Research Paper

Synthesis and Antimicrobial Activity of Fluorine Containing Pyrazole-clubbed Dihydropyrimidinones

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In the present communication synthesis of pyrazole-containing dihydropyrimidinone motifs (4a-o) and their antimicrobial activity and cytotoxicity were reported. The newly synthesized compounds were characterized using infrared, proton nuclear magnetic resonance, carbon-13 nuclear magnetic resonance and mass spectral techniques. Compounds 4b, 4c, 4f, 4g, 4i and 4j were the most active derivatives identified during antimicrobial activity screening. On the basis of antibacterial activities, it was observed that compounds 4b and 4c exhibited activity against methicillin resistant *Staphylococcus aureus* with minimum inhibitory concentrations of 12.5 and 6.25 µg/ml, respectively. From structure activity relationship studies, it could be concluded that electron withdrawing groups played a crucial role in enhancing antimicrobial and cytotoxic effects of title compounds. In addition, the results of the cytotoxicity studies indicated that compounds 4b, 4c, 4g and 4j possessed lower levels of cytotoxicity.

Key words: Pyrazole, dihydropyrimidinones, Biginelli adduct, antimicrobial screening, cytotoxicity

Continuing progress in the treatment of many infections is now threatened by the increasing number of pathogens resistant to antimicrobial drugs. There is a need to both stewards the use of existing drugs better and to develop new therapeutic antimicrobials. Presence of fluorine atom leads to modification of some physicochemical properties such as basicity or lipophilicity, bioavailability and increase in the binding affinity of drug molecules to the target protein[1]. Hybrid heterocycles containing fluorine atoms have many applications in pharmaceutical industry[2-7].

Recently dihydropyrimidones (DHPMs)-based compound like monastrol[8,9] has led to the devotion for efficient pharmacophore variation of Biginelli DHPMs. DHPMs are also used as orally active antihypertensive agents[10,11], as α1a adrenoceptor selective antagonists[12] and cyclooxygenase-2 inhibitors[13]. The batzelladine alkaloids A and B are used for the treatment of the epidemic, acquired immune deficiency syndrome (AIDS), which inhibited the binding of human immunodeficiency virus envelope protein gp-120 to human CD4 cells to become potential new leads for AIDS therapy[14]. In the past, a broad range of biological effects, including antibacterial[15], antitubercular[16,17], antitumor[18], antiinflammatory[19], antioxidant[20] and antiamoebic[21] activities have been ascribed to these partly reduced pyrimidine derivatives. Apart from synthetic DHPM derivatives, several marine natural products with fascinating biological activities containing the dihydropyrimidine-5-carboxylate core have been isolated[22].

The chemistry of pyrazole ring system has occupied prime position in medicinal chemistry for diverse biological activities such as antibacterial[23], antifungal[24], antioxidant[25], anticancer[26], BRAF($V^{600}E$) inhibitors[27] idiopathic or immune thrombocytopenic agents[28], antitubercular[29] and antiinflammatory. The pyrazole ring is present as a core in a variety of prominent drugs such as celecoxib, sildenafil, difenamizole, ionazolac and pyrazofurin.

In the present investigation the cytotoxicity of the title compounds has been evaluated. For the development of novel bioactive antimicrobial therapeutics, cytotoxicity

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test is very essential. Cytotoxicity is the quality of being toxic to cells; indicating the dose at which the cells are killed. Cytotoxicity assays are commonly used to screen chemical libraries. A compound to be tested for any biological activity, first of all should not be cytotoxic, so that cell can easily survive. Previously, our research group has synthesized various heterocyclic derivatives as potential antimicrobial agents[30-33]. Looking to the role of dihyropyrimidinones and pyrazole in the current drugs discovery; we have incorporated these two moieties in the core structure of title compounds.

MATERIALS AND METHODS

The required chemicals were purchased from E. Merck KG, Darmstadt, Germany. Melting points were recorded on Gallenkamp apparatus and were left uncorrected. Completion of reaction and purity of all compounds were checked on aluminium coated TLC plates 60, F_{245} (E. Merck KG) using n-hexane:ethyl acetate (7:3, v/v) as mobile phase and visualized under ultraviolet (UV) light, or iodine vapour. The compositions of elements (% C, H, N) for the synthesized compounds were determined by using Perkin-Elmer 2400 CHN analyser. Infrared (IR) spectra were also recorded on Perkin Elmer FT-IR spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance II 400 MHz and carbon-13 NMR spectra on a Varian Mercury-400 (100 MHz) in DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts were reported in parts per million (ppm). Mass spectra carried out using Shimadzu LCMS 2010 spectrophotometer.

Synthesis of ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1):

Synthesis of ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (Biginelli adduct) was achieved using previously published methods[34].

Synthesis of 4-(2-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (2):

Compound 1 (0.01 mol) was dissolved in 1,4-dioxane (20 ml) and to this hydrazine hydrate (99 %, 0.01 mol) was added followed by the addition of a catalytic amount of con. H_2SO_4 and allowed to stir for 3 h at 100°. After completion of reaction, the crude mass was allowed to cool and poured on crushed ice. Product obtained as yellowish precipitate, was filtered and dried. Purification was done by crystallization using ethanol (99 %) to give compound 2.

Percent yield: 69; melting point (MP): 198-200°, IR (KBr, ν_{max}, cm^{-1}): 3424 (–N–H, 1º amine), 3310 (N–H, 2º amine), 3080 (Ar–H), 2922 (C–H, –CH_3), 1717 (C=O, amide), 1676 (C=O, urea), 1574, 1520 (C=C), 1124 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.02 (s, 2H, –N–NH_2), 2.26 (s, 3H, Ar–CH_3), 5.50 (s, 1H, –CH of pyrimidine ring), 5.86 (s, 1H, –NH-N), 6.12 (s, 1H, –NH–C–Ph), 7.10–7.46 (m, 4H, Ar–H), 7.56 (s, 1H, –NH–C–CH_3); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 18.1 (–CH_3), 47.3 (–CH of pyrimidine ring), 150.3 (NH_2–CO–NH_2), 165.2 (–CONH), 159.4 (–C–F); LCMS (ESI); *m/z*: 264.10 (M^+). Ana. calcd. (%) for $C_{12}H_{13}FN_4O_2$: C, 54.54; H, 4.96; N, 21.20. Found: C, 54.44; H, 4.89; N, 21.15.

Synthesis of 4-(2-fluorophenyl)-6-methyl-5-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one (3):

To a mixture of compound 2 (0.01 mol) and ethyl acetoacetate (0.01 mol) in absolute ethanol (99 %, 20 ml), catalytic amount of triethylamine (1 ml) was added to a round bottom flask. The reaction mixture was refluxed for 12 h at 78° using reflux condenser equipped with magnetic stirrer. After completion of reaction, the resultant heavy reddish syrup was allowed to cool at room temperature. It was washed thoroughly with ether to remove impurities. The solid thus separated was filtered off under vacuum and recrystallized from ethanol (99 %) to give product 3.

Percent yield: 62; MP: 220-222°, IR (KBr, ν_{max}, cm^{-1}): 3082 (Ar–H), 2922, 2918 (C–H, pyrimidine and pyrazole –CH_3), 2882 (C–H, –CH_2), 1680 (C=O, urea), 1653 (C=O, 3° amide), 1574, 1520 (C=C), 1124 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.10 (s, 3H, pyrazole ring –CH_3), 2.20 (s, 2H, pyrazole ring –CH_2), 2.36 (s, 3H, pyrimidine ring –CH_3), 5.43 (s, 1H, –CH of pyrimidine ring), 6.20 (s, 1H, –NH–C–Ph), 7.10–7.50 (m, 4H, Ar–H), 7.55 (s, 1H, –NH–C–CH_3); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 16.4 (–CH_3 of pyrazole ring), 18.1 (–CH_3 of pyrimidine ring), 42.4 (–CH_2 of pyrazole ring), 47.3 (–CH of pyrimidine ring), 150.3 (NH_2–CO–NH_2), 165.6 (–C=O–N–), 159.6 (–C–F), 163.1 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 330.11 (M^+). Ana. calcd. (%) for $C_{16}H_{15}FN_4O_3$: C, 58.18; H, 4.58; N, 16.96. Found: C, 58.29; H, 4.55; N, 16.92.

General synthesis of 5-(4-arylidene-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(2-

fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-ones (4a-o):

A mixture of a solution of 4-(2-fluorophenyl)-6-methyl-5-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one 3 (0.01 mol) and different derivatives of aldehyde (0.01 mol) suspended in dry toluene were taken in a flask equipped with a Dean-Stark apparatus fitted with calcium guard tube. Catalytic amount of piperidine (0.5 ml) was added and the mixture was refluxed with stirring for 8 h. On cooling, the product was precipitated, filtered under vacuum and washed with cold methanol to give series of compounds 4a-o. Product was crystallized from ethanol/chloroform (1:1).

5-(4-benzylidene-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(2-fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4a):

Light yellowish crystals; % yield: 72; MP: 231-233°, IR (KBr, ν_{max}, cm^{-1}): 3080, 3025 (Ar–H), 2936 (C–H, $–CH_3$), 1713 (C=O, 3° amide), 1697 (C=O, urea), 1578 (C=N), 1545 (C=C), 1125 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.13 (s, 3H, pyrimidine ring $–CH_3$), 2.51 (s, 3H, pyrazole ring $–CH_3$), 5.50 (s, 1H, –CH of pyrimidine ring), 6.10 (s, 1H, –NH–C–Ph), 6.95 (s, 1H, ethylene >C=CH), 7.02–7.42 (m, 9H, Ar–H), 7.54 (s, 1H, –NH–C–CH_3); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 13.5 ($–CH_3$ of pyrazole ring), 16.3 ($–CH_3$ of pyrimidine ring), 53.5 (–CH of pyrimidine ring), 128.5, 145.1 (ethylene >C=CH), 155.5 (NH_2–CO–NH_2), 157.6 (–C=O–N–), 158.6 (–C–F), 165.1 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 418.14 (M^+). Ana. calcd. (%) for $C_{23}H_{19}FN_4O_3$: C, 66.02; H, 4.58; N, 13.39. Found: C, 66.07; H, 4.63; N, 13.35.

5-(4-(2-fluorobenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(2-fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4b):

Light yellowish crystals; % yield: 76; MP: 262-264°, IR (KBr, ν_{max}, cm^{-1}): 3082, 3030 (Ar–H), 2929 (C–H, $–CH_3$), 1710 (C=O, 3° amide), 1690 (C=O, urea), 1579 (C=N), 1548 (C=C), 1124 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.16 (s, 3H, pyrazole ring $–CH_3$), 2.48 (s, 3H, pyrimidine ring $–CH_3$), 5.53 (s, 1H, –CH of pyrimidine ring), 6.18 (s, 1H, –NH–C–Ph), 6.96 (s, 1H, ethylene >C=CH), 7.10–7.51 (m, 8H, Ar–H), 7.56 (s, 1H, –NH–C–CH_3); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 13.7 ($–CH_3$ of pyrazole ring), 16.1 ($–CH_3$ of pyrimidine ring), 52.5 (–CH of pyrimidine ring), 128.2, 144.4 (ethylene >C=CH), 155.2 (NH_2–CO–NH_2), 157.7 (–C=O–N–), 158.8 (–C–F), 166.0 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 436.13 (M^+). Ana. calcd. (%) for $C_{23}H_{18}F_2N_4O_3$: C, 63.30; H, 4.16; N, 12.84. Found: C, 63.37; H, 4.13; N, 12.80.

5-(4-(4-fluorobenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(2-fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4c):

Yellowish crystals; % yield: 73; MP: 275-277°, IR (KBr, ν_{max}, cm^{-1}): 3075, 3055 (Ar–H), 2925, 2920 (C–H, $–CH_3$), 1714 (C=O, 3° amide), 1693 (C=O, urea), 1577 (C=N), 1536 (C=C), 1102 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.24 (s, 3H, pyrazole ring $–CH_3$), 2.60 (s, 3H, pyrimidine ring $–CH_3$), 5.56 (s, 1H, –CH of pyrimidine ring), 6.15 (s, 1H, –NH–C–Ph), 6.98 (s, 1H, ethylene >C=CH), 6.99–7.45 (m, 8H, Ar–H), 7.53 (s, 1H, –NH–C–CH_3); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 13.8 ($–CH_3$ of pyrazole ring), 16.2 ($–CH_3$ of pyrimidine ring), 51.4 (–CH of pyrimidine ring), 126.2, 143.5 (ethylene >C=CH), 155.3 (NH_2–CO–NH_2), 158.5 (–C=O–N–), 159.3 (–C–F), 167.1 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 436.13 (M^+). Ana. calcd. (%) for $C_{23}H_{18}F_2N_4O_3$: C, 63.30; H, 4.16; N, 12.84. Found: C, 63.34; H, 4.09; N, 12.77.

4-(2-fluorophenyl)-5-(4-(2-hydroxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4d):

Light brown crystals; % yield: 57; MP: 221-223°, IR (KBr, ν_{max}, cm^{-1}): 3313 (O–H, Ar–OH), 3077, 3060 (Ar–H), 2926, 2919 (C–H, $–CH_3$), 1715 (C=O, 3° amide), 1686 (C=O, urea), 1575 (C=N), 1530 (C=C), 1125 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.25 (s, 3H, pyrazole ring $–CH_3$), 2.45 (s, 3H, pyrimidine ring $–CH_3$), 5.53 (s, 1H, –CH of pyrimidine ring), 6.74 (s, 1H, Ar–OH), 6.14 (s, 1H, –NH–C–Ph), 6.81 (s, 1H, ethylene >C=CH), 6.89–7.37 (m, 8H, Ar–H), 7.53 (s, 1H, –NH–C–CH_3); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.0 ($–CH_3$ of pyrazole ring), 16.0 ($–CH_3$ of pyrimidine ring), 49.5 (–CH of pyrimidine ring), 126.3, 143.4 (ethylene >C=CH), 154.1 (NH_2–CO–NH_2), 159.6 (–C=O–N–), 159.0 (–C–F), 167.0 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 434.14 (M^+); Ana. calcd. (%) for $C_{23}H_{19}FN_4O_4$: C, 63.59; H, 4.41; N, 12.90. Found: C, 63.62; H, 4.49; N, 12.94.

4-(2-fluorophenyl)-5-(4-(4-hydroxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-

carbonyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4e):

Light brown crystals; % yield: 65; MP: 252-254°, IR (KBr, ν_{max}, cm^{-1}): 3329 (O–H, Ar–OH), 3052, 3061 (Ar–H), 2924, 2920 (C–H, $–CH_3$), 1716 (C=O, 3° amide), 1682 (C=O, urea), 1568 (C=N), 1503 (C=C), 1133 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.24 (s, 3H, pyrazole ring $–CH_3$), 2.56 (s, 3H, pyrimidine ring $–CH_3$), 5.61 (s, 1H, –CH of pyrimidine ring), 6.04 (s, 1H, –NH–C–Ph), 6.75 (s, 1H, Ar–OH), 6.98 (s, 1H, ethylene >C=CH), 7.08–7.55 (m, 8H, Ar–H), 7.55 (s, 1H, $–NH–C–CH_3$); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.2 ($–CH_3$ of pyrazole ring), 16.5 ($–CH_3$ of pyrimidine ring), 49.4 (–CH of pyrimidine ring), 126.3, 143.3 (ethylene >C=CH), 154.3 ($NH_2–CO–NH_2$), 159.5 (–C=O–N–), 159.2 (–C–F), 167.9 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 434.14 (M^+). Ana. calcd. (%) for $C_{23}H_{19}FN_4O_4$: C, 63.59; H, 4.41; N, 12.90. Found: C, 63.61; H, 4.42; N, 12.80.

5-(4-(2-chlorobenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(2-fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4f):

Yellow crystals; % yield: 61; MP: 262-264°, IR (KBr, ν_{max}, cm^{-1}): 3071, 3063 (Ar–H), 2929, 2922 (C–H, $–CH_3$), 1719 (C=O, 3° amide), 1683 (C=O, urea), 1579 (C=N), 1518 (C=C), 1134 (C–F), 783 (C–Cl); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.26 (s, 3H, pyrazole ring $–CH_3$), 2.46 (s, 3H, pyrimidine ring $–CH_3$), 5.53 (s, 1H, –CH of pyrimidine ring), 6.05 (s, 1H, –NH–C–Ph), 6.80 (s, 1H, ethylene >C=CH), 7.10–7.66 (m, 8H, Ar–H), 7.52 (s, 1H, $–NH–C–CH_3$); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.6 ($–CH_3$ of pyrazole ring), 16.3 ($–CH_3$ of pyrimidine ring), 48.5 (–CH of pyrimidine ring), 126.2, 143.5 (ethylene >C=CH), 134.2 (–C–Cl), 153.5 ($NH_2–CO–NH_2$), 159.6 (–C–F), 160.5 (–C=O–N–), 167.2 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 452.11 (M^+). Ana. calcd. (%) for $C_{23}H_{18}ClFN_4O_3$: C, 61.00; H, 4.01; N, 12.37. Found: C, 61.04; H, 4.06; N, 12.29.

5-(4-(4-chlorobenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(2-fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4g):

Yellow crystals; % yield: 72; MP: 277-279°, IR (KBr, ν_{max}, cm^{-1}): 3076, 3065 (Ar–H), 2927, 2919 (C–H, $–CH_3$), 1720 (C=O, 3° amide), 1685 (C=O, urea), 1576 (C=N), 1519 (C=C), 1138 (C–F), 781 (C–Cl); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.24 (s, 3H, pyrazole ring $–CH_3$), 2.45 (s, 3H, pyrimidine ring $–CH_3$), 5.56 (s, 1H, –CH of pyrimidine ring), 6.07 (s, 1H, –NH–C–Ph), 6.84 (s, 1H, ethylene >C=CH), 7.06–7.72 (m, 8H, Ar–H), 7.56 (s, 1H, $–NH–C–CH_3$); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.8 ($–CH_3$ of pyrazole ring), 16.1 ($–CH_3$ of pyrimidine ring), 47.9 (–CH of pyrimidine ring), 126.4, 143.4 (ethylene >C=CH), 133.6 (–C–Cl), 153.2 ($NH_2–CO–NH_2$), 156.7 (–C–F), 160.4 (–C=O–N–), 167.3 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 452.11 (M^+). Ana. calcd. (%) for $C_{23}H_{18}ClFN_4O_3$: C, 61.00; H, 4.01; N, 12.37. Found: C, 60.59; H, 4.07; N, 12.28.

4-(2-fluorophenyl)-6-methyl-5-(3-methyl-4-(2-nitrobenzylidene)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one (4h)

Dark brown crystals; % yield: 68; MP: 204-206°, IR (KBr, ν_{max}, cm^{-1}): 3057 (Ar–H), 2918 (C–H, $–CH_3$), 1720 (C=O, 3° amide), 1680 (C=O, urea), 1549 (C=N), 1511 (C=C), 1384 (NO_2), 1107 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.34 (s, 3H, pyrazole ring $–CH_3$), 2.40 (s, 3H, pyrimidine ring $–CH_3$), 5.38 (s, 1H, –CH of pyrimidine ring), 6.04 (s, 1H, –NH–C–Ph), 6.19 (s, 1H, ethylene >C=CH), 7.21–7.47 (m, 8H, Ar–H), 7.48 (s, 1H, $–NH–C–CH_3$); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.9 ($–CH_3$ of pyrazole ring), 16.3 ($–CH_3$ of pyrimidine ring), 47.4 (–CH of pyrimidine ring), 126.2, 143.6 (ethylene >C=CH), 147.5 ($–C–NO_2$), 153.4 ($NH_2–CO–NH_2$), 156.5 (–C–F), 160.7 (–C=O–N–), 167.4 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 463.13 (M^+). Ana. calcd. (%) for $C_{23}H_{18}FN_5O_5$: C, 59.61; H, 3.92; N, 15.11. Found: C, 59.52; H, 3.97; N, 15.08.

4-(2-fluorophenyl)-6-methyl-5-(3-methyl-4-(3-nitrobenzylidene)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one (4i):

Dark brown crystals; % yield: 66, MP: 272-274°, IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar–H), 2916 (C–H, $–CH_3$), 1725 (C=O, 3° amide), 1677 (C=O, urea), 1550 (C=N), 1522 (C=C), 1460 (NO_2), 1121 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.32 (s, 3H, pyrazole ring $–CH_3$), 2.41 (s, 3H, pyrimidine ring $–CH_3$), 5.35 (s, 1H, –CH of pyrimidine ring), 6.06 (s, 1H, –NH–C–Ph), 7.16–7.50 (m, 8H, Ar–H), 6.15 (s, 1H, ethylene >C=CH), 7.47 (s, 1H, $–NH–C–CH_3$); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.8 ($–CH_3$ of pyrazole ring), 16.2 ($–CH_3$ of pyrimidine ring), 47.5 (–CH of pyrimidine ring), 126.2, 143.5 (ethylene >C=CH), 147.9 ($–C–NO_2$), 152.3 ($NH_2–CO–NH_2$),

157.6 (–C–F), 162.8 (–C=O–N–), 167.3 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 463.13 (M^+). Ana. calcd. (%) for $C_{23}H_{18}FN_5O_5$: C, 59.61; H, 3.92; N, 15.11. Found: C, 59.52; H, 3.84; N, 15.05.

4-(2-fluorophenyl)-6-methyl-5-(3-methyl-4-(4-nitro benzylidene)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one (4j):

Dark brown crystals; % yield: 58; MP: 275-277°, IR (KBr, ν_{max}, cm^{-1}): 3087, 3058 (Ar–H), 2925 (C–H, $–CH_3$), 1723 (C=O, 3° amide), 1691 (C=O, urea), 1591 (C=N), 1527 (C=C), 1481 (NO_2), 1097 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.27 (s, 3H, pyrazole ring $–CH_3$), 2.48 (s, 3H, pyrimidine ring $–CH_3$), 5.41 (s, 1H, –CH of pyrimidine ring), 6.01 (s, 1H, –NH–C–Ph), 7.20–7.77 (m, 8H, Ar–H), 7.71 (s, 1H, ethylene >C=CH), 7.81 (s, 1H, $–NH–C–CH_3$); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.7 ($–CH_3$ of pyrazole ring), 17.1 ($–CH_3$ of pyrimidine ring), 47.3 (–CH of pyrimidine ring), 126.2, 143.8 (ethylene >C=CH), 147.1 ($–C–NO_2$), 151.2 ($NH_2–CO–NH_2$), 157.5 (–C–F), 163.7 (–C=O–N–), 167.2 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 463.13 (M^+). Ana. calcd. (%) for $C_{23}H_{18}FN_5O_5$: C, 59.61; H, 3.92; N, 15.11. Found: C, 59.70; H, 3.90; N, 15.03.

5-(4-(2,6-dichlorobenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(2-fluoro phenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4k):

Light brown crystals; % yield: 55; MP: 215-217°, IR (KBr, ν_{max}, cm^{-1}): 3074, 3064 (Ar–H), 2930 (C–H, $–CH_3$), 1720 (C=O, 3° amide), 1688 (C=O, urea), 1574 (C=N), 1535 (C=C), 1135 (C–F), 779 (C–Cl); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.34 (s, 3H, pyrazole ring $–CH_3$), 2.43 (s, 3H, pyrimidine ring $–CH_3$), 5.42 (s, 1H, –CH of pyrimidine ring), 6.12 (s, 1H, –NH–C–Ph), 6.88 (s, 1H, ethylene >C=CH), 7.10–7.58 (m, 7H, Ar–H), 7.52 (s, 1H, $–NH–C–CH_3$); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.8 ($–CH_3$ of pyrazole ring), 16.2 ($–CH_3$ of pyrimidine ring), 53.4 (–CH of pyrimidine ring), 126.2, 143.5 (ethylene >C=CH), 132.6 (–C–Cl), 154.5 ($NH_2–CO–NH_2$), 156.8 (–C=O–N–), 159.5 (–C–F), 165.1 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 486.07 (M^+). Ana. calcd. (%) for $C_{23}H_{17}Cl_2FN_4O_3$: C, 56.69; H, 3.52; N, 11.50. Found: C, 56.71; H, 3.44; N, 11.45.

4-(2-fluorophenyl)-5-(4-(2-methoxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4l):

Dark yellowish crystals; % yield: 77; MP: 268-270°, IR (KBr, ν_{max}, cm^{-1}): 3074, 3036 (Ar–H), 2925 (C–H, $–CH_3$), 1715 (C=O, 3° amide), 1688 (C=O, urea), 1574 (C=N), 1535 (C=C), 1246 (C-O-C, Ar-O-CH_3), 1133 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.36 (s, 3H, pyrazole ring $–CH_3$), 2.45 (s, 3H, pyrimidine ring $–CH_3$), 3.83 (s, 3H, $–OCH_3$), 5.45 (s, 1H, –CH of pyrimidine ring), 6.13 (s, 1H, –NH–C–Ph), 6.89 (s, 1H, ethylene >C=CH), 7.10–7.67 (m, 8H, Ar–H), 7.50 (s, 1H, $–NH–C–CH_3$); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.7 ($–CH_3$ of pyrazole ring), 16.2 ($–CH_3$ of pyrimidine ring), 52.4 (–CH of pyrimidine ring), 56.5 ($–OCH_3$), 126.2, 143.9 (ethylene >C=CH), 153.2 ($NH_2–CO–NH_2$), 157.8 (–C=O–N–), 159.5 (–C–F), 166.4 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 448.15 (M^+). Ana. calcd. (%) for $C_{24}H_{21}FN_4O_4$: C, 64.28; H, 4.72; N, 12.49. Found: C, 64.16; H, 4.67; N, 12.40.

4-(2-fluorophenyl)-5-(4-(4-methoxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4m):

Dark yellowish crystals; % yield: 59; MP: 256-258°, IR (KBr, ν_{max}, cm^{-1}): 3075, 3030 (Ar–H), 2927 (C–H, $–CH_3$), 1716 (C=O, 3° amide), 1684 (C=O, urea), 1572 (C=N), 1540 (C=C), 1240 (C-O-C, Ar-O-CH_3), 1135 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.35 (s, 3H, pyrazole ring $–CH_3$), 2.47 (s, 3H, pyrimidine ring $–CH_3$), 3.80 (s, 3H,$–OCH_3$), 5.50 (s, 1H, –CH of pyrimidine ring), 6.15 (s, 1H, –NH–C–Ph), 6.84 (s, 1H, ethylene >C=CH), 7.13–7.46 (m, 8H, Ar–H), 7.55 (s, 1H, $–NH–C–CH_3$); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.6 ($–CH_3$ of pyrazole ring), 16.5 ($–CH_3$ of pyrimidine ring), 49.6 (–CH of pyrimidine ring), 55.7 ($–OCH_3$), 126.0, 143.6 (ethylene >C=CH), 154.4 ($NH_2–CO–NH_2$), 157.5 (–C=O–N–), 159.3 (–C–F), 169.2 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 448.15 (M^+). Ana. calcd (%) for $C_{24}H_{21}FN_4O_4$: C, 64.28; H, 4.72; N, 12.49. Found: C, 64.15; H, 4.63; N, 12.42.

4-(2-fluorophenyl)-6-methyl-5-(3-methyl-4-(2-methylbenzylidene)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one (4n):

Dark yellow crystals; % yield: 75; MP: 244-246°, IR (KBr, ν_{max}, cm^{-1}): 3075, 3034 (Ar–H), 2977 (C–H, Ar-CH_3), 2927 (C–H, $–CH_3$), 1751 (C=O, 3° amide), 1684 (C=O, urea), 1572 (C=N), 1534 (C=C), 1135 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.36

(s, 3H, pyrazole ring –CH_3), 2.40 (s, 3H, pyrimidine ring –CH_3), 2.50 (s, 3H, Ar–CH_3), 5.34 (s, 1H, –CH of pyrimidine ring), 6.20 (s, 1H, –NH–C–Ph), 6.89 (s, 1H, ethylene >C=CH), 7.01–7.56 (m, 8H, Ar–H), 7.56 (s, 1H, –NH–C–CH_3); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.9 (–CH_3 of pyrazole ring), 17.1 (–CH_3 of pyrimidine ring), 19.2 (Ar–CH_3), 49.3 (–CH of pyrimidine ring), 126.3, 143.8 (ethylene >C=CH), 153.1 (NH_2–CO–NH_2), 156.6 (–C=O–N–), 159.5 (–C–F), 165.2 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 432.16 (M^+). Ana. calcd. (%) for $C_{24}H_{21}FN_4O_3$: C, 66.66; H, 4.89; N, 12.96. Found: C, 66.72; H, 4.79; N, 12.90.

4-(2-fluorophenyl)-6-methyl-5-(3-methyl-4-(4-methylbenzylidene)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one (4o):

Dark yellow crystals; % yield: 78, MP: 234-236°, IR (KBr, ν_{max}, cm^{-1}): 3079, 3028 (Ar–H), 2980 (C–H, Ar–CH_3), 2925 (C–H, –CH_3), 1775 (C=O, 3º amide), 1689 (C=O, urea), 1575 (C=N), 1546 (C=C), 1131 (C–F); ^{1}H NMR (400 MHz, δ ppm, DMSO–d_6): 2.35 (s, 3H, Ar–CH_3), 2.38 (s, 3H, pyrazole ring –CH_3), 2.46 (s, 3H, pyrimidine ring –CH_3), 5.43 (s, 1H, –CH of pyrimidine ring), 6.18 (s, 1H, –NH–C–Ph), 6.85 (s, 1H, ethylene >C=CH), 7.10–7.55 (m, 8H, Ar–H), 7.56 (s, 1H, –NH–C–CH_3); ^{13}C NMR (100 MHz, δ ppm, DMSO–d_6): 14.8 (–CH_3 of pyrazole ring), 17.3 (–CH_3 of pyrimidine ring), 21.3 (Ar–CH_3), 50.5 (–CH of pyrimidine ring), 126.5, 143.5 (ethylene >C=CH), 153.0 (NH_2–CO–NH_2), 157.5 (–C=O–N–), 159.7 (–C–F), 165.0 (–C=O of pyrazole ring); LCMS (ESI); *m/z*: 432.16 (M^+). Ana. calcd (%) for $C_{24}H_{21}FN_4O_3$: C, 66.66; H, 4.89; N, 12.96. Found: C, 66.69; H, 4.79; N, 12.85.

***In vitro* antimicrobial screening:**

Antibacterial activity of newly synthesized compounds (4a-o) was carried out against the representative panel of bacteria such as *Escherichia coli* MTCC-443, *Pseudomonas aeruginosa* MTCC-1688, *Staphylococcus aureus* MTCC-96 and *Streptococcus pyogenes* MTCC-442 using ciprofloxacin as the standard antibacterial drug. Antifungal activity was screened against three fungal species, *Candida albicans* MTCC-227, *Aspergillus niger* MTCC-282, *Aspergillus clavatus* MTCC-1323, and griseofulvin was used as the standard antifungal drug. The minimal inhibitory concentration (MIC) of all the synthesized compounds was determined by the broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS)[35]. All the synthesized compounds (1, 2, 3 and 4a-o) were screened for antibacterial and antifungal activities in six sets (n=6) against bacteria and fungi used in the present protocol.

***In vitro* cytotoxicity studies:**

After identification of active antimicrobial agents, the next step was to determine the toxicity of drug contenders. *In vitro* cytotoxic activity of newly synthesized compounds (4a-o) were evaluated against 3T3 mouse embryonic fibroblast cell line and human cervical cancer cell line (HeLa) using the tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. The IC_{50} determination was achieved according to the NCCLS recommendations[36].

RESULTS AND DISCUSSION

We have synthesized new analogues in which pyrazole scaffold was linked to the DHPMs systems. The synthetic pathway for final compounds is illustrated in fig. 1. Compound 1 on reaction with hydrazine hydrate furnished 4-(2-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carbohydrazide 2. Cyclic condensation of compound 2 with ethyl acetoacetate resulted the compound 4-(2-fluorophenyl)-6-methyl-5-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one 3. This was further converted into 5-(4-(arylmethylene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(2-fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-ones (4a-o) by Knoevenagel condensation with different aryl aldehydes in the presence of piperidine catalyst.

IR spectrum of targeted compound 4c gave stretching vibrations at 3075 and 3055 cm^{-1} showed strong intensity absorption peaks corresponding to Ar-H groups. The absorption peaks at 2925 and 2920 cm^{-1} were assigned to stretching vibration corresponding to -CH_3 groups of pyrimidine and pyrazole ring system, respectively. The strong absorption at 1693 cm^{-1} observed due to the stretching vibration of >C=O group present in pyrimidine ring system. Apart from that >C=O group present in amide linkage, showed strong stretching absorption at 1714 cm^{-1}. Absorption bands at 1577 and 1536 cm^{-1} were observed due to the C=N and C=C stretching in aromatic rings. A strong intensity absorption band at 1102 cm^{-1} was shown due to -C-F stretching vibration (fig. 2).

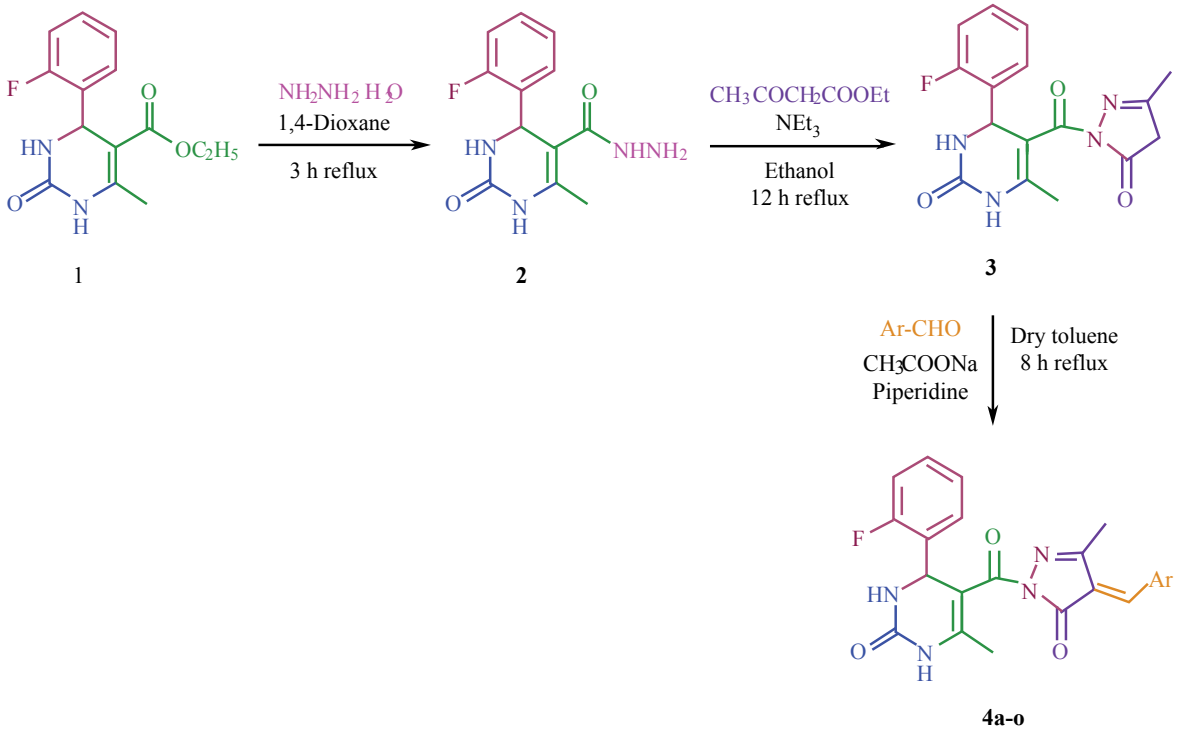

Fig. 1: Synthetic pathway of novel compounds 4a-o

Where, 4a. Ar=-C_6H_5; 4b. Ar=-2-F-C_6H_4; 4c. Ar=-4-F-C_6H_4; 4d. Ar=-2-OH-C_6H_4; 4e. Ar=-4-OH-C_6H_4; 4f. Ar=-2-Cl-C_6H_4; 4g. Ar=-4-Cl-C_6H_4; 4h. Ar=-2-NO_2-C_6H_4; 4i. Ar=-3-NO_2-C_6H_4; 4j. Ar=-4-NO_2-C_6H_4; 4k. Ar=-2,6$(Cl)_2$-C_6H_3; 4l. Ar=-2-OCH_3-C_6H_4; 4m. Ar=-4-OCH_3-C_6H_4; 4n. Ar=-2-CH_3-C_6H_4; 4o Ar=-4-CH_3-C_6H_4

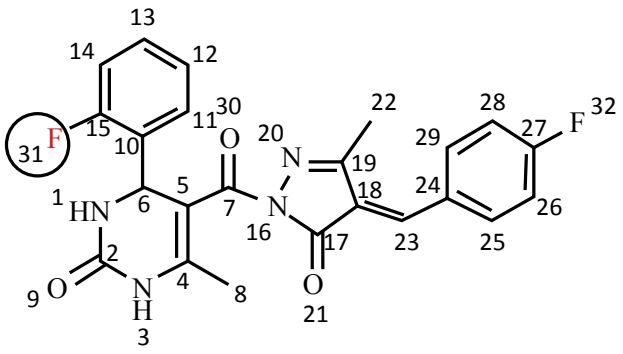

Fig. 2: Carbon enumerations of compound 4c

^{1}H NMR spectra of final compound 4c showed that protons of methyl groups present on pyrazole and pyrimidine ring systems were observed as a singlet, and it gave a shift at δ= 2.24 and δ= 2.60 ppm, respectively. Proton of the -CH-C- linkage, which was attached to C-6 appeared as a singlet at δ= 5.56 ppm. In pyrimidine ring proton group's linkage at -NH-C-Ph proton and -NH-C-CH_3 proton furnished a singlet values at δ= 6.15 ppm and δ= 7.53 ppm, respectively. Proton at ethylene group linkage at C-23 shown δ= 6.98 ppm as singlet. Looking to the ^{13}C NMR spectra, values of the chemical shift of final compound 4c was observed in between the range of δ= 167.1-13.8 ppm. In the carbon of methyl groups of at C-8 and C-22 appeared at δ= 16.2 and 13.8 ppm, respectively. The C-6 carbon of pyrimidine ring appeared at δ= 51.4 ppm. The C-18 and C-23 carbon of ethylene groups appeared at δ= 126.2 and 143.5 ppm, respectively. The carbonyl carbon of urea in pyrimidine ring, carbonyl in pyrazole ring and carbonyl in amide linkage appeared at δ= 155.3, 167.1 and 158.5 ppm, respectively. The C-15 and C-27 carbon of fluoro phenyl rings appeared at δ= 159.3 ppm due to the influence of fluoro groups. Carbon enumeration of compound 4c is described in fig. 2. Additionally, the mass spectrum of compound 4c showed a molecular ion peak at *m/z*= 436.13 (M^+) was also in harmony to the molecular formula $C_{23}H_{18}F_2N_4O_3$. The spectral values for all the compounds and C, H, and N analysis are listed in experimental section.

The outcome of antimicrobial activity evaluation of the synthesized compounds revealed that these compounds possessed antibacterial and antifungal activities. From antimicrobial activity data (Table 1), key precursor, fluoro hydrazide of tetrahydropyrimidine (2) showed poor antimicrobial activity at MIC= 1000 µg/ml. Compound (3) displayed moderate antibacterial activity at MIC= 25 and 50 µg/ml and good antifungal activity at MIC= 50 µg/ml against tested bacteria and fungi, respectively. Subsequently, when compound (3) was converted into final compounds (4a-o), these showed significant broad-spectrum antimicrobial activity.

Primary microbiological screening results showed that compounds 4a (-C_6H_5) and 4k (-2,6-$(Cl)_2$-C_6H_3) possessed moderate activity against *E. coli*. The antibacterial activity against *E. coli* improved when substitutions pattern was changed by the installation of fluoro and nitro groups in compounds 4b, 4c, 4i and 4j. Compound 4g was found to be most active against *E. coli* (MIC= 12.5 µg/ml). Compound 4f (-2-Cl-C_6H_4) possessed moderate activity against *P. aeruginosa*, while compounds 4a, 4b, 4c and 4g showed very good activity against *P. aeruginosa*. Moreover, when we introduced nitro group as a substituent at *meta* position in compound 4a, the activity was enhanced and showed excellent activity against *P. aeruginosa*. In case of *S. aureus*, electro withdrawing group at 2nd position like in 4b (-2-F-C_6H_4) obsessed moderate activity at MIC= 100 µg/ml, while electro withdrawing groups at 4th position like in 4g (-4-Cl-C_6H_4) and 4j (-4-NO_2-C_6H_4) displayed very good activity and highest inhibition (MIC= 12.5 µg/ml) observed in compound possessing fluoro group at *para* position i.e. compound 4c. It was observed from Table 1, compounds 4g (-4-Cl-C_6H_4) and 4i (-3-NO_2-C_6H_4) possessed moderate activity against *S. pyogenes*, while compounds 4a (-C_6H_4) and 4c (-4-F-C_6H_4) showed very good activity against *S. pyogenes* at 50 µg/ml MIC. The highest MIC inhibition at 12.5 µg/ml flaunted for *S. pyogenes* in compound 4b having fluoro group at *ortho* position.

MIC values of antifungal activity were observed against *C. albicans*, *A. niger* and *A. clavatus* by conventional broth micro dilution method using various concentrations for screening. On the basis of antifungal activity results, we concluded that compounds 4f (-2-Cl-C_6H_4) and 4j (-4-NO_2-C_6H_4) possessed moderate to very good activity against *C. albicans.* Again antifungal data revealed that the introduction of fluoro group as a substituent in targeted compound enhanced the activity against *C. albicans*. Furthermore, compounds 4c, 4h and 4j having -4-F-C_6H_4, -2-NO_2-C_6H_4, -4-NO_2-C_6H_4 functional groups, exhibited moderate activity against *A. niger*, while very good activity was shown against *A. niger* by -2-Cl-C_6H_4 (4f). *Ortho* fluoro substitution showed excellent results against *A. niger.* We have employed *A. clavatus* to check the activity of newly synthesized compounds. Compound 4h (-2-NO_2-C_6H_4) showed moderate activity against *A. clavatus*, on the other hand compound 4b (-2-F-C_6H_4) displayed very good activity against *A. clavatus.* The MIC= 25 µg/ml was demonstrated in compounds having electron withdrawing group, i.e. compounds 4f and 4j. The remaining compounds of the series showed feeble antifungal activity (Table 1).

TABLE 1: RESULTS OF ANTIBACTERIAL SCREENING OF COMPOUNDS 4a-o

Entry	-Ar	Minimum inhibitory concentration MIC (µg/ml)						
		Gram-negative bacteria		Gram-positive bacteria		Fungi		
		E. c.[a]	*P. a.*[b]	*S. a.*[c]	*S. p.*[d]	*C. a.*[e]	*A. n.*[f]	*A. c.*[g]
1	-	500	200	500	500	1000	500	>1000
2	-	500	500	500	200	500	1000	1000
3	-	100	25	200	50	500	50	500
4a	-C_6H_5	100	25	200	50	500	500	500
4b	-2-F-C_6H_4	50	50	100	12.5	1000	12.5	50
4c	-4-F-C_6H_4	25	50	12.5	50	25	100	200
4d	-2-OH-C_6H_4	500	200	500	500	500	1000	>1000
4e	-4-OH-C_6H_4	200	500	200	200	>1000	1000	1000
4f	-2-Cl-C_6H_4	100	100	200	200	100	50	25
4g	-4-Cl-C_6H_4	12.5	50	25	100	500	1000	1000
4h	-2-NO_2-C_6H_4	500	1000	>1000	1000	1000	100	100
4i	-3-NO_2-C_6H_4	50	12.5	200	100	500	1000	1000
4j	-4-NO_2-C_6H_4	50	1000	25	500	50	100	25
4k	-2,6-$(Cl)_2$-C_6H_3	100	500	500	1000	1000	500	500
4l	-2-OCH_3-C_6H_4	500	200	500	500	500	>1000	1000
4m	-4-OCH_3-C_6H_4	1000	>1000	1000	1000	1000	500	500
4n	-2-CH_3-C_6H_4	1000	200	1000	1000	1000	200	500
4o	-4-CH_3-C_6H_4	500	500	500	500	200	>1000	1000
Ciprofloxacin		25	25	50	50	-	-	-
Griseofulvin		-	-	-	-	500	100	100

[a]*E.c.*: *Escherichia coli* (MTCC-443); [b]*P.a.*: *Pseudomonas aeruginosa* (MTCC-1688); [c]*S.a.*: *Staphylococcus aureus* (MTCC-96); [d]*S.p.*: *Streptococcus pyogenes* (MTCC-442); [e]*C.a.*: *Candida albicans* (MTCC-227); [f]*A.n.*: *Aspergillus niger* (MTCC-282); [g]*A.c.*: *Aspergillus clavatus* (MTCC-1323)

The enhancement in activity of these compounds was endorsed to the presence of electron withdrawing groups like nitro and halogen in reported compounds. The most active compounds, 4b, 4c, 4g, and 4j against *S. aureus* and *S. pyogenes* (MIC= 12.5–25 µg/ml) were also tested against methicillin-resistant *S. aureus* (MRSA isolate ATCC 43300) and the results were given in Table 2. Compounds 4b and 4c exhibited more potent activity than the standard drugs against MRSA. Compound 4c, with MIC value of 6.25 µg/ml against MRSA, showed fourfold more potency than ciprofloxacin (MIC= 25 µg/ml) and eightfold more activity than chloramphenicol (MIC= 50 µg/ml). In addition, compound 4b endowed with fluoro group showed twofold more activity at MIC value of 12.5 µg/ml than ciprofloxacin and fourfold higher potency than chloramphenicol against MRSA.

The IC_{50} values achieved for these compounds were shown in Table 3. Cytotoxicity results displayed that the derivatives 4b, 4c, 4f, 4g, 4i and 4j accounted no toxicity at concentration of 100 µM (IC_{50}>100 µM), while other derivatives exhibited moderate toxicity against HeLa cell lines. It was established that none of the tested compounds revealed any significant cytotoxic effects on HeLa cell line, signifying that compounds were potential for their *in vivo* use as antimicrobial agents. Furthermore, none of the derivatives displayed cytotoxicity against 3T3 cell lines (IC_{50}>100 µM).

In the present investigation we have used the pyrazole bearing dihydropyrimidinone motif for the development of potential antimicrobial agents. Both the pharmacophores are therapeutically very important as dihydropyrimidines are potential inhibitors of dihydrofolate reductase, which is a promising drug target for treatment of mycobacterial infections. Similarly pyrazole moiety is also very promising as it possesses varieties of biological activities. This prompted us to use the hydride of both the pharmacophores for the present investigation[37,38]. SAR study helped in enlightening

TABLE 2: INHIBITORY ACTIVITY (MIC, µg/ml) OF COMPOUNDS 4b, 4c, 4g, AND 4j AGAINST METHICILLIN RESISTANT *S. AUREUS*

Entry	MRSA[a]
4b	12.5
4c	6.25
4g	>50
4j	>50
Ciprofloxacin	25
Chloramphenicol	50

[a]Methicillin-resistant *S. aureus* (ATCC 43300)

TABLE 3: LEVELS OF CYTOTOXICITY PROMPTED BY COMPOUNDS 4a-o ON HeLa CELLS AND 3T3 CELL LINES

Entry	Cytotoxicity (IC_{50} µM)[a]	
	HeLa[b]	3T3[c]
4a	60.95	>100
4b	>100	>100
4c	>100	>100
4d	87.56	>100
4e	65.57	>100
4f	>100	>100
4g	>100	>100
4h	94.94	>100
4i	>100	>100
4j	>100	>100
4k	96.34	>100
4l	77.98	>100
4m	56.65	>100
4n	80.23	>100
4o	70.68	>100

[a]IC_{50} is the concentration required to inhibit 50 % of cell growth, [b]HeLa human cervical cancer cell line, [c]Mouse embryonic fibroblast cell line

the use of different substitution and their electronic effect on microbial strains. Substitution pattern of pyrazole and dihydropyrimidine derivatives were chosen carefully for deliberating different electronic environment of the new molecules[37,38]. Electron donating groups on aromatic ring, such as methyl, methoxy and hydroxy, and electron withdrawing groups from aromatic ring, such as fluoro, chloro and nitro, were chosen as substituents in the molecular diversities of the targeted compounds. Results of antimicrobial activity of final compounds showed that the presence of hydrophobic substituent at *ortho* and *para* (-F, 4b and 4c) position of phenyl ring provided a positive impact on antimicrobial activity. The amplified activity was due to the hydrophobic nature of fluorine, which was responsible for the influence of substituent group's physicochemical properties. In addition, compounds 4b and 4c have two fluoro groups in structure which may also be responsible for the influence in antimicrobial activity. The antibacterial activity data (Table 1) revealed that the presence of electron withdrawing functional groups, specifically -F, -Cl and $-NO_2$ exhibited excellent activity against all type of bacterial strains, which is better than the electron donating groups like -OH, $-CH_3$ and $-OCH_3$. In nut shell, we can conclude that the incorporation of electron donating groups such as hydroxy, methyl, and methoxy diminished the antibacterial property. Effect of halogen group's as substitution was clearly

visible in activity enhancement (Table 1). It was noted that the formation of pyrazole ring (compound 3) from hydrazide precursor slightly improved the activity than its precursor but the drastic change was observed in antimicrobial activity of final Knoevenagel adduct (Table 1). The four compounds 4b, 4c, 4g, and 4j were screened against MRSA. Among these, compounds 4b and 4c showed superior activity than two representatives' ciprofloxacin and chloramphenicol. This may be due to presence of fluoro group in molecular framework. On the basis of SAR studies of compounds 4a-o, it was presumed that the presence of electron donating groups increased the cytotoxic activity.

The newly targeted compounds (4a-o) presented here clearly vary in their corresponding antimicrobial activity depending on the type of substituents. The antimicrobial activity data of the synthesized compounds indicated that electron withdrawing groups such as fluoro and chloro at *ortho* and *para* position in targeted molecule increased the antibacterial, antifungal and MRSA activities as well as negative cytotoxic effect. SAR study also support the positive evidence of fluoro substituent in hybrid molecule, showing the prime attention of fluorine atom in synthesis. Outcome of cytotoxicity study favours the role of electron withdrawing functional groups, specifically -F, -Cl and -NO_2 in antimicrobial and effect of cytotoxicity enhancement.

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Conflicts of interest:

There are no conflicts of interest.

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REFERENCES

1. Böhm HJ, Banner D, Bendels S, Kansy M, Kuhn B, Müller K, *et al*. Fluorine in medicinal chemistry. ChemBioChem 2004;5:637-43.
2. Mueller K, Faeh C, Diederich F. Fluorine in pharmaceuticals: looking beyond intuition. Science 2007;317:1881-86.
3. Gillis EP, Eastman KJ, Hill MD, Donnelly DJ, Meanwell NA. Applications of Fluorine in Medicinal Chemistry. J Med Chem 2015;58:8315-59.
4. Desai NC, Rajpara KM, Joshi VV. Microwave induced synthesis of fluorobenzamides containing thiazole and thiazolidine as promising antimicrobial analogs. J Fluorine Chem 2013;145:102-11.
5. Hagmann WK. The many roles for fluorine in medicinal chemistry. J Med Chem 2008;51:4359-69.
6. Liu P, Sharon A, Chu CK. Fluorinated Nucleosides: Synthesis and Biological Implication. J Fluor Chem 2008;129:743-66.
7. Berkowitz DB, Karukurichi KR, de la Salud-Bea R, Nelson DL, McCune CD. Use of Fluorinated Functionality in Enzyme Inhibitor Development: Mechanistic and Analytical Advantages. J Fluor Chem 2008;129:731-42.
8. DeBonis S, Skoufias DA, Indorato RL, Liger F, Marquet B, Laggner C, *et al*. Structure activity relationship of S-trityl-L-cysteine analogues as inhibitors of the human mitotic kinesin Eg5. J Med Chem 2008;51:1115-25.
9. Matsuno K, Sawada J, Sugimoto M, Ogo N, Asai A. Bis (hetero) aryl derivatives as unique kinesin spindle protein inhibitors. Bioorg Med Chem Lett 2009;19:1058-61.
10. Rovnyak GC, Atwal KS, Hedberg A, Kimball SD, Moreland S, Gougoutas JZ, *et al*. Dihydropyrimidine calcium channel blockers. 4. Basic 3-substituted-4-aryl-1,4-dihydropyrimidine-5-carboxylic acid esters Potent antihypertensive agents. J Med Chem 1992;35:3254-63.
11. Grover GJ, Dzwonczyk S, McMullen DM, Normandin DE, Parham CS, Sleph PG, *et al*. Pharmacologic profile of the dihydropyrimidine calcium channel blockers SQ 32,547 and SQ 32,946. J Cardiovasc Pharm 1995;26:289-94.
12. Barrow JC, Nantermet PG, Selnick HG, Glass KL, Rittle KE, Gilbert KF, *et al*. *In vitro* and *in vivo* evaluation of dihydropyrimidinone C-5 amides as potent and selective alpha (1A) receptor antagonists for the treatment of benign prostatic hyperplasia. J Med Chem 2000;43:2703-18.
13. Orjales A, Mosquera R, López B, Olivera R, Labeaga L, Núñez MT. Novel 2-(4-methylsulfonylphenyl)pyrimidine derivatives as highly potent and specific COX-2 inhibitors. Bio Med Chem 2008;16:2183-99.
14. Patil AD, Kumar NV, Kokke WC, Bean MF, Freyer AJ, De Brosse C, *et al*. Novel alkaloids from sponge batzella sp.: inhibitors of HIV gp120-Human CD4 binding. J Org Chem 1995;60:1182-88.
15. Kappe CO. Biologically active dihydropyrimidones of the Biginelli-type-a literature survey. Eur J Med Chem 2000;35:1043-52.
16. Singh K, Singh K, Wan B, Franzblau S, Chibale K, Balzarini J. Facile transformation of Biginelli pyrimidin-2(1*H*)-ones to pyrimidines *In vitro* evaluation as inhibitors of *Mycobacterium tuberculosis* and modulators of cytostatic activity. Eur J Med Chem 2011;46:2290-94.
17. Desai NC, Kotadiya GM, Trivedi AR. Studies on molecular

properties prediction, antitubercular and antimicrobial activities of novel quinoline based pyrimidine motifs. Bioorg Med Chem Lett 2014;24:3126-30.

18. Jyostna ST, Achaiah G. Synthesis of new Pyrrolo[2,3-*d*] pyrimidine derivatives and evaluation of their activities against human colon cancer cell lines. Eur J Med Chem 2010;45:1453-58.
19. Kwon OW, Moon E, Chari MA, Kim TW, Kim AJ, Lee P, *et al*. A substituted 3,4-dihydro pyrimidinone derivative (compound D22) prevents inflammation mediated neurotoxicity; role in microglial activation in BV-2 cells. Bioorg Med Chem Lett 2012;22:5199-03.
20. Attri P, Bhatia R, Gaur J, Arora B, Gupta A, Kumar N, Choi EH. Triethylammonium acetate ionic liquid assisted one-pot synthesis of dihydropyrimidinones and evaluation of their antioxidant and antibacterial activities. Arab J Chem 2014;114:1-9.
21. Gangjee A, Zhao Y, Raghavan S, Ihnat MA, Disch BC. Design, synthesis and evaluation of 2-amino-4-m-bromoanilino-6-arylmethyl-7*H*-pyrrolo[2,3-*d*]pyrimidines as tyrosine kinase inhibitors and antiangiogenic agents. Bioorg Med Chem 2010;18:5261-73.
22. Heys L, Moore CG, Murphy PJ. The guanidine metabolites of Ptilocaulisspiculifer and related compounds; isolation and synthesis. Chem Soc Rev 2000;29:57-67.
23. Ningaiah S, Bhadraiah UK, Doddaramappa SD, Keshavamurthy S, Javarasetty C. Novel pyrazole integrated 1,3,4-oxadiazoles: synthesis, characterization and antimicrobial evaluation. Bioorg Med Chem Lett 2014;24:245-48.
24. Gadakh AV, Pandit C, Rindhe SS, Karale BK. Synthesis and antimicrobial activity of novel fluorine containing 4-(substituted-2-hydroxybenzoyl)-1*H*-pyrazoles and pyrazolyl benzo[*d*]oxazoles. Bioorg Med Chem Lett 2010;20:5572-76.
25. Bole SB, Nargund LV, Nargund R, Devaraju KS, Vedamurthy AB. Synthesis of substituted fluoro pyrazoles for antibacterial and anti-oxidant activity. Int J Pharm Res Dev 2011;3:161-66.
26. Zhang H, Zhu P, Liu J, Lin Y, Yao H, Jiang J, *et al*. Synthesis, *in vitro* and *in vivo* antitumor activity of pyrazole-fused 23-hydroxybetulinic acid derivatives. Bioorg Med Chem Lett 2015;25:728-32.
27. Zhao MY, Yin Y, Yu XW, Sangani CB, Wang SF, Lu AM, *et al*. Synthesis, biological evaluation and 3D-QSAR study of novel 4,5-dihydro-1*H*-pyrazole thiazole derivatives as BRAF($V^{600}E$) inhibitors. Bioorg Med Chem 2015;23:46-54.
28. Purohit MK, Chakka SK, Scovell I, Neschadim A, Bello AM, Salum N, *et al*. Structure-activity relationships of pyrazole derivatives as potential therapeutics for immune thrombocytopenias. Bioorg Med Chem 2014;22:2739-52.
29. Alegaon SG, Alagawadi KR, Dadwe DH. Synthesis and antitubercular activity of novel 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives. Drug Res (Stuttg) 2014;64:553-58.
30. Desai NC, Somani HC, Trivedi AR, Bhatt KA, Nawale L, Khedkar VM, Jha PC, *et al*. Synthesis, biological evaluation and molecular docking study of some novel indole and pyridine based 1,3,4-oxadiazole derivatives as potential antitubercular agents. Bioorg Med Chem Lett 2016;26:1776-83.
31. Desai NC, Bhatt N, Dodiya A, Karkar T, Patel B, Bhatt M. Synthesis, characterization and antimicrobial screening of thiazole based 1,3,4-oxadiazoles heterocycles. Res Chem Intermed 2016;42:3039-53.
32. Desai NC, Shihory N, Bhatt M, Patel B, Karkar T. Studies on antimicrobial evaluation of some 1-((1-(1*H*-benzo[*d*] imidazol-2-yl)ethylidene)amino)-6-((arylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles. Synth Commun 2015;45:2701-11.
33. Desai NC, Trivedi AR, Vaghani HV, Somani HC, Bhatt KA. Synthesis and biological evaluation of 1,3,4-oxadiazole bearing dihydropyrimidines as potential antitubercular agents. Med Chem Res 2016;25:329-38.
34. Falsone FS, Kappe CO. The Biginelli dihydropyrimidone synthesis using polyphosphate ester as a mild and efficient cyclocondensation/dehydration reagent. ARKIVOC 2001;2:122-34.
35. Rattan A. Antimicrobials in Laboratory Medicine. New Delhi: B. I. Churchill Livingstone Pvt. Ltd.; 2000. p. 85.
36. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
37. Kaur R, Chaudhary S, Kumar K, Gupta MK, Rawal RK. Recent synthetic and medicinal perspectives of dihydropyrimidinones: A review. Eur J Med Chem 2017;132:108-34.
38. Trivedi AR, Bhuva VR, Dholariya BH, Dodiya DK, Kataria VB, Shah VH. Novel dihydropyrimidines as a potential new class of antitubercular agents. Bioorg Med Chem Lett 2010;20:6100-02.