Chemistry & Biology Interface, 2019, 9, 1, 21-31

RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Catalyst free three-component one-pot synthesis of dihydropyrazolo pyrimidine scaffolds as potent antibacterial and antifungal agents

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Received 25 June 2018; Accepted 30 October 2018

Abstract: Twenty novel dihydropyrazolo pyrimidine scaffolds were synthesized using one-pot MCRs. The reaction was employed using ethyl acetoacetate, 5-amino pyrazol and aldehydes in catalyst free condition to give dihydropyrazolo pyrimidine via non-classical Biginelli reaction. The current method describes non toxic medium, mild reaction condition as well as easy reaction work-up. All newly synthesized scaffolds characterize by microanalytical techniques like ^{1}H &^{13}C NMR, FT-IR and mass spectrometry. All synthesized scaffolds were screening *in-vitro* antibacterial and antifungal test against two gram-positive bacteria, two gram-negative bacteria and two fungal stains.

Keywords: Multi-component reaction; Dihydropyrazolo pyrimidine; Antibacterial; Antifungal

1. Introduction

Nitrogen contains fused heterocycles are core structure of numerous naturally founded molecules and number of biologically active molecules [1-3]. A huge range of dihydropyrimidine molecules synthesized using Biginelli reaction due to synthetic flexibility and numerous biological activities [4]. Now a day more potent class of antibacterial drugs required because microbes are gaining resistance against most of the anti microbial agents. Our interest to developed multi-component one-pot synthesis for dihydropyrazolo[1,5-*a*] pyrimidines (DPPMs) using non classical Biginelli reaction replacing nucleophilic moiety [5]. Pyrazolo[1,5-*a*]pyrimidines (PPMs) and their related scaffolds have also showed variety of biological activities in recent times, tyrosine kinase inhibition [6], anticancer activities in HeLa and $HepG_2$ cell lines [7], HMG-CoA inhibitor [8], B-Cell lymphoma binder [9], antibacterial [10, 11], antifungal [12, 13], antiviral HCV inhibitor [14], antidiabetic [15], DPP-4 inhibitors [16], PDE4 inhibitors [17], COX-2-selective inhibitors [18] and many other biological active class of PPMs [19]. Market available drugs Zaleplon as hypotonic

[20], Divaplon as anxiolytic and anticonvulsant [21] have PPMs analogs.

Various diverse path have been reported for the synthesis of pyrazolo[1,5-*a*]pyridine derivatives [22]. Especially, these three-component one-pot reactions gate the dihydroderivatives of pyrazolo[1,5-*a*]pyridine scaffolds. The reported method a number of limitations, such as long reaction time, several step syntheses, lengthy work-up, use toxic organic solvent and particular reaction condition [23]. We report simple, catalyst free and short reaction time, mild and realistic conditions is of great interest. Because of wide range of pharmacological activity of DPPMs scaffolds, we planned to synthesized ethyl 7-(Substituted phenyl)-5-methyl-2-phenyl-4,7-dihydropyrazolo[1,5-*a*] pyrimidine-6-carboxylate and ethyl 7-(Substituted phenyl)-2-(4-chlorophenyl)-5-methyl-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-6-carboxylate (**4a-t**) by non classical Biginelli condensation reaction of 1,3 diketo ester (**1**), 5 amino phenyl pyrazole (**2a-b**), and substituted aromatic aledehydes (**3a-j**). Synthesized all novel scaffolds studied by *in-vitro* antibacterial and antifungal activities.

2. Material and Methods

All the chemicals were of reagent grade and used as received. All the reactions were monitored by TLC (Merck TLC Silica gel PF_{254}) analysis using hexanes and ethyl acetate solvent system. 3-phenyl-1*H*-pyrazol-5-amine and 3-(4-chlorophenyl)-1*H*-pyrazol-5-amine were achieved by previously reported method [24, 25]. The column chromatography purification was performed in 60-120 mesh silica gel by gradient elution using hexanes in ethyl acetate. Microanalysis was carried out on Perkin Elmer 2400 CHNS analyzer, the FT-IR spectra were recorded from 400 to 4000 cm^{-1} with SHIMADZU FT-IR system using KBr pellet method. NMR ^{1}H and ^{13}Csamples were recorded on Bruker F113V (600 MHz) and referenced internally with TMS and DMSO-d_6 as a solvent. Splitting pattern was reported as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectrum was recorded on MS Micromass.

2.1 General synthesis procedure of 4,7-dihydropyrazolo[1,5-*a*]pyrimidine-6-carboxylate derivatives (4a-t)

A mixture of the ethyl acetoacetate (**1**, 1.2 mmol), 3-phenyl-1*H*-pyrazol-5-amine or 3-(4-chlorophenyl)-1H-pyrazol-5-amine (**2a-b**, 1 mmol) and an appropriate substituted aromatic aldehydes (**3a-j**, 1 mol) in MeOH (5 mL). The resulting mixture was reflux for 3 to 5 hr. The reaction completion was monitor by TLC. After completion of the reaction the reaction mixture was allowed to cool at room temperature. The precipitate was filtered off and washed with MeOH twice. Further purified by column chromatography using ethyl acetate/ hexane as eluent.

ethyl 5-methyl-2,7-diphenyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4a) Yield: 62%; mp: 170-172 ^{o}C; IR(KBr): υ 3405, 2990, 2940, 2815, 2732, 1740, 1630, 1590, 1485, 1208, 1180, 1095, 690 cm^{-1}; ^{1}H NMR (600 MHz, d_6DMSO): δ ppm 1.12 (t, J = 7.2 Hz 3H), 2.42 (s, 3H), 4.27 (q, 2H), 6.08 (s, 1H), 6.20 (s, 1H), 7.30-7.35 (m, 6H), 7.45-4.47 (d, J = 6.9 Hz 2H), 7.75-7.77 (d, J = 8.4 Hz 2H), 10.35 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO): δ ppm 165.90, 149.84, 146.86, 140.25, 137.24, 133.62, 129.26, 128.32, 127.15, 126.75, 125.32, 96.23, 87.21, 60.12, 59.17, 18.94, 14.63; MS (EI, *m/z*): 359.76 (M+1). Anal. Calc. For C, 73.52; H, 5.89; N, 11.69; O, 8.90; Found C, 73.49; H, 5.90; N, 11.68; O, 8.93.

ethyl 7-(3-chlorophenyl)-5-methyl-2-phenyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4b) Yield: 71%; mp: 191-193 ^{o}C;

IR (KBr, cm^{-1}): 3398, 2992, 2818, 2724, 1735, 1588, 1483, 1203, 1185, 1090, 810, 705; ^1H NMR (600 MHz, d_6DMSO): δ ppm 1.20 (t, *J* = 7.1 Hz 3H), 2.24 (s, 3H), 4.24 (q, 2H), 5.94 (s, 1H), 6.35 (s, 1H), 7.10-7.11 (d, *J* = 8.6 Hz 1H), 7.30-7.32 (m, 2H), 7.45-7.49 (m, 4H), 7.80-7.82 (d, *J* = 8.0 Hz 2H), 10.30 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 165.98, 149.68, 145.68, 141.87, 136.21, 133.67, 132.49, 132.14, 129.32, 128.01, 127.62, 126.42, 125.78, 96.23, 87.62, 60.12, 59.48, 19.85, 14.23; MS (EI, *m/z*): 393.74 [M+], 395.37 [M+2], Anal. calc. For $C_{22}H_{20}ClN_3O_2$: C, 67.09; H, 5.12; Cl, 9.00; N, 10.67; O, 8.12. Found: C, 67.12; H, 5.10; Cl, 9.03; N, 10.65; O, 8.18.

ethyl 7-(4-chlorophenyl)-5-methyl-2-phenyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4c) Yield: 80%; mp: 157-159 °C; IR (KBr, cm^{-1}): 3407, 2982, 2937, 2812, 2726, 1738, 1631, 1593, 1490, 1208, 1175, 1091, 698;^1H NMR (600 MHz, d_6DMSO): δ ppm 1.11 (t, *J* = 7.2 Hz 3H), 2.42 (s, 3H), 4.27 (q, 2H), 6.10 (s, 1H), 6.25 (s, 1H), 7.22-7.24 (d, *J* = 6.6 Hz 2H), 7.33-7.36 (m, 5H), 7.69-7.70 (d, *J* = 8.4 Hz 2H), 10.29 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 165.92, 150.94, 146.81, 138.87, 133.54, 132.49, 131.57, 131.03, 129.13, 129.01, 128.77,125.58, 125.53, 95.80, 85.46, 59.67, 59.18, 19.36, 14.61MS (EI, *m/z*): 393.45 [M+], 395.89 [M+2], Anal. calc. For $C_{22}H_{20}ClN_3O_2$: C, 67.09; H, 5.12; Cl, 9.00; N, 10.67; O, 8.12 Found: C, 67.11; H, 5.12; Cl, 8.95; N, 10.68; O, 8.14.

ethyl 7-(3-bromophenyl)-5-methyl-2-phenyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4d) Yield: 69%; mp: 201-203 °C; IR (KBr, cm^{-1}): 3394, 2982, 2821, 2724, 1737, 1584, 1480, 1210, 1180, 1095, 805, 702; ; ^1H NMR (600 MHz, d_6DMSO): δ ppm 1.15 (t, *J* = 7.4 Hz 3H), 2.20 (s, 3H), 4.31 (q, 2H), 5.98 (s, 1H), 6.21 (s, 1H), 7.20-7.22 (m, 2H), 7.48-7.52 (m, 5H), 7.84-7.86 (d, *J* = 8.0 Hz 2H), 10.21 (s, 1H);^{13}C NMR (150 MHz, d_6DMSO) δ166.45, 150.12, 147.23, 144.1, 135.48, 133.70, 132.47, 129.90, 129.31, 128.20, 128.02, 127.64, 124.23, 97.12, 88.12, 60.47, 59.68, 18.98, 14.23; MS (EI, *m/z*): 437.45 [M+], 439.12 [M+2], Anal. calc. For $C_{22}H_{20}BrN_3O_2$: C, 60.28; H, 4.60; Br, 18.23; N, 9.59; O, 7.30. Found: C, 60.33; H, 4.54; Br, 18.19; N, 9.60; O, 7.34.

ethyl 5-methyl-2-phenyl-7-(p-tolyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4e) Yield: 65%; mp: 183-185 °C; IR (KBr, cm^{-1}): 3410, 2976, 2875, 1734, 1581, 1475, 1212, 1170, 1090, 887, 810; ^1H NMR (600 MHz, d_6DMSO): δ ppm 1.18 (t, *J* = 7.2 Hz 3H), 2.30 (s, 3H), 2.40 (s, 3H), 4.14 (q, 2H), 6.08 (s, 1H), 6.21 (s, 1H), 6.98-7.00 (d, *J* = 8.2 Hz 2H), 7.15-7.17 (d, *J* = 8.2 Hz 2H), 7.47-7.50 (m, 3H), 7.69-7.70 (d, *J* = 8.0 Hz 2H), 10.32 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 166.45, 148.92, 146.78, 139.13, 135.13, 133.90, 132.51, 131.78, 129.87, 128.35, 128.23, 125.75, 96.40, 86.69, 6.12, 59.43, 21.56, 19.45, 14.61; MS (EI, *m/z*): 373.26 [M+], Anal. calc. For $C_{23}H_{23}N_3O_2$: C, 73.97; H, 6.21; N, 11.25; O, 8.57. Found: C, 74.00; H, 6.22; N, 11.26; O, 8.52.

ethyl 5-methyl-7-(4-nitrophenyl)-2-phenyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4f) Yield: 78%; mp: 195-197°C; IR (KBr, cm^{-1}): 3410, 3328, 2976, 2856, 1740, 1583, 1525, 1476, 1360, 1203, 1175, 1085, 890, 775; ^1H NMR (600 MHz, d_6DMSO): δ ppm 1.10 (t, *J* = 7.2 Hz 3H), 2.28 (s, 3H), 4.20 (q, 2H), 6.12 (s, 1H), 6.29 (s, 1H), 7.51-7.58 (m, 5H), 7.75-7.77 (d, *J* = 8.2 Hz 2H), 8.05-8.07 (d, *J* = 8.6 Hz 2H), 10.21 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 166.58, 151.92, 148.52, 147.12, 146.12, 134.42, 132.82, 130.52, 129.02, 128.90, 127.10, 125.87, 96.12, 88.75, 59.89, 58.63, 20.06, 15.23; MS (EI, *m/z*): 403.87 [M+], Anal. calc. For $C_{22}H_{20}N_4O_4$: C, 65.34; H, 4.98; N, 13.85; O, 15.82 Found: C, 65.27; H, 5.04; N, 13.81; O, 15.78.

ethyl 7-(4-hydroxy-3,5-dimethoxyphenyl)-5-

methyl-2-phenyl-4,7-dihydropyrazolo[1,5-a] pyrimidine-6-carboxylate (4g) Yield: 60%; mp: 164-166 °C; IR (KBr, cm^{-1}): 3580, 3408, 3334, 2945, 2843, 2768, 1736, 1583, 1480, 1212, 1195, 1098, 880, 720; ^{1}H NMR (600 MHz, d_6DMSO): δ ppm 1.12 (t, *J* = 7.2 Hz 3H), 2.21 (s, 3H), 3.88 (s, 6H), 4.22 (q, 2H), 5.14 (s, 1H), 5.89 (s, 1H), 6.20 (s, 1H), 6.58 (s, 2H), 7.38-7.40 (m, 1H), 7.54-7.56 (m, 2H), 7.90-7.92 (d, *J* = 8.6 Hz, 2H), 10.26 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 165.78, 152.23, 150.18, 147.19, 134.91, 133.90, 133.61, 132.11, 130.12, 128.77, 128.01, 125.58, 108.72, 96.76, 85.92, 61.13, 59.18, 57.34, 20.45, 15.23; MS (EI, *m/z*): 436.10 [M+], Anal. calc. For $C_{24}H_{25}N_3O_5$: C, 66.19; H, 5.79; N, 9.65; O, 18.37; Found: C, 66.10; H, 5.82; N, 9.70; O, 18.38.

ethyl 7-(3-ethoxy-4-methoxyphenyl)-5-methyl-2-phenyl-4,7-dihydropyrazolo[1,5-a] pyrimidine-6-carboxylate (4h) Yield: 63%; mp: 174-176 °C; IR (KBr, cm^{-1}): 3402, 3325, 2952, 2868, 2780, 1739, 1580, 1482, 1207, 1175, 1082, 874, 690; ^{1}H NMR (d_6DMSO, 600 MHz) δ ppm 1.18 (t, *J* = 7.2 Hz 3H), 2.21 (s, 3H), 3.81 (s, 3H), 4.08 (q, 2H), 4.25 (q, 2H), 5.92 (s, 1H), 6.26 (s, 1H), 6.68-6.69 (d, *J* = 4.2 Hz 1H), 6.75-6.77 (d, *J* = 8.2 Hz 1H), 6.84 (d, *J* = 2.2 Hz 1H), 7.41-7.46 (m, 3H), 7.90-7.92 (d, *J* = 8.2 Hz, 2H) 10.20 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 166.79, 157.61, 151.23, 150.75, 147.35, 138.90, 134.67, 132.58, 129.60, 128.70, 125.81, 122.60, 119.94, 114.62, 96.73, 87.68, 63.19, 60.62, 58.78, 20.22, 15.17, 14.70; MS (EI, *m/z*): 433.44 [M+], Anal. calc. For $C_{25}H_{27}N_3O_4$: C, 69.27; H, 6.28; N, 9.69; O, 14.76; Found: C, 69.30; H, 6.26; N, 9.64; O, 14.80.

ethyl 7-(2-chloro-4-hydroxy-5-methoxyphenyl)-5-methyl-2-phenyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4i) Yield: 65%; mp: 168-170 °C; IR (KBr, cm^{-1}): 3562, 3410, 3328, 2962, 2838, 2776, 1735, 1580, 1475, 1210, 1187, 1096, 888, 740; ^{1}H NMR (600 MHz, d_6DMSO): δ ppm 1.14 (t, *J* = 7.2 Hz 3H), 2.26 (s, 3H), 3.94 (s, 3H), 4.18 (q, 2H), 5.74 (s, 1H), 5.98 (s, 1H), 6.36 (s, 1H), 6.89 (s, 1H), 7.08 (s, 1H), 7.46-7.49 (m, 3H), 7.96-7.98 (d, *J* = 8.2 Hz, 2H), 10.31 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 167.23, 154.25 150.39, 149.78, 147.68, 134.99, 133.71, 132.60, 129.81, 128.59, 125.86, 125.08, 124.19, 118.29, 96.03, 86.25, 60.62, 59.59, 57.29, 20.12, 14.16; MS (EI, *m/z*): 440.16 [M+], 442.43 [M+2], Anal. calc. For $C_{23}H_{22}ClN_3O_4$: C, 62.80; H, 5.04; Cl, 8.06; N, 9.55; O, 14.55; Found: C, 62.85; H, 5.04; Cl, 7.99; N, 9.57; O, 14.60.

ethyl 7-(4-chloro-3-hydroxyphenyl)-5-methyl-2-phenyl-4,7-dihydropyrazolo[1,5-a] pyrimidine-6-carboxylate (4j) Yield: 67%; mp: 189-191 °C; IR (KBr, cm^{-1}): 3550, 3414, 3334, 2958, 2842,1733, 1565, 1445, 1202, 1170, 1084, 892, 795; ^{1}H NMR (d_6DMSO, 600 MHz) δ ppm 1.10 (t, *J* = 7.2 Hz 3H), 2.24 (s, 3H), 4.17 (q, 2H), 5.84 (s, 1H), 6.12 (s, 1H), 6.72-6.73 (d, *J* = 3.2 Hz 1H), 6.78-6.80 (d, *J* = 8.0 Hz 1H), 7.10 (d, *J* = 2.4 Hz 1H), 7.46-7.51 (m, 3H), 7.94-7.96 (d, *J* = 8.2 Hz, 2H) 9.68 (s, 1H), 10.22 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ166.80, 154.34, 147.12, 141.86, 135.66, 134.12, 132.16, 129.88, 127.20, 126.19, 121.16, 120.42, 119.95, 96.34, 87.41, 60.47, 59.39, 20.08, 14.16; MS (EI, *m/z*): 363.34 [M+], 365.23 [M+2], Anal. calc. For $C_{22}H_{20}ClN_3O_3$: C C, 64.47; H, 4.92; Cl, 8.65; N, 10.25; O, 11.71; Found: C, 64.52; H, 4.95; Cl, 8.67; N, 10.30; O, 11.66.

ethyl 2-(4-chlorophenyl)-5-methyl-7-phenyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4k) Yield: 64%; mp: 179-181 °C; IR (KBr, cm^{-1}): 3412, 3005, 2945, 2875, 1729, 1578, 1463, 1209, 1178, 1078, 883, 701; ^{1}H NMR (600 MHz, d_6DMSO): δ ppm 1.21 (t, *J* = 7.2 Hz 3H), 2.20 (s, 3H), 4.13 (q, 2H), 5.87 (s, 1H), 6.24 (s, 1H), 7.26-7.32 (m, 5H), 7.52-4.54 (d, *J* = 7.8 Hz 2H), 7.90-7.92 (d, *J* = 8.2 Hz 2H), 10.40 (s, 1H);^{13}C NMR (150 MHz, d_6DMSO) δ 167.23, 150.21, 147.23, 136.54, 134.63,

133.98, 132.24, 130.32, 129.54, 125.55, 97.21, 87.35, 60.31, 59.12, 19.81, 14.23; MS (EI, *m/z*): 393.78 [M+], 395.32 [M+2], Anal. calc. For $C_{22}H_{20}ClN_3O_2$: C, 67.09; H, 5.12; Cl, 9.00; N, 10.67; O, 8.12 Found: C, 66.99; H, 5.17; Cl, 9.05; N, 10.73; O, 8.18.

ethyl 7-(3-chlorophenyl)-2-(4-chlorophenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a] pyrimidine-6-carboxylate (4l) Yield: 79%; mp: 192-195 °C; IR (KBr, cm^{-1}): 3412, 3335, 2994, 2858, 1738, 1588, 1468, 1210, 1180, 1071, 892, 810, 790; ^{1}H NMR (600 MHz, d_6DMSO) δ ppm 1.19 (t, *J* = 7.2 Hz 3H), 2.24 (s, 3H), 4.18 (q, 2H), 5.94 (s, 1H), 6.21 (s, 1H), 7.08-7.10 (m, 1H), 7.21-7.24 (m, 2H), 7.43-7.43 (d, *J* = 3.2 Hz 2H), 7.57-4.59 (d, *J* = 8.4 Hz 2H),8.02-8.04 (d, *J* = 8.2 Hz 2H), 10.35 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 166.76, 151.23, 147.58, 141.23, 135.34, 133.78, 132.12, 130.27, 129.35, 126.59, 124.78, 97.29, 86.78, 60.09, 58.92, 20.04, 14.32; MS (EI, *m/z*): 426.91 [M+], 428.76 [M+2], Anal. calc. For $C_{22}H_{19}Cl_2N_3O_2$: C, 61.69; H, 4.47; Cl, 16.55; N, 9.81; O, 7.47; Found: C, 61.75; H, 4.45; Cl, 16.54; N, 9.83; O, 7.50.

ethyl 2,7-bis(4-chlorophenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4m) Yield: 82%; mp: 178-180 °C; IR (KBr, cm^{-1}): 3408, 3330, 2995, 2874, 1735, 1575, 1474, 1215, 1168, 1086, 895, 793; ^{1}H NMR (600 MHz, d_6DMSO) δ ppm 1.20 (t, *J* = 7.2 Hz 3H), 2.20 (s, 3H), 4.24 (q, 2H), 5.78 (s, 1H), 6.24 (s, 1H) 7.09-7.11 (d, *J* = 8.8 Hz, 2H), 7.28-7.30 (d, *J* = 8.2 Hz, 2H), 7.48-7.50 (d, *J* = 8.2 Hz, 2H), 7.92-7.94 (d, *J* = 7.8 Hz, 2H) 10.38 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 168.06, 150.87, 147.23,136.34, 135.71, 134.45, 132.13, 131.06, 129.34, 128.45, 127.86, 97.26, 87.36, 60.04, 59.34, 19.86, 14.19; MS (EI, *m/z*): 427.42 [M+], 429.02 [M+2], Anal. calc. For $C_{22}H_{19}Cl_2N_3O_2$: C, 61.69; H, 4.47; Cl, 16.55; N, 9.81; O, 7.47. Found: C, 61.72; H, 4.41; Cl, 16.62; N, 9.82; O, 7.52.

ethyl 7-(3-bromophenyl)-2-(4-chlorophenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a] pyrimidine-6-carboxylate (4n) Yield: 78%; mp: 212-214 °C; IR (KBr, cm^{-1}): 3432, 3331, 2990, 2878, 1740, 1578, 1469, 1230, 1195, 1096, 821, 739; ^{1}H NMR (600 MHz, d_6DMSO) δ ppm 1.20 (t, *J* = 7.2 Hz 3H), 2.28 (s, 3H), 4.23 (q, 2H), 5.68 (s, 1H), 6.13 (s, 1H), 7.12-7.15 (m, 2H), 7.40-7.43 (m, 2H), 7.57-7.59 (d, *J* = 8.2 Hz 2H), 7.94-7.96 (d, *J* = 8.2 Hz 2H), 10.31 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 167.17, 150.39, 146.51, 141.12, 133.34, 132.36, 130.12, 129.34, 128.67, 128.10, 124.8, 97.13, 86.54, 59.39, 58.56, 19.78, 14.12; MS (EI, *m/z*): 471.84 [M+], 473.62 [M+2], Anal. calc. For $C_{22}H_{19}BrClN_3O_2$: C, 55.89; H, 4.05; Br, 16.90; Cl, 7.50; N, 8.89; O, 6.77; Found: C, 55.93; H, 4.08; Br, 16.95; Cl, 7.53; N, 8.93; O, 6.73.

ethyl 2-(4-chlorophenyl)-5-methyl-7-(p-tolyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4o) Yield: 66%; mp: 198-200 °C; IR (KBr, cm^{-1}): 3410, 3321, 3023, 2986, 2862, 1738, 1565, 1450, 1222, 1196, 1070, 987, 758; ; ^{1}H NMR (600 MHz, d_6DMSO) δ ppm 1.12 (t, *J* = 7.2 Hz 3H), 2.19 (s, 3H), 4.28 (q, 2H), 5.68 (s, 1H), 6.20 (s, 1H) 7.10-7.12 (d, *J* = 6.8 Hz, 2H), 7.20-7.22 (d, *J* = 7.2 Hz, 2H), 7.58-7.60 (d, *J* = 7.4 Hz, 2H), 7.94-7.96 (d, *J* = 7.0 Hz, 2H) 10.31 (s, 1H);^{13}C NMR (150 MHz, d_6DMSO) δ 167.34, 150.87, 147.45, 136. 41, 136.07 134.15, 132.87, 129.27, 128.78, 128.27, 126.45, 97.23, 88.21, 59.82, 58.47, 22.34, 20.61, 15.30; MS (EI, *m/z*): 407.16 [M+], 409.23 [M+2], Anal. calc. For $C_{23}H_{22}ClN_3O_2$: C, 67.73; H, 5.44; Cl, 8.69; N, 10.30; O, 7.84; Found: C, 67.82; H, 5.40; Cl, 8.65; N, 10.34; O, 7.79.

ethyl 2-(4-chlorophenyl)-5-methyl-7-(4-nitrophenyl)-4,7-dihydropyrazolo[1,5-a] pyrimidine-6-carboxylate (4p) Yield: 81%; mp: 205-207 °C; IR (KBr, cm^{-1}): 3408, 3319, 3011, 2987, 2871, 1739, 1581, 1525, 1470, 1362, 1224, 1196, 1080, 890, 785; ; ^{1}H NMR (600 MHz, d_6DMSO): δ ppm 1.21 (t, *J* = 7.2 Hz 3H),

2.22 (s, 3H), 4.17 (q, 2H), 5.80 (s, 1H), 6.31 (s, 1H), 7.50-7.52 (d, *J* = 8.0 Hz, 2H), 7.62-7.64 (d, *J* = 8.2 Hz, 2H), 8.01-8.03 (d, *J* = 6.7 Hz, 2H), 8.20-8.22 (d, *J* = 8.4 Hz, 2H), 10.36 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 167.15, 150.67, 148.12, 147.19, 146.12, 136.42, 134.42, 132.82, 130.12, 129.25, 128.36, 124.64, 97.52, 87.35, 60.19, 59.61, 20.02, 14.33; MS (EI, *m/z*): 438.23[M+], 440.13[M+2], Anal. calc. For $C_{22}H_{19}ClN_4O_4$: C, 60.21; H, 4.36; Cl, 8.08; N, 12.77; O, 14.58; Found: C, 60.16; H, 4.32; Cl, 8.12; N, 12.80; O, 14.60.

ethyl 2-(4-chlorophenyl)-7-(4-hydroxy-3,5-dimethoxyphenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4q) Yield: 62%; mp: 199-201 °C; IR (KBr, cm^{-1}): 3561, 3407, 3326, 2990, 2878, 2764, 1735, 1578, 1487, 1227, 1208, 1190, 1068, 898, 751;^{1}H NMR (600 MHz, d_6DMSO) δ ppm 1.11 (t, *J* = 7.2 Hz 3H), 3.89 (s, 6H), 4.21 (q, 2H), 5.50 (s, 1H), 5.78 (s, 1H), 6.26 (s, 1H), 6.82 (s, 2H), 7.56-7.58 (d, *J* = 7.8 Hz, 2H), 8.05-8.07 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (150 MHz, d_6DMSO) δ 166.18, 153.21, 150.68, 147.28, 134.91, 134.28, 133.90, 133.61, 132.11, 129.17, 128.01, 109.34, 97.96, 87.42, 60.18, 59.48, 56.24, 20.12, 14.73; MS (EI, *m/z*): 470.19 [M+], 472.24 [M+2], Anal. calc. For $C_{24}H_{24}ClN_3O_5$: C, 61.34; H, 5.15; Cl, 7.54; N, 8.94; O, 17.02; Found: C, 61.30; H, 5.13; Cl, 7.58; N, 9.02; O, 17.08.

ethyl 2-(4-chlorophenyl)-7-(3-ethoxy-4-methoxyphenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4r) Yield: 65%; mp: 175-177 °C; IR (KBr, cm^{-1}): 3407, 3332, 2984, 2873, 2787, 1734, 1590, 1475, 1223, 1212, 1188, 1079, 892, 756; ; ^{1}H NMR (600 MHz, d_6DMSO) δ ppm 1.21 (t, *J* = 7.2 Hz 3H), 2.21 (s, 3H), 3.89 (s, 3H), 4.21 (q, 2H), 4.28 (q, 2H), 5.80 (s, 1H), 6.28 (s, 1H), 6.62-6.64 (d, *J* = 4.0 Hz 1H), 6.70-6.72 (d, *J* = 8.2 Hz 1H), 6.90 (d, *J* = 2.2 Hz 1H), 7.60-7.62 (d, *J* = 8.0 Hz, 2H), 7.99-8.00 (d, *J* = 8.8 Hz, 2H), 10.28 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 167.23, 153.61, 151.20, 149.73, 147.35, 137.90, 133.67, 132.58, 129.60, 128.70, 125.81, 120.14, 115.18, 97.53, 87.66, 62.21, 59.42, 58.18, 20.12, 16.11, 14.45; MS (EI, *m/z*): 467.78 [M+], 469.70 [M+2], Anal. calc. For $C_{25}H_{26}ClN_3O_4$: C, 64.17; H, 5.60; Cl, 7.58; N, 8.98; O, 13.68; Found: C, 64.22; H, 5.58; Cl, 7.62; N, 9.03; O, 13.70.

ethyl 7-(2-chloro-4-hydroxy-5-methoxyphenyl)-2-(4-chlorophenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4s) Yield: 71%; mp: 186-188 °C; IR (KBr, cm^{-1}): 3552, 3412, 3323, 3015, 2980, 2871, 2768, 1734, 1580, 1470, 1218, 1186, 1074, 912, 862,750; ; ^{1}H NMR (600 MHz, d_6DMSO): δ ppm 1.13 (t, *J* = 7.2 Hz 3H), 2.21 (s, 3H), 3.82 (s, 3H), 4.28 (q, 2H), 5.49 (s, 1H), 5.88 (s, 1H), 6.26 (s, 1H), 6.81 (s, 1H), 7.12 (s, 1H), 7.54-7.56 (d, *J* = 7.8 Hz, 2H), 8.02-8.04 (d, *J* = 8.4 Hz, 2H), 10.36 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 166.83, 153.21 150.25, 149.14, 146.56, 134.73,134.78, 133.13, 132.61, 129.81, 128.59, 127.86, 122.29, 118.23, 97.35, 87.21, 60.21, 59.39, 56.78, 19.19, 14.11;MS (EI, *m/z*): 473.26 [M+], 475.31 [M+2], Anal. calc. For $C_{23}H_{21}Cl_2N_3O_4$: C, 58.24; H, 4.46; Cl, 14.95; N, 8.86; O, 13.49; Found: C, 58.17; H, 4.50; Cl, 14.97; N, 8.90; O, 13.46.

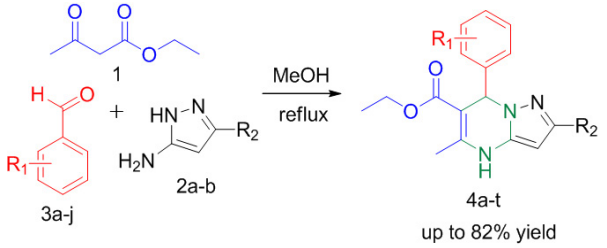
ethyl 7-(4-chloro-3-hydroxyphenyl)-2-(4-chlorophenyl)-5-methyl-4,7-dihydropyrazolo[1,5-a]pyrimidine-6-carboxylate (4t) Yield: 68%; mp: 190-192°C; IR (KBr, cm^{-1}): 3521, 3402, 3328, 2978, 2879, 1738, 1589, 1478, 1236, 1198, 1086, 908, 786; ^{1}H NMR (600 MHz, d_6DMSO) δ ppm 1.14 (t, *J* = 7.2 Hz 3H), 2.19 (s, 3H), 4.25 (q, 2H), 5.78 (s, 1H), 6.27 (s, 1H), 6.77-6.78 (d, *J* = 3.2 Hz 1H), 6.96-6.98 (d, *J* = 8.0 Hz 1H), 7.21 (d, *J* = 2.4 Hz 1H), 7.58-7.60 (d, *J* = 8.0 Hz, 2H), 7.90-7.92 (d, *J* = 8.2 Hz, 2H), 9.45 (s, 1H), 10.31 (s, 1H); ^{13}C NMR (150 MHz, d_6DMSO) δ 167.10, 153.34, 150.13, 146.18, 135.61, 134.78, 132.14,

130.56, 129.88, 128.25, 128.09, 120.34, 119.40, 97.31, 87.25, 60.61, 59.75, 19.78, 14.68;MS (EI, *m/z*): 443.15 [M+], 445.21 [M+2], Anal. calc. For $C_{22}H_{19}Cl_2N_3O_3$: C, 59.47; H, 4.31; Cl, 15.96; N, 9.46; O, 10.80; Found: C, 59.55; H, 4.27; Cl, 16.01; N, 9.48; O, 10.78.

3. Result and discussion

3.1 Chemistry

To set the protocol we have started the reaction using ethyl acetoacetate **(1)** with 3-phenyl-1*H*-pyrazol-5-amine **(2a)** and benzaldehyde **(3a)** in MeOH to our pleasant surprise we obtain positive results (scheme 1). Twenty examples of DPPMs **(4a-t)** through one-pot, three component reaction in high efficiency and fairly good yield, reaction time, melting points and yields 60-82% are shown in Table 1. In our study, Para –Cl substituted derivatives and electron withdrawing groups like $–NO_2$ gives a maximum yields and remain electron donating group's gives moderate yields.



Scheme 1. Catalyst free non classical Biginelli type reaction.

Table 1. Physicochemical Parameters of the Synthesized Scaffolds (4a-t)

Sr. No.	Sample code	-R_1	-R_2	Time (hr)	M.P. (°C)	Yield (%)
1	4a	H	-C_6H_5	5	170-172	62
2	4b	3-Cl	-C_6H_5	4	191-193	71
3	4c	4-Cl	-C_6H_5	3	157-159	80
4	4d	3-Br	-C_6H_5	4	201-203	69
5	4e	4-CH_3	-C_6H_5	5	183-185	65
6	4f	4-NO_2	-C_6H_5	4	195-197	78
7	4g	3,5-OCH_3,4-OH	-C_6H_5	5	164-166	60
8	4h	3-OC_2H_5,4-OCH_3	-C_6H_5	5	174-176	63
9	4i	2-Cl,4-OH,5-OCH_3	-C_6H_5	4	168-170	65
10	4j	3-OH 4-Cl	-C_6H_5	4	189-191	67
11	4k	H	-4-Cl-C_6H_4	4	179-181	64
12	4l	3-Cl	-4-Cl-C_6H_4	3	192-193	79
13	4m	4-Cl	-4-Cl-C_6H_4	3	178-180	82
14	4n	3-Br	-4-Cl-C_6H_4	3	212-215	78
15	4o	4-CH_3	-4-Cl-C_6H_4	4	198-200	66
16	4p	4-NO_2	-4-Cl-C_6H_4	3	205-207	81
17	4q	3,5-OCH_3,4-OH	-4-Cl-C_6H_4	5	194-196	62
18	4r	3-OC_2H_5,4-OCH_3	-4-Cl-C_6H_4	4	175-177	65
19	4s	2-Cl,4-OH,5-OCH_3	-4-Cl-C_6H_4	4	186-188	71
20	4t	3-OH 4-Cl	-4-Cl-C_6H_4	4	190-192	68

The plausible reaction mechanism for formation of DPPMs shown in Fig 1. Here, initially reaction carried via keto-enol tautomerization of 1, 3 diketo ester **1** [26] with substituted aromatic aldehyde through knowengel condensation to gives α, β unsaturated molecules, than *N, N* dinucleophilic addition of 5-amino pyrazole gives intermediate, they immediate remove water molecules, transfer proton –NH to carbanion to give desire DPPMs scaffolds. Different derivatives of DPPMs are synthesized using different base catalyst and reaction condition to gives regioisomers of PPMs [27]. Though, we found the simple MCRs reaction was carried out under reflux condition. Major product is non classical Biginelli-type DPPMs was reputable that the most advantageous synthetic rout.

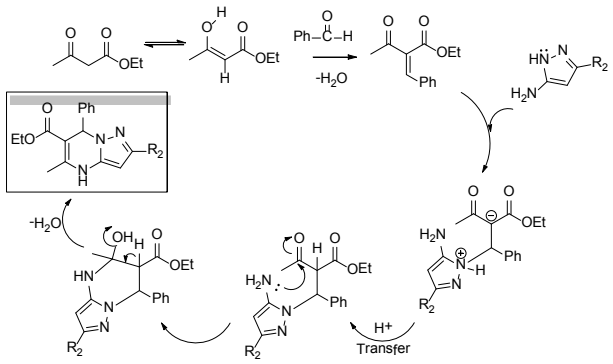


Fig 1. Proposed reaction mechanism

The newly synthesized scaffolds were confirmed on the basis of their spectral data; for example, the ^{1}H-nuclear magnetic resonance (NMR) spectrum of **4m** exhibited characteristic peak triplet at 1.12 ppm and quartet at 4.26 ppm for ester group. Cyclic Sp^3 hybridized carbon of hydro pyrimidine shows singlet at 6.11 ppm. Pyrimidine –NH siglet shows at 10.29 ppm. The ^{13}C NMR spectrum, ester C=O shows at 165.9 ppm and pyrazole ring fused with pyrimidine Sp^2 hybridized carbon shows 138.8 ppm. Cyclic –CH for hydro pyrimidine shows at 59.6 ppm. FT-IR spectrum shows singlet at 3407 cm^{-1} for cyclic –NH and ester C=O shows at 1738 cm^{-1}. Mass spectrum of compound **4n** molecular ion peak m/z shows 473.56 (M+2). All the synthesized scaffolds were conforming by their characteristic value.

3.2 Biological activities

All newly synthesized 20 scaffolds were filtered out with the Lipinski filter [28]. There was no single violation in 17 scaffolds from 20 scaffolds, reaming 3 there is MLogP > 4.15. All the theoretical calculation calculated by Swiss ADME [29]. All the synthesized scaffolds were screened for their *in-vitro* antibacterial and antifungal activity against following strains: two Gram positive bacteria, *Staphylococcus aureus* (MTCC 96) and *Enterococcus faecalis* (MTCC 439); two Gram negative bacteria, *Escherichia coli* (MTCC 443) and *Salmonella typhi* (MTCC 98) and two fungi, *Aspergillus niger* (MTCC 282) and *Candida albicans* (MTCC 227). *In-vitro* antibacterial and antifungal activity of synthesized scaffolds using agar well diffusion methods, performed using nutrient agar medium, as per the described by reported methods [30-34].

After making agar media to make well using 5mm sterilize cork borer. Bacterial and fungal test were carried out using 10 mL of tested scaffolds solutions making 1 mg of the synthesized scaffolds in 1 mL of dimethyl sulfoxide (DMSO). Clarithromycin, Cefixime and Ketoconazole were used as standard references for bacteria and fungus respectively. The results were recorded for each tested scaffolds as the average diameter of zone of inhibition. The zone of inhibition attributes to 1 mg/mL as a primary testing. The % inhibition calculated using equation-1.

Newly synthesized 20 scaffolds of DPPMs *in-vitro* antibacterial and antifungal evaluation results are shown in Table 1.

Table 2. Zone of inhibition (mm) antibacterial and antifungal activity of synthesized scaffolds

Sample Code	Bacterial strains				Fungal strains	
	Gram +Ve bacteria		Gram -Ve bacteria			
	S. aureus	*E. faecalis*	*E. coli*	*S. typhi*	*A. niger*	*C. albicans*
4a	17.6	14.7	15.2	16.5	13.4	18.3
4b	10.1	13.1	19.4	18.2	22.4	25.2
4c	19.4	20.7	12.1	14.7	24.6	27.7
4d	11.6	12.3	16.2	15.1	15.1	13.5
4e	16.2	17.2	18.7	17.6	21.3	19.2
4f	16.7	10.3	20.6	11.7	16.7	16.1
4g	23.6	21.2	24.5	25.2	13.6	15.6
4h	16.2	14.7	12.9	13.6	18.2	16.7
4i	21.8	22.8	21.4	23.2	12.4	10.3
4j	24.1	23.4	21.3	22.8	14.5	13.6
4k	14.5	13.2	14.5	12.2	17.2	14.4
4l	18.7	12.1	16.7	16.7	28.3	22.2
4m	9.7	10.3	15.1	10.6	23.6	29.3
4n	11.6	13.6	12.2	15.4	26.8	20.8
4o	15.2	17.7	11.2	13.2	21.2	19.4
4p	22.4	24.4	23.3	20.7	18.4	19.3
4q	21.4	20.7	24.6	23.3	17.3	15.5
4r	19.7	17.4	9.1	11.3	13.2	12.2
4s	19.3	16.5	13.4	14.2	18.4	13.4
4t	21.1	20.7	19.8	21.6	19.2	17.8
Clarithromycin	26.8	25.3	27.4	26.4	-	-
Cefixime	25.4	27.1	28.5	30.1	-	-
Ketoconazole	-	-	-	-	30.5	34.2

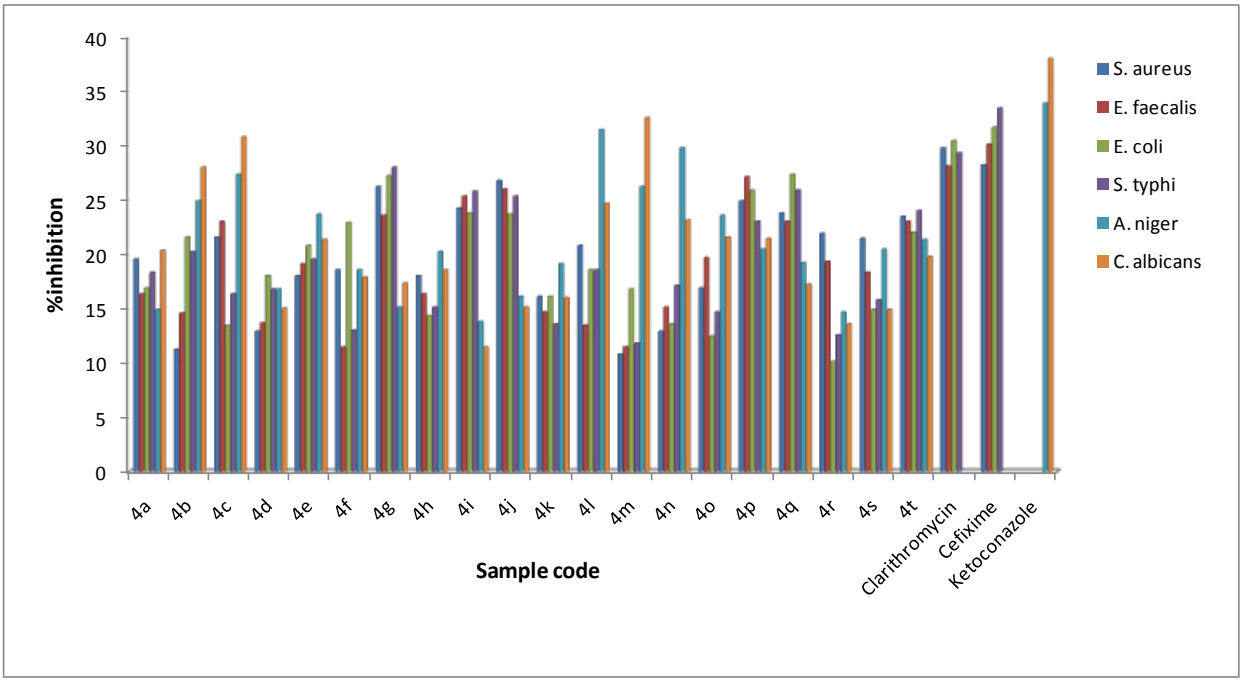


Fig 2. The % inhibitions of antibacterial and antifungal activity were comparison with standard drugs against different bacterial and fungal strain.

$$\%\text{Inhibition} = \frac{I}{P}(100) \ldots\ldots\ldots (1)$$

Where, I= Diameter zone of inhibition (mm)
P= Diameter of petridis (90 mm)

The results for **4g**, **4i**, **4j**, **4p** and **4q** are exhibited the excellent antibacterial activity against most of the bacterial organisms, these scaffolds exhibited activities near to the references drugs (Clarithromycin and Cefixime). Scaffold **4t** shows good activity against gram +Ve bacteria and moderate active against gram –Ve bacteria. A scaffold **4c** is moderate active against gram +Ve bacteria and poor active against gram –Ve bacteria. In general, most of the studied scaffolds revealed better activity against both bacterial strains. All the active scaffolds are lipophilic in nature and MlogP vale is < 4.15.

On the other hand results of fungal stains are totally opposite of antibacterial results. Scaffolds **4b**, **4c, 4l** and **4m** shows excellent antifungal activities against both fungal stains, these scaffolds %inhibition are close to standard drugs Ketoconazole shows fig 2. Scaffolds **4e** and **4o** shows good antifungal activity against *A. niger* and poor active against *C. albicans*. The % inhibition of all screened scaffolds is comparing with all bacterial and fungal stains are shown in fig 2. Remaining scaffolds shows moderate to poor active against both bacterial and fungal stains.

4. Conclusion

In summary, a facile synthetic protocol of the synthesis of 4,7-dihydropyrazolo[1,5-*a*] pyrimidine derivatives by the reaction of 1,3 diketo, aldehyde and 5-aminopyrazole in methanol at reflux condition. This method provides simple reaction without any catalyst, less reaction time and simple reaction work-up. The result of this work exhibited excellent antimicrobial and antifungal activity. Higly lipophilic nature scaffolds shows good antibacterial activity and halogenated scaffolds shows good antifungal activity.

Acknowledgement

We thankful to Department of Microbiology, Grow more Institute of Science for providing laboratory facilities and biological analysis. Heartily thanks A. Ansari for spectroscopic analysis.

References

1. L. D. Ouin, J. A. Tyrell, Fundamentals of heterocyclic chemistry, importance in nature and in the synthesis of pharmaceuticals John Wiley & Sons, Inc., Hoboken, New Jersey, 2010.
2. Y. Xu, B. G. Brenning, S. G. Kultgen, J. M. Foulks, A. S. Clifford, Lai, A. Chan, S. Merx, M. V. McCullar, S. B. Kanner K. K. Ho, ACS Med. Chem. Lett., 6, 2015, 63.
3. B. Jismy, G. Guillaumet, H. Allouchi, M. Akssira, M. Abarbri, Eur. J. Org. Chem., 2017, 41, 6168.
4. J. E. Biggs-Houck, A. Younai, J. T. Shaw, Curr. Opin. Chem. Biol., 14, 2010, 371.
5. M. A. Kolosov, D. A. Beloborodov, V. D. Orlova, V. V. Dotsenko, New J.Chem., 40, 2016, 7573
6. M. T. El Sayed, H. A. R. Hussein, N. M. Elebiary, G. S. Hassan, S. M. Elmessery, A. R. Elsheakh, M. Nayel, H. A. Abdel-Aziz, Bioorganic Chemistry, 78, 2018, 312.
7. A. Sasikumar, V. Mohanasrinivasan, Ajeesh A. K. Kumar, D. Krishnaswamy, J. Heterocyclic Chem., 55, 2017, 214.
8. M. Suzuki, H. Iwasaki, Y. Fujikawa, M. Sakashita, M. Kitahara, R. Sakoda, Bioorg. Med. Chem. Lett., 11, 2001, 1285.
9. W. McCoull, R. D. Abrams, E. Anderson, K.Blades, P. Barton, M. Box, J. Burgess, K. Byth, Q. Cao, C. Chuaqui, R. J. Carbajo, T. Cheung, E. Code, A. D. Ferguson, S. Fillery, N. O. Fuller, E. Gangl, N. Gao, M. Grist, D. Hargreaves, M. R. Howard, J. Hu, P. D. Kemmitt, J. E. Nelson, N. O'Connell, D. B. Prince, P. Raubo, P. B. Rawlins, G. R. Robb, J. Shi, M. J. Waring, D. Whittaker, M. Wylot, X. Zhu, J. Med. Chem., 60, 2017, 4386.
10. A. S. Hassan, D. M. Masoud, F. M. Sroor, A. A. Askar, Med Chem. Res., 26, 2017, 2909.
11. M. H. Hamdy, M. A. Salem, H. M, Aly, J. Heterocyclic Chem., 54, 2017, 2614.
12. J. Zhang, J. F. Peng, Y. B. Bai, P. Wang, T. Wang, J. M. Gao, Z. T. Zhang, Mol. Divers., 20, 2016, 887.
13. J. Zhang, J. F. Peng, T. Wang, P. Wang, Z. T. Zhang, J. Mol. Structure., 1120, 2016, 228.

14. J. P. Muller, G. W. Shipps, K. E. Rosner, Y. Deng, T. Wang, P. J. Curran, M. A. Brown, M. A. Siddiqui, A. B. Cooper, J. Duca, M. Cable, V. Girijavallabhan, Bioorg. Med. Chem. Lett., 19, 2009, 6331.
15. O. M. Ahmed, A. M. Hussein, R. R. Ahmed, Med. Chem., 2, 2012, 20.
16. X. Deng, J. Shen, H. Zhu, J. Xiao, R. Sun, F. Xie, C. Lam, J. Wang, Y. Qiao, M. S. Tavallaie, Y. Hu, Y. Du, J. Li, L. Fu, F. Jiang, Bioorg. Med. Chem., 26, 2018, 903.
17. J. L. Roux, C. Leriche, P. Chamiot-Clerc, J. Feutrill, F. Halley, D. Papin, N. Derimay, C. Mugler, C. Grépin, L. Schio, Bioorg. Med. Chem. Lett., 26, 2016, 454.
18. C. Almansa, A. F. de Arriba, F. L. Cavalcanti, L.A. Gomez, A. Miralles, M. Merlos, J. G. Rafanell, J. Forn, J. Med. Chem. 44, 2001, 350.
19. S. Cherukupalli, R. Karpoormath, B. Chandrasekaran, G. A. Hampannavar, N. Thapliyal, V. N. Palakollu, Eur. J. Med. Chem., 126, 2017, 298.
20. P. Golubev, E. A. Karpova, A. S. Pankova, M. Sorokina, M. A. Kuznetsov, J. Org. Chem., 81, 2016, 11268.
21. L. Li, H. Xu, L. Dai, J. Xi, L. Gao, L. Rong, Tetrahedron, 2017, 73, 5358.
22. (a)Y. I. Sakhno, A. V. Kozyryev, S. M. Desenko, S. V. Shishkina, V. I. Musatov, D. O. Sysoiev and V. A. Chebanov, Tetrahedron, 74, 2018, 564. (b) S. K. Krishnammagari, B. G. Cho, J. T. Kim and Y. T. Jeong, Synth. Commun., 2018, 1–12.
23. (a) P. Kaswan, K. Pericherla, D. Purohit, A. Kumar, Tetrahedron Lett., 56, 2015, 549. (b) Y. I. Sakhno, A. V. Kozyryev, S. M. Desenko, S. V. Shishkina, V. I. Musatov, D. O. Sysoiev and V. A. Chebanov, Tetrahedron, 74, 2018, 564. (c) M. P. Dwyer, K. Keertikar, K. Paruch, C. Alvarez, M. Labroli, C. Poker, T. O. Fischmann, R. Mayer-Ezell, R. Bond, Y. Wang, R. Azevedo and T. J. Guzi, Bioorganic Med. Chem. Lett., 23, 2013, 6178.
24. J. H. Kim, C. M. Song, J. W. Park, H. R. Jeong, J. R. Kim, Z. Ha, Y. L. No, Y. S. Hyun, N. Cho, S. Kang, D. J. Jeon, Bioorg. Med. Chem. Lett., 20, 2010, 922.
25. R. E. Khidre and B. F. Abdelwahab, Turkish J. Chem., 37, 2013, 685.
26. V. A. Chebanov, V. E. Saraev, S. M. Desenko, V. N. Chernenko, I. V. Knyazeva, U. Groth, T. N. Glasnov, C. O. Kappe, J. Org. Chem., 73, 2008, 5110.
27. V. A. Chebanov, Y. I. Sakhno and S. M. Desenko, Ultrason. Sonochem., 19, 2012, 707.
28. C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, Adv. Drug Deliv. Rev., 23, 1997, 3.
29. A. Daina, O. Michielin, V. Zoete, J. Chem. Inf. Model., 54, 2014, 3284.
30. M. F. El Shehry, M. M. Ghorab, S. Y. Abbas, E. A. Fayed, S. A. Shedid, Y. A. Ammar, Eur. J. Med. Chem., 143, 2018, 1463.
31. R. M. Gol, K. M. Khokhani, T. T. Khatri, J. J. Bhatt, J. Korean Chem. Soc., 58, 2014, 49.
32. R. E. Cooper, F.W. Kavangeh, Analytical Microbiology, vols. 1&2, Academic Press, New York and London, 1972.
33. K. M. Khokhani1, R. M.Gol, T. T .Khatri, P. K. Patel, Chemistry & Biology Interface, 4, 2014, 119.
34. Clinical and Laboratory Standards Institute, Performance Standards for AntimicrobialDisk Susceptibility Test. Approved Standard, ninth ed. CLSI, Wayne, PA, USA, 2006.