (s, 3H, O-CH3), 3.6 (t, 4H, $(CH_2)_2$), 3.4 (t, 4H, $(CH_2)_2$), 3.1 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{17}H_{18}N_4O_3$ C, 62.57%; H, 5.56%; N, 17.17%; Found; C, 62.54%; H, 5.52%; N, 17.15%.

1-Methyl-2-morpholino-4-(*p*-chlorophenyl)-5-cyano-1,6-dihydropyrimidine-6-one. (5c).Yield 60%; mp 225 °C; IR (KBr) ν (cm^{-1}): 2212 (CN), 1695 (C=O), 1585 (C=C), 1519 (C=N) 2175 (C-O-C); ^{1}H NMR δ 7.3 (d, 2H, Ar-H), 7.1 (d, 2H, Ar-H), 3.2 (t, 4H, $(CH_2)_2$), 3.0 (t, 4H, $(CH_2)_2$), 2.2 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{16}H_{15}ClN_4O_2$ C, 58.10%; H, 4.57%; N, 16.94%; Found; C, 58.05%; H, 4.54%; N, 16.90%.

1-Methyl-2-morpholino-4-(*m*-nitrophenyl)-5-cyano-1,6-dihydropyrimidine-6-one.

(5d). Yield 60%; mp 228°C; IR (KBr) ν (cm^{-1}): 2202 (CN), 1680 (C=O), 1570 (C=C), 1505 (C=N), 2170 (C-O-C); ^{1}H NMR δ 8.4 to 8.0 (m, 4H, Ar-H), 3.4 (t, 4H, $(CH_2)_2$), 3.1 (t, 4H, $(CH_2)_2$), 2.5 (s, 3H, N-CH_3); *Analysis*. Calcd. For $C_{16}H_{15}N_5O_4$ C, 56.30%; H, 4.43%; N, 20.52%; Found; C, 56.27%; H, 4.41%; N, 20.49%.

Biological Evaluation

***In vitro* Antibacterial activity**

Cup plate method using Hi-Media agar medium has been employed to study the antibacterial activity of compounds **3a-d, 4a-d** and **5a-d** against *S. aureus*, and *B. subtilis*, Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound was dissolved in Dimethylformamide, making a concentration of 100µg/ml and 50µg/ml. These were used as sample solution. Sample size for all the compounds was fixed as 0.1ml. The cups are made by scooping out agar medium with sterilized cork borer in a petridish, which was previously inoculated with the microorganisms. The solution of each test compound (0.1ml) was added in the cups and petridishes were subsequently incubated at 37 ^{0}C for 48hrs. Trimethoprim and Streptomycin were used as standard drugs and Dimethylformamide as a control. Zones of inhibition produced by each compound, was measured in mm, as shown in **Table 1.**

***In Vitro* Antifungal activity**

The antifungal activity of compounds **3a-d, 4a-d** and **5a-d** was tested using potato dextrose agar medium, against two different fungi such as *C. albicans*, and *A. niger* by filter paper disc technique. The concentration of test compounds was 100µg/ml and 50µg/ml. After 48hrs of treatment, zones of inhibition produced by all compound, were measured in mm, and is shown in **Table 2.** Griseofulvin was used as the standard antifungal agent and dimethylformamide as a control. All the tested compounds showed significant antifungal activity.

Table 1. Antibacterial activity of the compounds 3a-d, 4a-d **and** 5a-d.

Compound	*S. aureus*		*B. subtilis*	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
3a	09	12	08	10
3b	07	10	08	13
3c	11	14	12	15
3d	09	13	14	14
4a	10	12	11	12
4b	17	17	11	15
4c	16	18	14	16
4d	14	18	14	19
5a	07	10	09	11
5b	12	14	14	16
5c	12	13	10	14
5d	10	14	12	14
Trimethoprim	19	22	21	23
streptomycin	21	23	21	24

Table 2. Antifungal activity of the compounds 3a-d, 4a-d **and** 5a-d.

Compound	*C. albicans*		*A.niger*	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
3a	17	19	15	16
3b	14	17	14	17
3c	15	18	14	18
3d	14	18	15	20
4a	15	17	11	16
4b	16	19	14	18
4c	14	18	15	19
4d	16	20	14	17
5a	17	19	15	18
5b	17	20	18	21
5c	16	19	17	20
5d	16	21	18	22
Griseofulvin	19	23	20	24

Results and discussion

The most general and widely employed route to prepare pyrimidines, involves the combination of a reagent containing the N-C-N skeleton with C-C-C unit. This approach is a typical example for the bis-nucleophilic plus bis-electrophilic method of constructing heterocycles. Both the nitrogen atoms of the N-C-N reagent act as nucleophiles and both the terminal carbon atoms of C-C-C reagent act as electrophiles. Urea, thiourea and guanidine are commonly used as N-C-N reagents and 1,3-diketones, diesters and dinitriles are the typical C-C-C reagents.

As depicted in the **scheme 1,** ethylcyanoacetate (C-C unit) on condensation with thiourea (N-C-N unit) in presence of diverse aryl aldehydes (-C- unit) in presence of potassium-carbonate in absolute alcohol yielded 6-(Substituted)-5-cyano-2-thiouracils **1a-d**. The structural analysis of the products retains cyano group of ethylcyanoacetate. This suggests that the requisite (C-C-C) functionality for the construction of pyrimidine ring uses only two carbon centers of these esters and

the third carbon being provided by the aldehydic function of the aldehyde employed. The infrared spectra of these compounds showed characteristic absorption bands, one of which appearing at 2225cm^{-1} was attributed to CN group, and 1270cm^{-1} was assigned for C=S. The enolisible C-SH group has shown absorption band at 3620cm^{-1}.The compounds **1a-d** were further treated with methyl iodide in presence of potassium carbonate and N,N-dimethyl formamide as solvent to get **2a-d.** The ^{1}H NMR spectra of these synthesized compounds **2a-d** showed two characteristic broad signals in the range of δ 2.3 to 2.8 and 3.2 to 3.5 due to N-CH_3 and S-CH_3 protons respectively. These 1-methyl-2-methylthio-4-(substituted)-5-cyano-1,6-dihydropyrimidine-6-ones **2a-d** were treated with heterocyclic secondary amines like N-methyl-piperazine, piperazine and morpholine which` affords a different pyrimidine analogs **3a-d, 4a-d and 5a-d,** with elimination of S-CH_3 group. ^{1}H NMR spectra of the synthesized compounds showed two triplet signals in the range of δ 2.0 to 3.5 due to piperazine and morpholine ring protons respectively.

All the newly synthesized compounds showed good antibacterial activity against *S. aureus, B. subtilis*, and significant antifungal activity against *C. albicans* and *A. niger*. The data of these studies is summarized in **Table 1** and **Table 2.**

Conclusion

In conclusion, a new class of piperazine and morpholine encompassing pyrimidine derivatives were synthesized and evaluated as antibactrial agents. The newly synthesized hetreocyclics exhibited mordarate antibactrial activity aganist *S. aureus,* and *B. subtilis,* and significant antifungal activity against *C. albicans* and *A. niger*. It can be concluded that these class of compounds certainly holds great promise towards good active leads in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress.

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