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Synthesis and antimicrobial activity of some 4-morpholino-6-trifluoromethyl pyrimidine derivatives based on Schiff base

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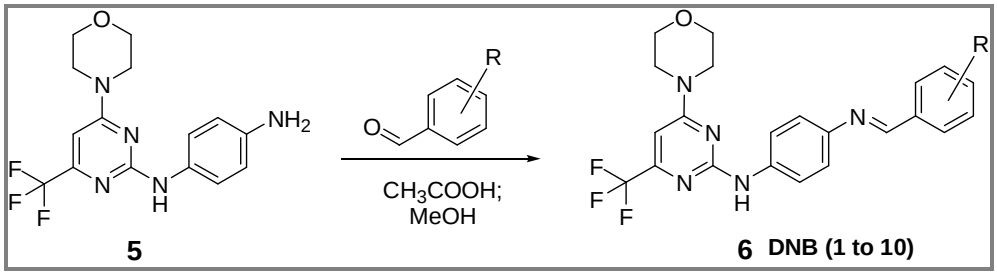
ABSTRACT

Somenovel4-morpholino-6-trifluoromethyl pyrimidine derivatives based on Schiff base have been synthesized and characterized by IR and NMR. The synthesized compounds have been tested for their antimicrobial activity.

Graphical Abstract



Synthesis of compounds.

Keywords: Morpholine, Pyrimidine, Substituted thiourea, Antibacterial activity and antifungal activity.

INTRODUCTION

Due to the importance of heterocyclic compounds as versatile therapeutic agents, heterocyclic compounds have attracted the attention of chemists and druggists to the investigation of their biological activity. Heterocyclic compounds are cyclic compounds having at least one heteroatom. Nitrogen, oxygen and sulfur are the most abundant heteroatoms [**1**]. The heterocycles are abundant in nature and are very important in our lives because of their existence in many natural molecules such as hormones, antibiotics, caffeine [**2**], etc. The pyrimidine ring is a heterocyclic aromatic compound present in nature. Pyrimidines are one of the two major biological groups of nitrogenous molecules called nitrogenous bases. The pyrimidines are known for their formation as essential components of the nucleic acid.

In addition, the synthesis of prebiotic nucleic acid bases is a central topic in the world hypothesis of RNA, one of the major proposals for the emergence of life based on self-assembly of nucleic acid monomers [3]. Possible scenarios of nucleic acid synthesis are still under discussion, and despite the abiotic synthesis of several nucleobases, the importance of these syntheses for the origin of life is not adequately demonstrated [4]. The pyrimidine nucleus is located as an inner framework in the nucleic acid components; Uracil, thymine and cytosine. The literature review has shown that compounds containing pyrimidine nucleons have a wide range of pharmacological activities. The pyrimidine and its derivatives have a promising anticoagulant [5], antioxidant [6, 7], antituberculosis [8], antileukemic [9], antiallergic [10], antipyretic [11], antidiabetic [12], antibiotics [13], anti-inflammatory [14, 15], Anti-HIV [16], analgesics [17, 18], anticancer [19-23], antihypertensive [20, 21], anticonvulsants [22, 23], antileishmanial [24], anti-platelets [25], antihistaminic [26], antifungals [27-29], herbicidal [28], antiviral [29, 30], antibacterial [31-35], malaria [32], antinociceptive [33], and many of pyrimidines derivatives are reported to possess potential central nervous system (CNS) depressant properties [24, 34] and also act as calcium channel blockers [35].

Pyrimidine is a central skeleton that is part of biologically active natural products. Pyrimidine is naturally found in substances such as vitamins such as thiamine, riboflavin (in milk, eggs and liver), folic acid (liver and yeast), barbituric acid (2,4,6-trihydroxy-pyrimidine), the components of nucleic acids (uracil, cytosine and thymine), coenzymes, purines, pterins, nucleotides, alkaloids of tea, coffee, cocoa, and essential components, many drug molecules [36]. Vicin is possibly the first simple pyrimidine derivative found in nature. It was discovered in 1870 in the seed Vetch (*Vicia sativa*, *Vicia faba* L.) by Ritthausen. Among the nucleic acids, pyrimidines, uracil and dihydrouracil isolated from cattle spleen were found in free form. A number of related pyrimidines are also produced in small quantities in some nucleic acids [37]. The other common natural pyrimidines are orotic acid and thiamine. These findings encouraged us to explore the synthesis of pyrimidines containing morpholine and trifluoromethyl moieties and to examine their antibacterial and antifungal properties. In this paper we present the syntheses, characterization and in-vitro antibacterial and antifungal activities of the some 4-morpholino-6-trifluoromethyl pyrimidine derivatives. The structures of these compounds have been confirmed by spectral analysis like FTIR and ^{1}H NMR (400MHz) analysis.

MATERIALS AND METHODS

Reagent and solvents used were obtained from commercial sources. The ^{1}H NMR spectra were obtained on The FT-NMR Spectrometer System (400 MHz) in DMSO-$d_6$with TMS as internal standard. The purity was determined on Agilent liquid chromatography. Analytical thin layer chromatography was carried out on TLC plates of 3×15 cm coated with silica gel-G for reaction monitoring and for determination of retardation factor, UV-254 were used for detection.

Caution: Materials and reaction vapors have a strong effect on eyes and skin. Skin adsorption and exposure to vapors may cause systemic poisonous effects. Contact with liquids and vapors should be avoided by appropriate ventilation and appropriate protective clothing.

The 6-(trifluoromethyl)pyrimidine with different substituent in the 2-position and 4-substituted with imidazole were synthesized by the typical route indicated in the figure 1. The various imine Compounds-(6) were prepared by condensation of N^1-4-morpholino-6-(trifluoromethyl)pyrimidin-2-yl)benzene-1,4-diamine (5) with various substituted aldehydes in methanol using acetic acid as catalyst. The treatment of ethyl 4,4,4-trifluoro-3-oxobutanoate (1) with 1-(4-nitrophenyl) guanidine sulphate in 2-propanol using K_2CO_3 yielded 2-((4-nitrophenyl)amino)-6-(trifluoromethyl)pyrimidin-4(3H)-one (2) which was then reacted with phosphorous oxy chloride to obtain 4-chloro-N-(4-nitrophenyl)-6-(trifluoromethyl) pyrimidin-2-amine (3). The Compound-(3) was reacted with morpholine in presence of inorganic base to obtain4-morpholino-N-(4-nitrophenyl)-6-(trifluoro methyl) pyrimidin-2-amine as compound (4). The reduction of nitro group in compound (4) with stannous chloride dihydrate in isopropyl alcohol gave N^1-4-morpholino-6-(trifluoromethyl)

pyrimidin-2-yl)benzene-1,4-diamine (5) which is the key starting material for the synthesis of N-(4-(substitutedbenzylideneamino)phenyl)-4-morpholino-6-(trifluoromethyl)pyrimidin-2-amine (6).

Synthesis of 2-((4-nitrophenyl)amino)-6-(trifluoromethyl)pyrimidin-4(3H)-one (2)[14]: The ethyl 4,4,4-trifluoro-3-oxobutanoate (50.0g, 271.57mmol) was added to a solution of 1-(4-nitrophenyl) guanidine sulphate (67.9 g, 296.01 mmol) and sodium hydroxide (16.29 g, 407.36 mmol) in 2-propanol (250 mL). The solution was stirred under reflux for 8 h. The reaction mixture was cooled to 0°C, filtered, washed with isopropanol, water, ethanol and dried to give 67.8g of 2-((4-nitrophenyl) amino)-6-(trifluoromethyl)pyrimidin-4(3H)-one. Yield 83.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27(s, 1H), 8.93 (s, 1H), 7.89 (d, 2H), 7.03 (d, 2H).

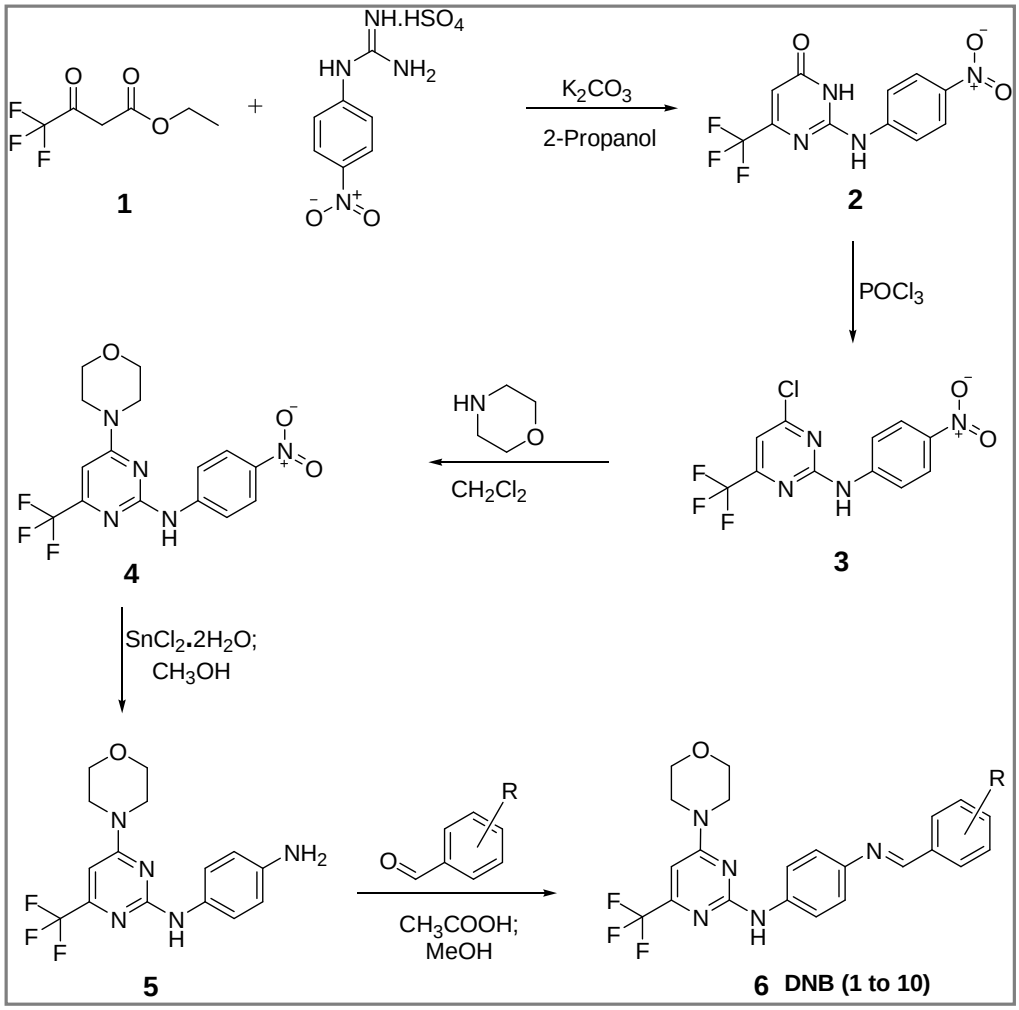


Figure 1. Reaction procedure

4-chloro-N-(4-nitrophenyl)-6-(trifluoromethyl)pyrimidin-2-amine (3): 2-((4-nitrophenyl)amino)-6-(trifluoromethyl)pyrimidin-4(3H)-one (70g, 234mmol) and phosphorous oxychloride (109.0 mL, 1170 mmol) were charged to a 3lit. 4-neck flask equipped with overhead stirrer, a reflux condenser, an addition funnel and an internal thermocouple. Heating was initiated and the orange reaction mass was maintained at 100°C for 2 h. Once the reaction was completed on TLC, external heating was removed and the reaction mass was cooled to room temperature and excess of $POCl_3$ was removed by distillation under vacuum. The residue was then diluted with CH_2Cl_2 (250 mL) and stirred with Sat. $NaHCO_3$ (3x75 mL) keeping the temperature below 15°C. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (1x50 mL). The combined organic layers were washed with water

until the washes were neutral (5x50mL), dried with $MgSO_4$ and concentrated to provide 61.3g of yellow plate. Yield: 82%. R_f = 0.6 (EtOAc: Hexanes = 3:7)

4-morpholino-N-(4-nitrophenyl)-6-(trifluoromethyl)pyrimidin-2-amine (4): 4-chloro-N-(4-nitro phenyl)-6-(trifluoromethyl) pyrimidin-2-amine (25.0 g, 78.46 mmol), morpholine (8.20 g, 94.154 mmol), and dichloromethane (100 mL) were mixed, and the mixture heated on the oil-bath for 2 hours. After cooling, the crystals which had been deposited were filtered off, suspended in alcohol, and treated with ammonia. After addition of water, precipitated product was filtered off, washed with water, and crystallized from alcohol. Yield: 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 8.62 (t, J = 1.7 Hz, 1H), 8.05 (d, 2H), 7.96 (dd, J = 5.0, 1.7 Hz, 1H), 7.39 (d, 2H), 7.18 (dd, J = 5.0, 1.7 Hz, 1H), 6.40 (s, 1H).

N^1-(4-morpholino-6-(trifluoromethyl)pyrimidin-2-yl)benzene-1,4-diamine (5): 4-morpholine-(4-nitrophenyl)-6-(trifluoromethyl)pyrimidin-2-amine (**4**) (25.0 g, 67.69 mmol) and stannous chloride dehydrate (76.4 g, 338.47 mmol) were dissolved in a solvent mixture of ethyl acetate and ethanol (250 mL, 10/1, v/v), and the reaction solution was refluxed for 4 h. The solution was cooled to room temperature, washed with 10% aqueous sodium hydroxide solution, and concentrated to give 21 g of N^1-(4-morpholino-6-(trifluoromethyl)pyrimidin-2-yl)benzene-1,4-diamine. Yield: 87.07 %, TLC: R_f = 0.45 (Methylene chloride: Methanol = 9:1).^{1}H-NMR (400MHz,DMSO-d_6): 2.04 (s, 3H), 6.30-6.34 (m, 1H), 6.76-6.77 (m, 1H), 6.84-6.87 (d, 1H), 7.34-7.35 (m, 1H), 7.50-7.56 (m, 1H), 8.38-8.47 (m, 1H), 8.53-8.57 (m, 2H), 8.66-8.70 (m, 1H), 9.23-9.24 (d, 1H).

Representative procedure for the imine preparation. (General Method): To a well stirred solution of N-4-morpholine-6-(trifluoromethyl)-pyrimidin-2-yl)benzene-1,4-diamine **(5)** (0.1 mol) and benzaldehyde (0.1 mol) in ethanol (100 mL), was added 2-3drops of glacial acetic acid. The reaction mixture was stirred at 70°C for 2 h and left over night. After the completion of reaction, it was poured into ice water, filtered and recrystallized from ethanol to get the title compounds.

Synthesis of(E)-N-(4-(benzylideneamino)phenyl)-4-morpholino-6-(trifluoromethyl)pyrimidin-2-amine (DNB-1): Preparation is described in general method, pale yellow solid. Yield82%; ^{1}H NMR(400 MHz, DMSO-d_6) δ 9.67 (bs, 1H,-NH), 8.62 (s, 1H,imine), 7.85 (d, *J* = 7.5 Hz, 2H), 7.41-7.33 (m, 2H), 7.33-7.24 (m, 3H), 7.19 (d, *J* = 7.6 Hz, 2H), 6.94 (s, 1H,pyrimidine), 3.80-3.69 (m, 4H,morpholine), 3.68-3.57 (m, 4H,morpholine);IR (KBr, cm^{-1}) :3350.51 (N-H str., secondary amine),3065.60 (C-H str., imine), 1657.81(C=N str., imine), 1616.69 (C-N str., morpholine), 1565.38 (C=N str., pyrimidine),1524.62 (C=C str., Ar.), 1291.32 (C=N pyrimidine), 1140.53 (C-F str., trifluromethyl), 1086.77 (C-O str., morpholine).

Synthesis of (E)-N-(4-((2-chlorobenzylidene)amino)phenyl)-4-morpholino-6-(trifluoromethyl) pyrimidin-2-amine (DNB-2): Preparation is described in general method, off-white solid. Yield 61%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (bs, 1H,-NH), 8.68 (s, 1H,imine), 7.67 (d, 7.4Hz, 1H), 7.44-7.37 (m, 3H), 7.28 (d, *J* = 7.6 Hz, 2H), 7.21 (d, *J* = 7.6 Hz, 2H), 6.92 (s, 1H,pyrimidine), 3.83-3.69 (m, 4H,morpholine), 3.69-3.57 (m, 4H,morpholine);IR(KBr, cm^{-1}): 3348.44 (N-H str., secondary amine),3056.58 (C-H str., imine), 1657.81(C=N str., imine), 1616.69 (C-N str., morpholine), 1565.38(C=N str., pyrimidine), 1524.62(C=C str., Ar.), 1291.32 (C=N pyrimidine), 1175.46(C-F str., trifluromethyl), 1086.77(C-O str., morpholine), 681.83 (C-Cl str., Ar-Cl).

Synthesis of (E)-N-(4-((4-chlorobenzylidene)amino)phenyl)-4-morpholino-6-(trifluoromethyl) pyrimidin-2-amine (DNB-3): Preparation is described in general method, off-white solid. Yield69%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (bs, 1H, -NH), 8.68 (s, 1H, imine), 7.75 (d, J=7.5 Hz, 2H), 7.62 (dd, J=7.7, 1.3 Hz, 2H), 7.27 (d, J=7.6 Hz, 2H), 7.20 (d, J=7.6 Hz, 2H), 6.92 (s, 1H,pyrimidine), 3.79–3.70 (m, 4H,morpholine), 3.69-3.61 (m, 4H,morpholine). IR (KBr, cm^{-1}):3339.85 (N-H str., secondary amine), 3035.50 (C-H str., imine), 1653.81(C=N str., imine), 1618.73 (C-N str.,

morpholine), 1525.38(C=N str., pyrimidine), 1182.56(C-F str., trifluromethyl), 1086.77(C-O str., morpholine), 695.43 (C-Cl str., Ar-Cl).

Synthesis of (E)-N-(4-((2-fluorobenzylidene)amino)phenyl)-4-morpholino-6-(trifluoromethyl) pyrimidin-2-amine (DNB-4): Preparation is described in general method, yellow solid. Yield 74%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H,-NH), 8.69 (s, 1H,imine), 7.76 (ddd, J = 6.8, 5.0, 1.3 Hz, 1H), 7.48-7.36 (m, 1H), 7.30-7.17 (m, 5H), 7.12 (td, J =7.9, 1.5 Hz, 1H), 6.92 (s, 1H,pyrimidine), 3.80-3.69 (m, 4H,morpholine), 3.69-3.60 (m, 4H,morpholine). IR (KBr, cm^{-1}): 3348.61 (N-H str., secondary amine), 3062.34 (C-H str., imine), 1655.81(C=N str., imine), 1610.83 (C-N str., morpholine), 1562.80(C=N str., pyrimidine),1514.62(C=C str., Ar.), 1271.33 (C=N pyrimidine), 1192.56(C-F str., trifluromethyl), 1086.77(C-O str., morpholine), 1056.23 (C-F str., Ar-F).

Synthesis of (E)-N-(4-((4-fluorobenzylidene)amino)phenyl)-4-morpholino-6-(trifluoromethyl) pyrimidin-2-amine (DNB-5): Preparation is described in general method, yellow solid. Yield79% ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (bs, 1H,-NH), 8.70 (s, 1H,imine), 7.96-7.85 (m, 2H), 7.27 (d, J = 7.6 Hz, 2H), 7.20 (d, J = 7.6 Hz, 2H), 7.18-7.12 (m, 2H), 6.92 (s, 1H,pyrimidine), 3.83-3.70 (m, 4H,morpholine), 3.68-3.58 (m, 4H,morpholine).IR (KBr, cm^{-1}): 3350.51(N-H str., secondary amine), 3065.60(C-H str., imine), 1657.81(C=N str., imine), 1616.69 (C-N str., morpholine), 1565.38 (C=N str., pyrimidine),1524.62(C=C str., Ar.),1291.32(C=N pyrimidine), 1182.56(C-F str., trifluromethyl), 1086.77(C-O str., morpholine), 1036.05(C-F str., Ar-F), 832.58(C-H bending, para substitution).

Synthesis of (E)-N-(4-((4-methylbenzylidene)amino)phenyl)-4-morpholino-6-(trifluoromethyl) pyrimidin-2-amine (DNB-6): Preparation is described in general method, greenish yellow solid. Yield64%;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (bs, 1H,-NH), 8.62 (s, 1H,imine), 7.70 (d, J = 7.5 Hz, 2H), 7.27 (d, J=7.6 Hz, 2H), 7.24-7.16 (m, 4H), 6.92 (s, 1H,pyrimidine), 3.91-3.69 (m, 4H,morpholine), 3.69-3.55 (m, 4H,morpholine),2.40 (s, 3H); IR (KBr, cm^{-1}): 3351.45 (N-H str., secondary amine), 3128.87 and 3092.44 (C-H str., methylene), 3035.71(C-H str., imine),1654.33 (C=N str., imine), 1571.35(C=N str., pyrimidine), 1465.38(C=C str., Ar.),1307.13 (C=N pyrimidine), 1206.18(C-F str., trifluromethyl), 1118.18 (C-O str., morpholine).

Synthesis of (E)-N-(4-((4-methoxybenzylidene)amino)phenyl)-4-morpholino-6-(trifluoromethyl) pyrimidin-2-amine (DNB-7): Preparation is described in general method, orange solid. Yield59%;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H,-NH), 8.59 (s, 1H,imine), 7.76 (d, *J* = 7.5 Hz, 2H), 7.27 (d, *J* = 7.6 Hz, 2H), 7.20 (d, *J* = 7.6 Hz, 2H), 7.04 –6.98 (m, 2H), 6.92 (s, 1H,pyrimidine), 3.82 (s, 3H), 3.79 –3.71 (m, 4H,morpholine), 3.69 – 3.62 (m, 4H,morpholine).IR (KBr, cm^{-1}): 3328.87 (N-H str., secondary amine), 3108.57& 3078.34 (C-H str., methylene),3045.51(C-H str., imine),1656.13 (C=N str., imine), 1575.73(C=N str., pyrimidine),1454.37(C=C str., Ar.), 1298.07 (C=N pyrimidine), 1186.27 (C-F str., trifluromethyl), 1118.18 (C-O str., morpholine), 1022.18 (C-O str., OCH_3).

Synthesis of (E)-4-morpholino-N-(4-((4-nitrobenzylidene)amino)phenyl)-6-(trifluoromethyl) pyrimidin-2-amine (DNB-8): Preparation is described in general method, yellow solid. Yield86%;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H,-NH), 8.70 (s, 1H,imine), 8.30 (dd, J = 7.7, 1.3 Hz, 2H), 8.10 (d, J = 7.5 Hz, 2H), 7.27 (d, J = 7.6 Hz, 2H), 7.19 (d, J = 7.6 Hz, 2H), 6.91 (s, 1H,pyrimidine), 3.80–3.71 (m, 4H,morpholine), 3.70–3.60 (m, 4H,morpholine). IR (KBr, cm^{-1}) :3310.17 (N-H str., secondary amine), 3025.51(C-H str., imine), 1643.23 (C=N str., imine), 1548.43(C=N str., pyrimidine),1498.33(C=C str., Ar.), 1301.47 (C=N pyrimidine), 1156.85 (C-F str., trifluromethyl), 1108.43 (C-O str., morpholine), 1289.79 and 1452.57 (N-O str., NO_2).

Synthesis of (E)-N-(4-((2,4-dichlorobenzylidene)amino)phenyl)-4-morpholino-6-(trifluoromethyl) pyrimidin-2-amine (DNB-9): Preparation is describedin general method, off-white solid. Yield79%;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (bs, 1H,-NH), 8.70 (s, 1H,imine), 7.86 (d, J=7.5 Hz, 1H), 7.54 (d, J=1.5 Hz, 1H), 7.43 (dd, J = 7.5, 1.5 Hz, 1H), 7.27 (d, J = 7.6 Hz, 2H), 7.21 (d, J=7.6 Hz, 2H), 6.91 (s, 1H,pyrimidine), 3.79-3.70 (m, 4H,morpholine), 3.70-3.59 (m,

4H,morpholine). IR (KBr, cm^{-1}) :3388.54 (N-H str., secondary amine), 3045.31 (C-H str., imine), 1627.51(C=N str., imine), 1626.13 (C-N str., morpholine), 1545.08(C=N str., pyrimidine), 1524.62(C=C str., Ar.), 1157.14(C-F str., trifluromethyl), 1106.49 (C-O str., morpholine), 5671.44 (C-Cl str., Ar-Cl).

Synthesis of (E)-4-morpholino-N-(4-((3-phenoxybenzylidene)amino)phenyl)-6-(trifluoromethyl) pyrimidin-2-amine (DNB-10): Preparation is described in general method, pale yellow solid. Yield72%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74 (s, 1H,-NH), 8.64 (s, 1H,imine), 7.51 (dt, *J*= 7.5, 1.3 Hz, 1H), 7.38-7.29 (m, 4H), 7.27-7.18 (m, 4H), 7.11 (tt, *J* = 7.8, 1.5 Hz, 1H), 6.98 (m, *J* = 9.0, 7.5, 1.5 Hz, 3H), 6.91 (s, 1H,pyrimidine), 3.77-3.70 (m, 4H,morpholine), 3.70-3.63 (m, 4H,morpholine). IR (KBr, cm^{-1}): 3344.81 (N-H str., secondary amine), 3119.13 and 3082.41 (C-H str., methylene), 1656.13 (C=N str., imine), 1585.12(C=N str., pyrimidine), 1498.15(C=C str., Ar.), 1255.11 (C-O str., OPh), 1198.45 (C-F str., trifluromethyl), 1120.03 (C-O str., morpholine).

RESULTS AND DISCUSSION

All newly synthesized compounds were screened for the antimicrobial activity using Broth dilution method [**38, 39**]. All necessary controls like drug control, vehicle control, agar control, organism control and known antibacterial drugs control were used. Mueller Hinton Broth was used as nutrient medium to grow and dilute the drug suspension for the test bacteria. The standard strains used for screening of antibacterial and antifungal activities were: *Staphylococcus aureus*, *Streptococcus pyogenes* as Gram positive bacteria, *Escherichia coli*, *Pseudomonas aeruginosa* as Gram negative bacteria, *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus* as fungi. The standard drugs used in the present study are Norfloxacin, Ciprofloxacin, Chloramphenicol and Ampicillin for evaluating antibacterial activity. Nystatin and Greseofulvin are used as the standard drugs for evaluating antifungal activity which showed MIC against *C.albicans*, *A.niger* and *A.clavatus*. Mueller Hinton Broth was used as nutrient medium for bacteria and Sabouraud Dextrose Broth for fungal to grow. Inoculums size for test strain was adjusted to 108 CFU [Colony Forming Unit] per milliliter by comparing the turbidity. DMSO was used as diluents/vehicle to get desired concentration of compounds and reference drugs to test against standard bacterial strains. Inoculums size for test strain was adjusted to 108 CFU mL^{-1} by comparing the turbidity with McFarland standards. Each synthesized compound and standard drugs were diluted with DMSO to obtain 2000 µg mL^{-1} concentration, as a stock solution. In primary screening 1000 µg mL^{-1}, 500 µg mL^{-1}, 250 µg mL^{-1}, 125 µg mL^{-1}, 62.5 µg mL^{-1} 62.5 µg mL^{-1}, 31.25 µg mL^{-1} and 15.62 µg mL^{-1} concentrations of the synthesized compounds were prepared and the drugs found active in primary screening were similarly diluted to obtain 200 µg mL^{-1}, 100 µg mL^{-1}, 50 µg mL^{-1}, 25 µg mL^{-1}, 12.5 µg mL^{-1}, and 6.250 µg mL^{-1} concentrations. The 10 µL suspensions from each well were further inoculated on appropriate media and growth was noted after 24 and 48 hours at 37°C. The lowest concentration, which showed no growth after spot subculture was considered as MIC for each drug. The result of this test is affected by the size of inoculums.

APPLICATION

Antimicrobial Screening: The in-vitro antimicrobial activities of the above synthesized compounds were tested at10 µg mL^{-1} concentration under similarly controlled condition of experiments carried out by using 40 µg mL^{-1} concentrations of standard drugs for a comparison and the results are shown in table 1.

From the Antibacterial screening results, compound DNB-6 is having excellent activity against *S.aureus* while better activity against *S.pyogenes*.DNB-10 is having excellent activity against *S.pyogenes* while better activity against *S.aureus*. The compound DNB-2 showed better activity against *S.pyogenes* and *E.coli*. The compound DNB-8 is having excellent activity against *E.coli* while

Table1. *In-vitro* Antimicrobial activityof4-morpholino-6-trifluoromethyl pyrimidine derivatives

Compounds	Antibacterial Activity Minimal Inhibition Concentration (µg mL^{-1})				Antifungal Activity Minimal Inhibition Concentration (µg mL^{-1})		
	S.aureus	*S.pyogenes*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
DNB-1	500	1000	250	500	250	1000	500
DNB -2	500	125	125	250	500	1000	500
DNB -3	500	125	250	250	1000	250	250
DNB -4	250	500	250	250	1000	500	500
DNB -5	250	500	500	500	500	250	500
DNB -6	62.5	125	250	250	250	500	500
DNB -7	250	500	125	62.5	125	250	1000
DNB -8	250	250	62.5	125	250	250	500
DNB -9	125	1000	250	250	1000	500	250
DNB -10	125	62.5	500	500	1000	500	500
Nystatin	--	--	--	--	**100**	**100**	**100**
Greseofulvin	--	--	--	--	**500**	**100**	**100**
Norfloxacin	**10**	**10**	**10**	**10**	--	--	--
Ciprofloxacin	**50**	**50**	**25**	**25**	--	--	--
Chloramphenicol	**50**	**50**	**50**	**50**	--	--	--
Ampicillin	**250**	**100**	**100**	**100**	--	--	--

better activity against *P.aeruginosa*. The compound DNB-7 is having excellent activity against *P.aeruginosa* while better activity against *E.coli*. The compound DNB-3 showed better activity against *S.pyogenes*, while DNB-9 showed better activity against *S.aureus.*

From the Antibacterial screening results, compound DNB-7 showed very good activity against *C.albican* while compound DNB-1, DNB-6, DNB-8 showed good activity against *C.albican.* The rest of compound showed moderate and good activity against remaining fungal species.

CONCLUSION

The newly synthesized (E)-N-(4-((substitutedbenzylidene)amino)phenyl)-4-morpholino-6-(trifluoro methyl) pyrimidin-2-amines **[6-(DNB 1-10)]** were characterized by ^{1}H NMR and IR analysis and screened for the antimicrobial activity. The screening result showed that most of newly synthesized compounds exhibited promising activities against gram positive, gram negative bacterial and fungal strains.

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