Approach for the Synthesis of Potent Antimicrobials Containing Pyrazole, Pyrimidine and Morpholine Analogues

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Abstract. The present study is in the interest of some synthesized novel derivatives containing 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-*N*-(morpholinomethyl)-6-arylpyrimidin-2-amines pooled with different bio-active heterocycles such as pyrazole, pyrimidine and morpholine derivatives. The structures of newly synthesized compounds were elucidated by IR, ¹H NMR, ¹³C NMR and mass spectral data. The synthesized compounds were evaluated for their *in vitro* antimicrobial activity against different bacterial and fungal strains using Mueller-Hinton Broth dilution method. On the basis of SAR studies, it was observed that the presence of electron withdrawing groups remarkably enhanced the antimicrobial activity of synthesized compounds.

1. Introduction

Pyrazoles and several *N*-substituted pyrazoles are known to possess numerous chemical, biological and medicinal applications because of their adaptable biological activities such as antimicrobial [1], antileukemia [2], antidepressant [3], anticonvulsant, antipyretic, antineoplastic and antitubercular [4]. It is considered a typical model of pyrazole containing, diaryl-heterocyclic template that is known to selectively inhibit (cyclooxygenase enzyme) COX-2 [5]. Some of the example of pyrazole based drug are: Lonazolac (Non-steroidal anti-inflammatory drug), Mepiprazole (Minor tranquilizer), Rimonabant (Anorectic antiobesity drug), Ramifenazone (Analgesic, antipyretic and anti-inflammatory agent), Fipronil (Broad-spectrum insecticide) (Fig. 1). A literature survey revealed that a number of biologically active compounds have been synthesized using pyrazole-3(4)-carbaldehydes and therefore in first step we have selected 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde as a starting material.

The pyrimidine base is found in thymine, cytosine and uracil; which are the essential building blocks of nucleic acids and is also found in Vitamin B₁. The literature indicated that compounds having pyrimidine nucleus possess broad range of biological activities, like 5-fluorouracil as anticancer, idoxuridine and trifluridine as antiviral, zidovudine and stavudine as anti-HIV, trimethoprim, sulphamethiazine and sulphadiazine as antibacterial, sulphadoxin as antimalarial. Pyrimidine derivatives have remarkable pharmaceutical importance because of their biological activity as anti-HIV [6], antitubercular, antimicrobial [7], antidiabetic [8] and anti-inflammatory [9]. It has been found that several DHPM derivatives possess biological activity, which has led to their use as antiplasmodial [10] and anticancer [11]. Substituted aminopyrimidine nuclei is the key structure marketed drugs such as antiatherosclerotic aronixil, anti-histaminic thonzylamine, antianxiolytic buspirone, antihypertensive minoxidil and prazosin, anti-psoriatic enazadrem, Common examples of pyrimidine based drugs are: Bacimethrin (Antibiotics), Viomycin (Antitubercular drugs), Alobarbitol (Sedative/hypnotic), Retrovir (Antiviral and anti-HIV), etc (Fig. 1).

Promising diverse pharmacological activities are shown by various N-functionalized morpholines. They are reported to exert a number of important physiological activities, such as antibacterial, antifungal [12], platelet aggregation inhibitors [13], antiemetic [14] and antidepressants [15]. Phenmetrazine was used as an appetite suppressant. It was firstly replaced by its equivalent phendimetrazine which functions as a prodrug (Fig. 1). In accordance with our previous work [16-18] and medicinal significance of pyrazole, pyrimidine and morpholine motifs, we decided to combine all these moieties in to one single frame with hybrid approach as potential antimicrobial agents.

Fig. 1 Pyrazole, pyrimidine and morpholine-based commercially drugs

2. Experimental

2.1. Materials and methods

The required chemicals were purchased from E. Merck. Melting points were recorded on Gallenkamp apparatus and were left uncorrected. The completion of reaction and the purity of all compounds was checked on aluminum-coated TLC plates 60, F₂₄₅ (E. Merck) using various solvent systems as mobile phase and visualized under ultraviolet (UV) light, or iodine vapor. Elemental analysis (% C, H, N) was carried out by a Perkin-Elmer 2400 CHN analyzer. IR spectra were also recorded on Perkin Elmer FT-IR spectrophotometer. ¹H NMR and spectra were recorded on Varian Gemini 400 MHz and ¹³C NMR spectra on Varian Mercury-400, 100 MHz in DMSO-*d*₆ as a solvent and tetramethylsilane (TMS) as an internal standard. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer.

Synthesis of 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde (1)

1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde (**1**) was prepared according to the literature method [19].

Synthesis of 3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1-arylprop-2-en-1-ones (3a-o)

3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1-arylprop-2-en-1-ones (**3a-o**) were prepared according to the literature method [20].

Synthesis of 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-arylpyrimidin-2-amines (4a-o)

4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-arylpyrimidin-2-amines (**4a-o**) were prepared according to the literature method [21].

General synthesis of 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-*N*-(morpholinomethyl)-6-arylpyrimidin-2-amines (5a-o)

A mixture of compounds (**4a-o**) (0.01 mol), morpholine (0.01 mol) and formaldehyde (0.02 mol) was refluxed in ethanol for 10 h. Excess of solvent was removed under reduced pressure and after cooling, the residue was poured onto crushed ice. Obtained solid product was filtered off, washed with water and crystallized from ethanol (95%).

4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-*N*-(morpholinomethyl)-6-phenylpyrimidin-2-amine (5a)

Yield: 70%; mp 194°C; IR (cm⁻¹): 3311, 3236 (-N-H stretching), 3062 (-C-H aromatic stretching), 2956, 2914, 2852 (-C-H, CH₂ stretching), 1597 (-C=N stretching), 1330 (-C-N stretching), 1222 (>C-O stretching), 1036 (-C-O-C stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.62 (t, 4H, N(CH₂)₂), 3.49 (t, 4H, O(CH₂)₂), 3.93 (s, 2H, CH₂), 6.75 (s, 1H, pyrimidine-H), 7.40-8.45 (m, 15H, Ar-H),

8.32 (s, 1H, CH of pyrazole), 10.20 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 53.5, 66.4, 74.8, 101.2, 103.8, 119.8, 126.3, 127.6, 128.7, 129.3, 129.4, 131.6, 133.0, 135.6, 143.5, 145.8, 162.1, 162.3, 165.2; MS: *m/z* = 488.23; Anal. Calcd. for C₃₀H₂₈N₆O: C, 73.75; H, 5.78; N, 17.20; Found: C, 73.81; H, 5.74; N, 17.26 %.

4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-*N*-(morpholinomethyl)-6-(*o*-tolyl)pyrimidin-2-amine (5b)

Yield: 68%; mp 185°C; IR (cm⁻¹): 3313, 3229 (-N-H stretching), 3060 (-C-H aromatic stretching), 2962, 2919, 2850 (-C-H, CH₂ stretching), 2930 (C-H, CH₃), 1610 (-C=N stretching), 1321 (-C-N stretching), 1034 (-C-O-C stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.46 (s, 3H, CH₃), 2.60 (t, 4H, N(CH₂)₂), 3.52 (t, 4H, O(CH₂)₂), 3.94 (s, 2H, CH₂), 6.94 (s, 1H, pyrimidine-H), 7.37-7.99 (m, 14H, Ar-H), 8.15 (s, 1H, CH of pyrazole), 10.19 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 18.7, 53.4, 66.3, 74.8, 101.2, 103.9, 119.9, 122.7, 126.2, 127.4, 128.6, 128.7, 129.2, 129.5, 129.7, 131.7, 133.0, 136.6, 143.5, 145.8, 162.0, 162.2, 165.2; MS: *m/z* = 502.25; Anal. Calcd. for C₃₁H₃₀N₆O: C, 74.08; H, 6.02; N, 16.72; Found: C, 73.96; H, 6.06; N, 16.62 %.

4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-*N*-(morpholinomethyl)-6-(*p*-tolyl)pyrimidin-2-amine (5c)

Yield: 65%; mp 180°C; IR (cm⁻¹): 3311, 3232 (-N-H stretching), 3061 (-C-H aromatic stretching), 2960, 2914, 2851 (-C-H, CH₂ stretching), 2938 (C-H, CH₃), 1612 (-C=N stretching), 1324 (-C-N stretching), 1031 (-C-O-C stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.36 (s, 3H, CH₃), 2.62 (t, 4H, N(CH₂)₂), 3.50 (t, 4H, O(CH₂)₂), 3.92 (s, 2H, CH₂), 6.91 (s, 1H, pyrimidine-H), 7.35-7.96 (m, 14H, Ar-H), 8.14 (s, 1H, CH of pyrazole), 10.16 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 21.7, 53.5, 66.5, 74.7, 101.3, 103.7, 119.9, 123.2, 126.2, 127.4, 128.6, 128.7, 129.5, 129.7, 131.5, 131.7, 132.8, 133.0, 143.5, 145.8, 162.0, 162.2, 165.2; MS: *m/z* = 502.25; Anal. Calcd. for C₃₁H₃₀N₆O: C, 74.08; H, 6.02; N, 16.72; Found: C, 74.00; H, 6.09; N, 16.76 %.

4-(2-chlorophenyl)-6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-*N*-(morpholinomethyl)pyrimidin-2-amine (5d)

Yield: 66%; mp 174°C; IR (cm⁻¹): 3314, 3230 (-N-H stretching), 3058 (-C-H aromatic stretching), 2963, 2915, 2856 (-C-H, CH₂ stretching), 1610 (-C=N stretching), 1322 (-C-N stretching), 1035 (-C-O-C stretching), 756 (C-Cl stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.61 (t, 4H, N(CH₂)₂), 3.53 (t, 4H, O(CH₂)₂), 3.90 (s, 2H, CH₂), 6.89 (s, 1H, pyrimidine-H), 7.34-7.98 (m, 14H, Ar-H), 8.12 (s, 1H, CH of pyrazole), 10.17 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 53.6, 66.3, 75.0, 101.3, 103.8, 119.9, 126.2, 127.4, 128.7, 128.9, 129.2, 129.7, 129.9, 130.1, 130.5, 131.5, 132.2, 132.5, 133.0, 133.2, 143.5, 145.7, 162.0, 162.2, 165.1; MS: *m/z* = 522.19; Anal. Calcd. for C₃₀H₂₇ClN₆O: C, 68.89; H, 5.20; N, 16.07; Found: C, 68.96; H, 5.26; N, 16.10 %.

4-(4-chlorophenyl)-6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-*N*-(morpholinomethyl)pyrimidin-2-amine (5e)

Yield: 67%; mp 168°C; IR (cm⁻¹): 3316, 3237 (-N-H stretching), 3062 (-C-H aromatic stretching), 2958, 2919, 2857 (-C-H, CH₂ stretching), 1615 (-C=N stretching), 1320 (-C-N stretching), 1034 (-C-O-C stretching), 764 (C-Cl stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.60 (t, 4H, N(CH₂)₂), 3.56 (t, 4H, O(CH₂)₂), 3.94 (s, 2H, CH₂), 6.88 (s, 1H, pyrimidine-H), 7.36-8.00 (m, 14H, Ar-H), 8.10 (s, 1H, CH of pyrazole), 10.15 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 53.6, 66.3, 74.8, 101.3, 104.2, 119.7, 126.2, 127.6, 128.8, 128.9, 129.2, 129.5, 131.7, 133.0, 133.9, 134.3, 143.5, 145.9, 162.0, 162.2, 165.1; MS: *m/z* = 522.19; Anal. Calcd. for C₃₀H₂₇ClN₆O: C, 68.89; H, 5.20; N, 16.07; Found: C, 68.83; H, 5.29; N, 16.12 %.

4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-(4-fluorophenyl)-*N*-(morpholinomethyl)pyrimidin-2-amine (5f)

Yield: 72%; mp 159°C; IR (cm⁻¹): 3320, 3234 (-N-H stretching), 3060 (-C-H aromatic stretching), 2955, 2922, 2854 (-C-H, CH₂ stretching), 1616 (-C=N stretching), 1321 (-C-N stretching), 1118 (C-F stretching), 1037 (-C-O-C stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.65 (t, 4H, N(CH₂)₂), 3.54 (t, 4H, O(CH₂)₂), 3.97 (s, 2H, CH₂), 6.87 (s, 1H, pyrimidine-H), 7.36-8.07 (m, 14H, Ar-H), 8.12 (s,

1H, CH of pyrazole), 10.16 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 53.6, 66.5, 74.9, 101.3, 103.8, 116.2, 119.8, 126.2, 127.6, 128.8, 128.9, 129.2, 129.4, 130.6, 131.4, 131.7, 133.0, 134.3, 143.5, 145.9, 162.0, 162.2, 162.9, 165.1; MS: *m/z* = 506.22; Anal. Calcd. for C₃₀H₂₇N₆O: C, 71.13; H, 5.37; N, 16.59; Found: C, 71.06; H, 5.29; N, 16.64 %.

4-(4-bromophenyl)-6-(1,3-diphenyl-1H-pyrazol-4-yl)-N-(morpholinomethyl)pyrimidin-2-amine (5g)

Yield: 60%; mp 147°C; IR (cm⁻¹): 3324, 3235 (-N-H stretching), 3061 (-C-H aromatic stretching), 2957, 2922, 2851 (-C-H, CH₂ stretching), 1617 (-C=N stretching), 1324 (-C-N stretching), 1034 (-C-O-C stretching), 663 (-C-Br stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.62 (t, 4H, N(CH₂)₂), 3.57 (t, 4H, O(CH₂)₂), 3.94 (s, 2H, CH₂), 6.84 (s, 1H, pyrimidine-H), 7.41-8.03 (m, 14H, Ar-H), 8.12 (s, 1H, CH of pyrazole), 10.16 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 53.6, 66.5, 74.7, 101.3, 103.8, 119.8, 123.2, 126.2, 127.6, 128.3, 128.8, 129.2, 129.4, 131.7, 132.1, 133.0, 134.8, 143.5, 145.9, 162.0, 162.2, 165.3; MS: *m/z* = 566.14; Anal. Calcd. for C₃₀H₂₇BrN₆O: C, 63.50; H, 4.80; N, 14.81; Found: C, 63.56; H, 4.87; N, 14.86 %.

4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-(2-methoxyphenyl)-N-(morpholinomethyl)pyrimidin-2-amine (5h)

Yield: 68%; mp 214°C; IR (cm⁻¹): 3325, 3239 (-N-H stretching), 3067 (-C-H aromatic stretching), 2951, 2924, 2857 (-C-H, CH₂ stretching), 2840 (-OCH₃ stretching), 1615 (-C=N stretching), 1325 (-C-N stretching), 1032 (-C-O-C stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.63 (t, 4H, N(CH₂)₂), 3.54 (t, 4H, O(CH₂)₂), 3.83 (s, 3H, OCH₃), 3.96 (s, 2H, CH₂), 6.82 (s, 1H, pyrimidine-H), 7.37-8.04 (m, 14H, Ar-H), 8.14 (s, 1H, CH of pyrazole), 10.17 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 53.6, 56.1, 66.7, 74.7, 101.3, 103.8, 111.5, 118.9, 119.7, 121.5, 126.2, 127.6, 128.3, 128.7, 129.2, 129.4, 129.7, 131.1, 131.7, 132.1, 133.0, 143.4, 145.9, 157.4, 162.0, 162.2, 165.3; MS: *m/z* = 518.24; Anal. Calcd. for C₃₁H₃₀N₆O₂: C, 71.79; H, 5.83; N, 16.20; Found: C, 71.82; H, 5.76; N, 16.28 %.

4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-(4-methoxyphenyl)-N-(morpholinomethyl)pyrimidin-2-amine (5i)

Yield: 63%; mp 205°C; IR (cm⁻¹): 3323, 3233 (-N-H stretching), 3064 (-C-H aromatic stretching), 2950, 2926, 2854 (-C-H, CH₂ stretching), 2844 (-OCH₃ stretching), 1617 (-C=N stretching), 1327 (-C-N stretching), 1034 (-C-O-C stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.62 (t, 4H, N(CH₂)₂), 3.52 (t, 4H, O(CH₂)₂), 3.88 (s, 3H, OCH₃), 3.97 (s, 2H, CH₂), 6.84 (s, 1H, pyrimidine-H), 7.34-8.07 (m, 14H, Ar-H), 8.11 (s, 1H, CH of pyrazole), 10.12 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 53.6, 55.8, 66.6, 74.7, 101.3, 103.8, 111.5, 118.9, 119.9, 121.5, 126.1, 127.6, 128.4, 128.7, 129.2, 129.3, 129.8, 131.0, 131.4, 132.1, 133.1, 143.5, 145.7, 160.6, 162.1, 162.3, 165.3; MS: *m/z* = 518.24; Anal. Calcd. for C₃₁H₃₀N₆O₂: C, 71.79; H, 5.83; N, 16.20; Found: C, 71.83; H, 5.79; N, 16.26 %.

2-(6-(1,3-diphenyl-1H-pyrazol-4-yl)-2-((morpholinomethyl)amino)pyrimidin-4-yl)phenol (5j)

Yield: 61%; mp 187°C; IR (cm⁻¹): 3408 (-OH stretching), 3326, 3239 (-N-H stretching), 3067 (-C-H aromatic stretching), 2951, 2927, 2858 (-C-H, CH₂ stretching), 1614 (-C=N stretching), 1328 (-C-N stretching), 1036 (-C-O-C stretching); ¹H NMR (DMSO-*d*₆) δ ppm: 2.60 (t, 4H, N(CH₂)₂), 3.54 (t, 4H, O(CH₂)₂), 3.95 (s, 2H, CH₂), 6.87 (s, 1H, pyrimidine-H), 7.30-8.12 (m, 14H, Ar-H), 8.14 (s, 1H, CH of pyrazole), 9.16 (s, 1H, OH), 10.17 (s, 1H, NH, D₂O exch.); ¹³C NMR (DMSO-*d*₆) δ ppm: 53.6, 66.4, 74.9, 101.3, 103.9, 117.9, 119.9, 120.8, 121.8, 126.1, 127.6, 128.7, 129.2, 129.3, 131.1, 131.5, 131.6, 133.1, 143.5, 145.7, 155.7, 162.2, 162.3, 165.2; MS: *m/z* = 504.23; Anal. Calcd. for C₃₀H₂₈N₆O₂: C, 71.41; H, 5.59; N, 16.66; Found: C, 71.49; H, 5.62; N, 16.60 %.

3-(6-(1,3-diphenyl-1H-pyrazol-4-yl)-2-((morpholinomethyl)amino)pyrimidin-4-yl)phenol (5k)

Yield: 59%; mp 231°C; IR (cm⁻¹): 3411 (-OH stretching), 3324, 3235 (-N-H stretching), 3069 (-C-H aromatic stretching), 2952, 2929, 2856 (-C-H, CH₂ stretching), 1616 (-C=N stretching), 1326

(-C-N stretching), 1037 (-C-O-C stretching); ^1H NMR (DMSO- d_6) δ ppm: 2.63 (t, 4H, $\text{N}(\text{CH}_2)_2$), 3.57 (t, 4H, $\text{O}(\text{CH}_2)_2$), 3.91 (s, 2H, CH_2), 6.80 (s, 1H, pyrimidine-H), 7.28-8.02 (m, 14H, Ar-H), 8.16 (s, 1H, CH of pyrazole), 9.29 (s, 1H, OH), 10.15 (s, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO- d_6) δ ppm: 53.6, 66.4, 74.7, 101.4, 103.9, 115.9, 119.9, 120.1, 126.2, 127.6, 128.7, 129.2, 129.3, 130.5, 131.6, 133.1, 138.6, 143.5, 145.7, 155.7, 157.6, 162.2, 162.4, 165.2; MS: m/z = 504.23; Anal. Calcd. for $\text{C}_{30}\text{H}_{28}\text{N}_6\text{O}_2$: C, 71.41; H, 5.59; N, 16.66; Found: C, 71.48; H, 5.58; N, 16.64 %.

4-(6-(1,3-diphenyl-1H-pyrazol-4-yl)-2-((morpholinomethyl)amino)pyrimidin-4-yl)phenol (5l)

Yield: 73%; mp 216°C; IR (cm^{-1}): 3418 (-OH stretching), 3321, 3232 (-N-H stretching), 3071 (-C-H aromatic stretching), 2953, 2930, 2854 (-C-H, CH_2 stretching), 1618 (-C=N stretching), 1324 (-C-N stretching), 1036 (-C-O-C stretching); ^1H NMR (DMSO- d_6) δ ppm: 2.65 (t, 4H, $\text{N}(\text{CH}_2)_2$), 3.57 (t, 4H, $\text{O}(\text{CH}_2)_2$), 3.92 (s, 2H, CH_2), 6.81 (s, 1H, pyrimidine-H), 7.24-8.07 (m, 14H, Ar-H), 8.13 (s, 1H, CH of pyrazole), 9.34 (s, 1H, OH), 10.11 (s, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO- d_6) δ ppm: 53.6, 66.6, 74.7, 101.4, 103.9, 116.5, 119.8, 120.1, 126.2, 127.6, 128.4, 128.7, 128.9, 129.2, 129.3, 131.6, 132.8, 143.5, 145.7, 158.6, 162.2, 162.4, 165.2; MS: m/z = 504.23; Anal. Calcd. for $\text{C}_{30}\text{H}_{28}\text{N}_6\text{O}_2$: C, 71.41; H, 5.59; N, 16.66; Found: C, 71.45; H, 5.55; N, 16.62 %.

4-(1,3-diphenyl-1H-pyrazol-4-yl)-N-(morpholinomethyl)-6-(3-nitrophenyl)pyrimidin-2-amine (5m)

Yield: 64%; mp 241°C; IR (cm^{-1}): 3321, 3232 (-N-H stretching), 3071 (-C-H aromatic stretching), 2953, 2930, 2854 (-C-H, CH_2 stretching), 1618 (-C=N stretching), 1485, 1351 (N-O asymmetric, symmetric stretching, $-\text{NO}_2$ group), 1321 (-C-N stretching), 1039 (-C-O-C stretching); ^1H NMR (DMSO- d_6) δ ppm: 2.69 (t, 4H, $\text{N}(\text{CH}_2)_2$), 3.53 (t, 4H, $\text{O}(\text{CH}_2)_2$), 3.91 (s, 2H, CH_2), 6.85 (s, 1H, pyrimidine-H), 7.26-8.26 (m, 14H, Ar-H), 8.16 (s, 1H, CH of pyrazole), 10.13 (s, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO- d_6) δ ppm: 53.4, 66.4, 74.7, 101.4, 103.7, 119.8, 122.7, 123.9, 126.2, 127.6, 128.4, 128.9, 129.3, 129.5, 129.7, 131.6, 132.9, 143.5, 145.7, 158.6, 162.2, 162.4, 165.3; MS: m/z = 533.22; Anal. Calcd. for $\text{C}_{30}\text{H}_{27}\text{N}_7\text{O}_3$: C, 67.53; H, 5.10; N, 18.38; Found: C, 67.50; H, 5.08; N, 18.36 %.

4-(1,3-diphenyl-1H-pyrazol-4-yl)-N-(morpholinomethyl)-6-(4-nitrophenyl)pyrimidin-2-amine (5n)

Yield: 62%; mp 262°C; IR (cm^{-1}): 3321, 3236 (-N-H stretching), 3070 (-C-H aromatic stretching), 2950, 2936, 2857 (-C-H, CH_2 stretching), 1621 (-C=N stretching), 1481, 1350 (N-O asymmetric, symmetric stretching, $-\text{NO}_2$ group), 1326 (-C-N stretching), 1041 (-C-O-C stretching); ^1H NMR (DMSO- d_6) δ ppm: 2.70 (t, 4H, $\text{N}(\text{CH}_2)_2$), 3.51 (t, 4H, $\text{O}(\text{CH}_2)_2$), 3.93 (s, 2H, CH_2), 6.83 (s, 1H, pyrimidine-H), 7.24-8.31 (m, 14H, Ar-H), 8.21 (s, 1H, CH of pyrazole), 10.17 (s, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO- d_6) δ ppm: 53.4, 66.4, 74.9, 101.4, 103.8, 119.8, 124.4, 126.2, 127.6, 128.4, 128.9, 129.3, 129.5, 131.6, 133.0, 141.7, 143.5, 145.7, 147.5, 162.2, 162.4, 165.3; MS: m/z = 533.22; Anal. Calcd. for $\text{C}_{30}\text{H}_{27}\text{N}_7\text{O}_3$: C, 67.53; H, 5.10; N, 18.38; Found: C, 67.48; H, 5.12; N, 18.40 %.

4-(2,4-dichlorophenyl)-6-(1,3-diphenyl-1H-pyrazol-4-yl)-N-(morpholinomethyl)pyrimidin-2-amine (5o)

Yield: 69%; mp 227°C; IR (cm^{-1}): 3314, 3233 (-N-H stretching), 3067 (-C-H aromatic stretching), 2958, 2914, 2861 (-C-H, CH_2 stretching), 1615 (-C=N stretching), 1324 (-C-N stretching), 1034 (-C-O-C stretching), 739 (-C-Cl stretching); ^1H NMR (DMSO- d_6) δ ppm: 2.61 (t, 4H, $\text{N}(\text{CH}_2)_2$), 3.58 (t, 4H, $\text{O}(\text{CH}_2)_2$), 3.96 (s, 2H, CH_2), 6.87 (s, 1H, pyrimidine-H), 7.30-8.08 (m, 12H, Ar-H), 8.10 (s, 1H, CH of pyrazole), 10.18 (s, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO- d_6) δ ppm: 53.6, 66.3, 74.8, 101.3, 104.2, 119.7, 126.2, 127.4, 128.0, 128.8, 128.9, 129.2, 129.5, 130.3, 130.9, 131.7, 133.0, 133.9, 135.3, 143.5, 145.9, 162.0, 162.2, 165.1; MS: m/z = 556.15; Anal. Calcd. for $\text{C}_{30}\text{H}_{26}\text{Cl}_2\text{N}_6\text{O}$: C, 64.64; H, 4.70; N, 15.08; Found: C, 64.69; H, 4.67; N, 15.01 %.

3. Results and discussion

3.1. Chemistry

Synthetic strategies adopted to achieve the target compounds are depicted in Scheme 1. Here, scaffold **5** is a part of synthesis of new chemical entities in the form of antimicrobial agents. According to Scheme 1, the key chalcone derivatives 3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1-arylprop-2-en-1-ones (**3a-o**) are used as precursors for the synthesis of title compounds (**5a-o**). Compounds (**3a-o**) were synthesized through the Claisen-Schmidt condensation of equimolar amounts of different acetophenone derivatives (**2a-o**) and compound 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde (**1**) (Vilsmeier-Haack reaction adduct) by stirring the reactants in aqueous alcoholic solution containing potassium hydroxide at room temperature. To study the structure activity relationship with respect to antimicrobial properties, chalcone compounds (**3a-o**) on cyclocondensation reaction with guanidine hydrochloride in the presence of potassium hydroxide yielded compounds (**4a-o**). ¹H NMR spectrum of compound (**4a**) revealed a singlet characteristic peak for primary amine protons at $\delta = 5.65$ ppm which was confirmed by disappearance of peak when exchange with D₂O. The IR spectra for (**3a**) showed the specific bands for the primary amine at 3344 and 3450 cm⁻¹. After that, on Mannich reaction of 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-arylpyrimidin-2-amines (**4a-o**) with morpholine and formaldehyde in ethanol furnished 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-*N*-(morpholinomethyl)-6-arylpyrimidin-2-amines (**5a-o**). Structures of the isolated products (**5a-o**) were confirmed on the basis of their spectral data.

Where R = -H, -2-CH₃, -4-CH₃, -2-Cl, -4-Cl, -4-F, -4-Br, -2-OCH₃, -4-OCH₃,
-2-OH, -3-OH, -4-OH, -3-NO₂, -4-NO₂, -2,4-(Cl)₂

Scheme 1. Synthetic pathway for the synthesis of title compounds (**5a-o**)

IR spectrum of compounds (**5a-o**) revealed an absorption bands at 3325-3311, 3237-3229 and 1621-1597 cm⁻¹ characteristic to secondary amine and cyanide group, respectively. Absorption bands at 2964-2950, 2936-2914 and 2861-2850 cm⁻¹ were due to stretching vibration corresponding to methylene group. ¹H NMR spectrum revealed two triplet signals at $\delta = 2.60$ -2.70 and 3.49-3.57 ppm with coupling constants of nearly 4.5 and 4.8 Hz assignable to N(CH₂)₂ and O(CH₂)₂ of morpholine ring, respectively. Protons of methylene group showed as a singlet at $\delta = 3.90$ -3.98 ppm. The ¹³C NMR spectrum of compounds (**5a-o**) revealed two important characteristic signals of carbons. One signals were displayed at $\delta = 53.4$ -53.6 and 66.3-66.7 ppm for N(CH₂)₂ and O(CH₂)₂,

respectively, while methylene carbon were appeared downfield at $\delta = 74.7\text{--}75.0$ ppm. The mass spectrum revealed a molecular ion peaks were in agreement with proposed molecular weight and elemental analysis.

Compounds (**5a-o**) have pyrimidine core nucleus and are generated through cyclization, migration of α , β unsaturated double bonds, and diminishing of carbonyl group. A plausible mechanistic pathway for the formation of compounds (**5a-o**) is proposed in Scheme 2.

Scheme 2. Plausible mechanistic pathway of targeted compounds (**5a-o**)

3.2. *In vitro* antimicrobial evaluation

The activity of compounds was determined as per the National Committee for Clinical Laboratory Standards (NCCLS) protocol using Mueller–Hinton Broth (Becton–Dickinson, USA) [22–25]. All the newly synthesized compounds were evaluated against Gram-positive bacteria [*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442)], Gram-negative bacteria *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688)] and fungi [*C. albicans* (MTCC-227), *A. niger* (MTCC-282) and *A. clavatus* (MTCC-1323)] strains.

Table 1 Antimicrobial screening of the compounds **5a-o**

Sr. No.	-R	MINIMUM INHIBITORY CONCENTRATIONS						
		FOR BACTERIA (MIC) in $\mu\text{g ml}^{-1}$				FOR FUNGI (MIC) in $\mu\text{g ml}^{-1}$		
		Gram-negative		Gram-positive		Fungi		
		<i>E.c.</i>	<i>P.a.</i>	<i>S.a.</i>	<i>S.p.</i>	<i>C.a.</i>	<i>A.n.</i>	<i>A.c.</i>
5a	-H	500	250	500	500	1000	500	1000
5b	-2-CH ₃	250	500	>1000	250	1000	500	250
5c	-4-CH ₃	250	250	500	>1000	1000	250	1000
5d	-2-Cl	250	250	250	500	250	100	50
5e	-4-Cl	50	100	50	12.5	>1000	250	250
5f	-4-F	100	12.5	250	250	1000	250	250
5g	-4-Br	250	250	500	250	250	50	100
5h	-2-OCH ₃	1000	250	500	500	1000	500	250
5i	-4-OCH ₃	1000	500	>1000	500	>1000	500	250
5j	-2-OH	500	250	500	250	>1000	250	1000
5k	-3-OH	250	>1000	500	1000	1000	1000	500
5l	-4-OH	500	250	>1000	250	1000	500	250
5m	-3-NO ₂	250	250	500	100	500	250	250
5n	-4-NO ₂	12.5	100	100	100	500	500	500
5o	-2,4-(Cl) ₂	100	250	25	250	1000	25	50
Ampicillin		100	100	250	100	-	-	-
Griseofulvin		-	-	-	-	500	100	100

E.c.- *Escherichia coli* MTCC 443; *P.a.*- *Pseudomonas aeruginosa* MTCC 1688, *S.a.*- *Staphylococcus aureus* MTCC 96; *S.p.*- *Staphylococcus pyogenes* MTCC 442; *C.a.*- *Candida albicans* MTCC 227; *A.n.*- *Aspergillus niger* MTCC 282; *A.c.*- *Aspergillus clavatus* MTCC 1323

Amongst synthesized compounds (**5a-o**), many compounds exhibited antimicrobial potency that diverged from good to excellent. In preliminary screening, compounds **5a-o** against the test microbes are listed in Table 1 along with MIC values of reference compounds ampicillin (for bacteria) and griseofulvin (for fungi). On the basis of antibacterial screening results from Table 1, compounds **5e**, **5f**, **5n** and **5o** were noticeably displayed outstanding antibacterial activity. For several pathogens, these compounds were more active than the reference drugs and the remaining compounds of the series possessed feeble antibacterial activity. Tabular data revealed that the presence of functional group at *para* position enhanced antibacterial activity as compared to *ortho* and *meta* substituted compounds.

We have discussed and compared antifungal activities based on standard drug Griseofulvin shown in Table 1. Compounds **5m** (-3-NO₂) and **5n** (-4-NO₂) were exhibited same MIC value as standard drug against *C. albicans*. Good activity possessed against *C. albicans* by compounds **5d** (-2-Cl) and **5g** (-4-Br) at MIC= 250 $\mu\text{g ml}^{-1}$. Same compounds **5d** and **5g** also showed very good activity against *A. niger*, while highest inhibition (25 $\mu\text{g ml}^{-1}$) exists in di-chloro substituted compounds, i.e. **5o** (-2,4-(Cl)₂). Moreover, compounds **5d** and **5o** having -4-Cl, -2,4-(Cl)₂ functional groups respectively, obsessed excellent activity against *A. clavatus*. Compounds having electron withdrawing groups in structure proves its significance to bacterial and fungal strains that makes a great imaginary impact in antimicrobial activity.

3.3. Structure-activity relationship studies

SAR studies revealed that the antimicrobial activity in heterocyclic class of bioactive molecules depends on the nature of the peripheral substituents and their spatial relationship within this skeleton. The presence of chloro, fluoro and nitro substituents at *para* position on substituted ring system had enriched the antibacterial activity of compounds compared to those of electron donating substituents. Compounds tend to lose its potency by changing the substitution pattern to *ortho* and *meta* position. Integration of electron donating groups such as methyl, methoxy and hydroxy

reduced the antibacterial property. In rationality with the above results, electron withdrawing groups like fluoro and chloro on *ortho* and *para* substituted position showed optimal activity. It is determined from Table 1 that, a compound without any substitution does not display antimicrobial activity against a panel of all microorganisms.

4. Conclusion

We have synthesized newly compounds (**5a-o**) by conventional method with enlargement in yield of reactions. The synthesized compounds were screened for their *in vitro* antibacterial and antifungal activity against several bacterial and fungal strains. It is concluded from biological activity table that structural and electronic diversity of these products affects their biological activities. Compounds **5d**, **5e**, **5f**, **5g**, **5n** and **5o** are the most characteristic derivatives identified in present study because of their prominent *in vitro* antimicrobial potency. SAR studies reveal that when *ortho* and *para* positions are substituted by electron withdrawing group's fluoro, chloro, bromo and nitro; compounds revealed effective antimicrobial activity.

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