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Synthesis and Antimicrobial Screening of Pyrimidine Annulated Dihydropyrano[2, 3-*c*]pyrazole Derivatives

Hemant Hegde[†], Chuljin Ahn[‡], Preeti Waribam[†], and Nitinkumar S. Shetty[†,*]

[†]*Department of Chemistry, Manipal Institute of Technology, Manipal, Karnataka 576104, India.*
[*]*E-mail: snitinshetty@gmail.com*
[‡]*Department of Chemistry, Changwon National University, Changwon 51140, Korea.*
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ABSTRACT. A series of pyrimidine annulated dihydropyrano[2, 3-*c*]pyrazole derivatives were synthesized and screened for their antimicrobial activity. The precursor, dihydropyrano[2, 3-*c*]pyrazole was synthesised under catalyst free condition using PEG-400 as reaction medium which facilitated improved yield compared to base catalyzed reaction. Wide scope of substrates, simple workup procedure and high yield even in the absence of catalyst are the major highlights of the protocol. Dihydropyrano[2, 3-*c*]pyrazoles on condensation with formic acid formed pyrimidine annulated dihydropyrano[2, 3-*c*]pyrazole derivatives. All the products are characterized using FTIR, ^{1}H-NMR and ^{13}C-NMR spectroscopic techniques. The molecules have shown good to moderate activity as antimicrobial agents when compared to the standard drug ciprofloxacin.

Key words: Dihydropyrano[2, 3-*c*]pyrazole, PEG-400, Green medium, Annulation, Antimicrobial agents

INTRODUCTION

Fused pyran derivatives represent promising class of fused heterocycles which constitute structural unit of many therapeutic agents[1] and natural products.[2–4] Pyrano[2, 3-*c*]pyrazole derivatives are such compounds gained major attention due to their potent biological activities such as antimicrobial,[5] anti-inflammatory,[6] analgesic,[7] molluscicidal[8] and anticancer[9] activity. Also, they are well known as biodegradable agrochemicals[10] and pharmaceutical ingredients.[11]

The synthesis of pyrano[2, 3-*c*]pyrazole has been first reported by Junek and co-workers by the reaction between 3-methyl-1-phenylpyrazolin-5-one and tetracyano ethylene in the presence of triethylamine in ethanol.[12] Thereafter, several synthetic strategies have been introduced for the synthesis of this nucleus by employing various catalysts which includes acidic[13,14] or basic[15–18] catalyst, organocatalyst,[19] biocatalyst,[20] heterogenous catalyst[21] and phase transfer catalyst.[22] Also, catalyst free synthesis of pyrano[2, 3-*c*]pyrazoles is reported by Mandha and his coworkers.[23] In spite of reporting many synthetic methods for the synthesis of dihydropyrano[2, 3-*c*]pyrazole, there is a need for clean and efficient protocols to develop wide variety of such nucleus.

Fused pyrimidines attracted the major interest of synthetic chemist due to their resemblance with the nitrogen bases. Hence, the annulated pyrimidines have emerged as an important pharmacophore for many life-threatening diseases such as cancer, tuberculosis etc.[24] Pyrimidine fused pyrano[2, 3-*c*]pyrazoles have gained considerable interest among such derivatives. Many researchers have reported the synthesis of pyrimidine fused pyrano[2, 3-*c*]pyrazoles which found to exhibit significant biological activities such as antimicrobial, anti-inflammatory and anticancer agents.[25,26]

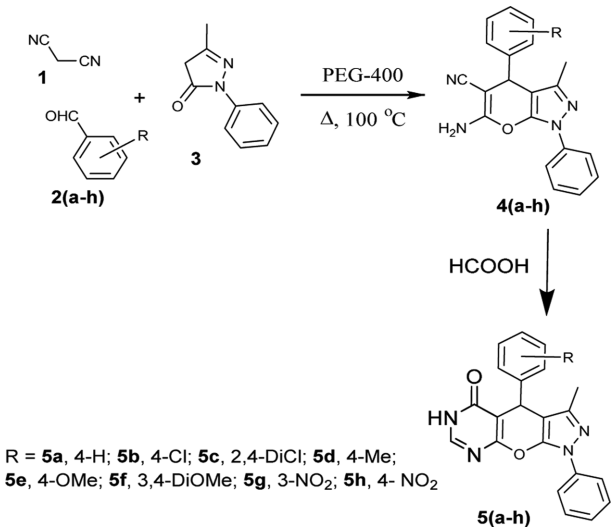
The use of greener and safe reaction media in place of volatile solvents have been considered as an essential part of organic synthesis. To address the problems caused by volatile organic solvents liquid polymers or low melting polymers are developed as an alternate medium for the organic synthesis.[27,28] In this direction, PEG-400 is emerged as an important reaction medium due to its thermal stability, non-toxicity, recyclability, and water solubility.[29-31]

Herewith, we are reporting facile synthesis of dihydropyrano[2, 3-*c*]pyrazole under catalyst free condition using PEG-400 as a medium. Also, further derivatization was done on the dihydropyrano[2, 3-*c*]pyrazole using formic acid to obtain pyrimidine fused pyrano-pyrazoles and were screened for their activity as antimicrobial agents against *M. smegmatis*, *E. coli* and *S. aureus*.

EXPERIMENTAL

General

Chemicals are procured commercially and are used as such without any purification. Progress of the reaction is monitored using thin layer chromatographic technique



***Scheme* 1.** Synthesis of pyrimidine annulated dihydropyrano[2, 3-*c*]pyrazole derivatives.

(TLC) using pre-coated aluminum sheets alichrosep silica gel-60/UV_{254} with I_2 and UV light as visualizing agents. Melting point is determined using Thiele's tube in open capillary tube and is uncorrected. IR spectra are recorded on Shimadzu Infrared spectrometer-8400s using KBr as background. ^1H and ^{13}C NMR analysis is done with Bruker avance NMR-400 MHz and 100 MHz respectively using TMS as an internal standard. Elemental analysis is performed by using Perkin Elmer 2400 CHN elemental analyzer.

General Procedure for the Synthesis of 1, 4-dihydropyrano[2, 3-*c*]pyrazole Derivatives 4(a-h)

Mixture of malononitrile (1 mmol) and appropriate aromatic aldehyde (1 mmol) in PEG-400 (5 mL) was stirred at room temperature for 30 min. To this mixture 3-methyl-1-phenyl-2-pyrazolin-5-one (1 mmol) was added and refluxed at 100 °C for 40-45 minute to get the titled product. The reactions were monitored by TLC. The reaction mixture was then cooled to room temperature and added to crushed ice. It was stirred for about 30 min. and solid thus formed was filtered and washed with 70% ethanol. The crude products were recrystallised from ethyl acetate. The spectral data of the synthesized molecules are given below.

6-Amino-3-methyl-1, 4-diphenyl-1, 4-dihydropyrano[2, 3-*c*]pyrazole-5-carbonitrile (4a): yield, 302 mg (92%); m.p. 168–170 °C, (Lit. 171–173 °C[32a]); FTIR (γ_{max}, cm^{-1}): 3450 (N-H), 2195 (CN), 1589 (C=N), 1256 (C-O-C). ^1H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 1.79 (s, 3H, CH_3), 4.92 (s, 1H, 4-H), 7.65 (s, 2H, NH_2), 7.26–7.81 (m, 10H, Ar-H). ^{13}C NMR (100 MHz) - spectrum in DMSO-d_6 (δ, ppm): 162.4, 145.7, 144.9, 140.5, 138.1, 133.8, 133.1, 132.3, 130.3, 129.8, 128.7, 127.6, 120.3, 120.1, 97.9, 56.9, 12.5. Anal. Calcd. for $C_{20}H_{16}N_4O$: C, 73.15; H, 4.91; N, 17.06. Found: C, 73.17; H, 4.97; N, 17.09.

6-Amino-4-(4-chlorophenyl)-3-methyl-1-phenyl-1, 4-dihydropyrano[2, 3-*c*]pyrazole-5 carbonitrile (4b): yield, 304 mg (94%); m.p. 170–172 °C, (Lit. 173–174 °C[32a]); FTIR (KBr): ν_{max} = 3456 (N-H), 3195 (Ar-C-H), 2202 (CN), 1595 (C=N), 752 (C-Cl); ^1H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 1.79 (s, 3H, CH_3), 4.73 (s, 1H, 4-H), 7.28–7.38 (m, 3H, Ar-H), 7.42 (d, 2H, *J*=8.0 Hz, Ar-H), 7.48–7.52 (m, 2H, Ar-H), 7.51 (s, 2H, NH_2), 7.77–7.79 (d, 2H, *J*=8.0 Hz, Ar-H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.9, 145.6, 144.4, 143.1, 137.9, 132.0, 130.1, 129.1, 126.7, 120.5, 120.3, 98.6, 58.2, 36.5, 13.0 ppm. Anal. Calcd. for $C_{20}H_{15}ClN_4O$: C, 66.21; H, 4.17; N, 15.44. Found: C, 66.24; H 4.21; N, 15.45.

6-Amino-4-(2, 4-dichlorophenyl)-3-methyl-1-phenyl-1, 4-dihydropyrano[2, 3-*c*]pyrazole-5-carbonitrile (4c): yield, 368 mg (93%); m.p. 184–186 °C, (Lit. 185–187 °C[32a]); FTIR (γ_{max}, cm^{-1}): 3458 (N-H), 2198 (CN), 1593 (C=N), 756 (C-Cl). ^1H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 1.77 (s, 3H, CH_3), 5.15 (s, 1H, 4-H), 7.31–7.47 (m, 5H, ArH) 7.50–7.52 (d, *J*=8 Hz, ArH) 7.63 (s, 2H, NH_2), 7.77–7.79 (d, 2H, *J*=8 Hz, ArH) ppm. ^{13}C NMR (100 MHz) - spectrum in DMSO-d_6 (δ, ppm):160.4, 145.3, 144.7, 139.7, 137.6, 133.5, 133, 132.9, 129.8, 129.8, 128.5, 126.7, 120.4, 120, 97.7, 56.6, 12.8. Anal. Calcd. for $C_{20}H_{14}Cl_2N_4O$: C, 60.47; H, 3.55; N, 14.10. Found: C, 60.50; H, 3.57; N, 14.13.

6-Amino-3-methyl-4-(4-methylphenyl)-1-phenyl-1, 4-dihydropyrano[2, 3-*c*]pyrazole-5-carbonitrile (4d): yield, 312 mg (91%); m.p. 176–178 °C, (Lit. 177–179 °C[31b]); FTIR (γ_{max}, cm^{-1}): 3451 (N-H), 2201(CN), 1591 (C=N). ^1H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 1.80 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 5.21 (s, 1H, 4-H), 7.28–7.44 (m, 5H, Ar-H), 7.46–7.48 (d, 2H, *J*=8.4 Hz, Ar-H), 7.52 (s, 2H, NH_2), 7.58–7.60 (d, 2H, *J*=8.0 Hz, Ar-H) ppm. ^{13}C NMR (100 MHz) - spectrum in DMSO-d_6 (δ, ppm): 161.3, 145.7, 144.5, 140.4, 136.9, 133.9, 133.3, 132.7, 130.2, 129.7, 128.4, 126.3, 120.9, 120.4, 96.9, 57.2, 15.6, 13.2 ppm. Anal. Calcd. for $C_{21}H_{18}N_4O$: C, 73.67; H, 5.30; N, 16.36. Found: C, 73.70; H, 5.35; N, 16.39.

6-Amino-4-(4-methoxyphenyl)-3-methyl-1-phenyl-1, 4-dihydropyrano[2, 3-*c*]pyrazole-5-carbonitrile (4e): yield, 331 mg (95%); m.p. 172–174 °C, (Lit. 172–173 °C[32b]); FTIR (γ_{max}, cm^{-1}): 3395 (N-H), 2192 (CN), 1595 (C=N). ^{1}H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 1.78 (s, 3H, CH_3), 3.72 (s, 3H, OCH3), 4.88 (s, 1H, 4-H), 6.82 (d, 2H, *J*=8.0 Hz, Ar-H), 6.96 (s, 2H, NH_2), 7.04 (d, 2H, *J*=8.0 Hz, Ar-H), 7.20–7.24 (m, 1H, Ar-H), 7.40 (d, 2H, *J*=8.0 Hz, Ar-H), 7.58 (d, 2H, *J*=8.0 Hz, Ar-H). ^{13}C NMR (100 MHz) - spectrum in DMSO-d_6 (δ, ppm): 163.2, 145.1, 144.4, 139.2, 137.9, 133.9, 133.2, 132.4, 130.2, 129.5, 128.9, 126.8, 120.9, 120.5, 98.2, 72.4, 56.9, 13.1. Anal. Calcd. for $C_{21}H_{18}N_4O_2$: C, 70.38; H, 5.06; N, 15.63. Found: C, 70.40; H, 5.10; N, 15.66.

6-Amino-4-(3, 4-dimethoxyphenyl)-5-cyano-3-methyl-1-phenyl-1, 4-dihydropyrano[2, 3-*c*]pyrazole (4f): yield, 337 mg (89%); m.p. 192–194 °C, (Lit. 193–195 °C[32b]); FTIR (γ_{max}, cm^{-1}): 3386, 3323, 2198, 1658, 1633, 1595, 1515, 1388, 1257, 1022 cm^{-1}. ^{1}H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 1.82 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.63 (s, 1H, 4-H), 7.18–7.38 (m, 5H, ArH), 7.47 (s, 2H, NH_2), 7.49 (d, 2H, *J*=8.0 Hz, ArH), 7.78 (d, 2H, *J*=8.0 Hz, ArH). ^{13}C NMR (100 MHz) - spectrum in DMSO-d_6 (δ, ppm):159.7, 149.0, 148.2, 145.8, 144.2, 138.0, 136.5, 129.8, 128.5, 120.5, 120.3, 112.2, 112.0, 99.1, 58.8, 56.3, 55.5, 34.8, 13.2. Anal. Calcd. for $C_{20}H_{15}N_4O$: C, 66.21; H, 4.17; N, 15.44. Found: C, 66.25; H, 4.21; N, 15.50.

6-Amino-3-methyl-4-(3-nitrophenyl)-1-phenyl-1, 4-dihydropyrano[2, 3-*c*]pyrazole-5-carbonitrile (4g): yield, 345 mg (91%); m.p. 190–192 °C, (Lit. 188–190 °C[32b]); FTIR (γ_{max}, cm^{-1}): 3429 (N-H), 2117 (CN), 1599 (C=N). ^{1}H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 1.79 (s, 3H, CH_3), 5.02 (s, 1H, 4-H), 7.25 (s, 2H, NH_2), 7.30–7.36 (m, 1H, Ar-H), 7.44–7.46 (m, 2H, Ar-H), 7.58 (d, 2H, *J*=8.4 Hz, Ar-H), 7.78 (d, 2H, *J*=8.4 Hz, Ar-H), 8.15 (d, 2H, Ar-H). ^{13}C NMR (100 MHz) - spectrum in DMSO-d_6 (δ, ppm):159.7, 144.8, 143.2, 139.4, 138.6, 133.4, 132.9, 132.3, 129.8, 129.1, 128.6, 126.5, 120.9, 120.4, 98.4, 57.5, 13.7. Anal. Calcd. for $C_{20}H_{15}N_5O_3$: C, 64.34; H, 4.05; N, 18.76. Found: C, 64.31; H, 4.03; N, 18.77.

6-Amino-3-methyl-4-(4-nitrophenyl)-1-phenyl-1, 4-dihydropyrano[2, 3-*c*]pyrazole-5-carbonitrile (4h): yield, 349 mg (90%); m.p. 194–196 °C, (Lit. 194–196 °C[32c]); FTIR (γ_{max}, cm^{-1}): 3430, 3340, 2116, 1665, 1596, 1354, 1124, 832, 754 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80 (s, 3H, CH_3), 4.96 (s, 1H, 4-H), 6.98 (s, 2H, NH_2), 7.32–7.36 (m, 1H, ArH), 7.48–7.52 (m, 2H, Ar-H), 7.58 (d, 2H, *J*=8.4 Hz, Ar-H), 7.80 (d, 2H, *J*=8.4 Hz, Ar-H), 8.24 (d, 2H, Ar-H) ppm. ^{13}C NMR (100 MHz) - spectrum in DMSO-d_6 (δ, ppm): 162.4, 146.1, 145.2, 140.1, 138, 133.9, 133.2, 132.7, 129.9, 129.5, 128.2, 126.3, 120.8, 120.1, 98.2, 57.1, 13.4. Anal. Calcd. for $C_{20}H_{15}N_5O_3$: C, 64.34; H, 4.05; N, 18.76. Found: C, 64.40; H, 4.08; N, 18.80.

General Procedure for the Synthesis of Pyrano[2, 3-*c*] pyrazolopyrimidinone Derivatives

Pyrano[2, 3-*c*]pyrazole derivatives and formic acid (3 ml) were refluxed at 90 °C for 8 hours. On completion (confirmed by TLC) of the reaction, reaction mixture was cooled at room temperature. Further, the mixture was poured in the beaker and crushed ice was added and stirred it at room temperature, until separation of solid takes place. Then, the crude precipitate was filtered using buchner funnel. Crude solid was recrystallized using ethanol:DMF (1:10).

4-(4-Chlorophenyl)-3-methyl-1-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2, 3-*d*] pyrimidin-5(1H)-one (5b): yield, 348 mg (89%); m.p. 196–198 °C; FTIR (KBr): ν_{max}= 3464 (NH), 1681 and 1724 (C=O of amide), 1600 (C=N), 756 (C-Cl) cm^{-1}. ^{1}H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm):2.16(s,3H, CH_3 proton), 4.23(s, 1H, CH proton), 7.17 (s, 1H, imine CH), 7.31–7.33 (d, 2H, *J*= 8 Hz), 7.39–7.46 (m, 5H, Ar-H), 7.68–7.70 (d, 2H, *J*=8 Hz, Ar-H), 10.90 (s, 1H, NH) ppm. ^{13}C NMR (100 MHz) - spectrum in DMSO-d_6 (δ, ppm): 162.1, 161.4, 153.7, 150.4, 140.4, 137.5, 130.6, 130.2, 129.3, 129.1, 126.6, 125.8, 125.4, 120.7, 119.1, 107.2, 35.7, 13.4. Elemental Anal. Clacd. for $C_{21}H_{15}ClN_4O_2$: C, 64.54; H, 3.87; N, 14.34; Found: C, 64.57; H, 3.92; N, 14.29.

4-(2,4-Dichlorophenyl)-3-methyl-1-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2, 3-*d*]pyrimidin-5(1H)-one (5c): yield, 358 mg (84%); m.p. 208–210 °C; FTIR (KBr): ν_{max}=3456 (NH), 1684 and 1729 (C=O of amide), 1596 (C=N), 762 (C-Cl) cm^{-1}. ^{1}H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 2.13 (s,3H, CH_3 proton), 4.25 (s, 1H, CH proton), 7.19 (s, 1H, imine CH), 7.29–7.31 (d, 1H, *J*=8 Hz), 7.35–7.42 (m, 5H, Ar-H), 7.62–7.64 (d, 1H, *J*=8 Hz, Ar-H), 7.78 (s, 1H, Ar-H), 11.10 (s, 1H, NH) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): 162.6, 161.3, 153.9, 150.7, 140.5, 137.6, 131.5, 130.9, 129.5, 129.3, 127.1, 126.2, 125.8, 121.3, 119.5, 107.6, 36.2, 13.6. Elemental Anal. Calcd. for $C_{21}H_{14}Cl_2N_4O_2$: C, 59.31; H, 3.32; N, 13.17; Found: C, 59.27; H, 3.30; N, 13.20.

3-Methyl-4-(3-nitrophenyl)-1-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-*d*]pyrimidin-5(1H)-one (5g): yield, 325 mg (81%); m.p. 218–220 °C; FTIR (KBr): ν_{max} =3452 (NH), 1679 and 1731 (C=O of amide), 1604 (C=N), 1589 and 1378 (N-O str.) cm^{-1}. ^1H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 2.09 (s, 3H, CH_3 proton), 4.27 (s, 1H, CH proton), 7.17 (s, 1H, imine CH), 7.24–7.26 (d, 1H, *J*=8 Hz), 7.31–7.40 (m, 5H, Ar-H), 7.59–7.61 (d, 1H, *J*=8 Hz, Ar-H), 7.83 (s, 1H, Ar-H), 11.17 (s, 1H, NH) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): 162.9, 161.6, 154.2, 150.9, 140.7, 137.8, 131.6, 131.4, 129.7, 129.5, 127.5, 126.4, 121.0, 119.9, 119.3, 107.3, 36.6, 13.9. Elemental Anal. Calcd. for $C_{21}H_{15}N_5O_4$: C, 62.84; H, 3.77; N, 17.45; Found: C, 62.81; H, 3.81; N, 17.49.

3-Methyl-4-(3-nitrophenyl)-1-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2, 3-*d*]pyrimidin-5(1H)-one (5h): yield, 341 mg (85%); m.p. 164–166 °C; FTIR (KBr): ν_{max} =3449 (NH), 1679 and 1731 (C=O of amide), 1604 (C=N), 1589 and 1378 (N-O str.) cm^{-1}. ^1H NMR (400 MHz) - spectrum in DMSO-d_6 (δ, ppm): 2.09 (s, 3H, CH_3 proton), 4.27 (s, 1H, CH proton), 7.17 (s, 1H, imine CH), 7.24–7.26 (d, 1H, *J*=8 Hz), 7.31–7.40 (m, 5H, Ar-H), 7.59–7.61 (d, 1H, *J*=8 Hz, Ar-H), 7.83 (s, 1H, Ar-H), 11.17 (s, 1H, NH) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): 162.9, 161.6, 154.2, 150.9, 140.7, 137.8, 131.6, 131.4, 129.7, 129.5, 127.5, 126.4, 121.0, 119.9, 119.3, 107.3, 36.6, 13.9. Elemental Anal. Calcd. for $C_{21}H_{15}N_5O_4$: C, 62.84; H, 3.77; N, 17.45; Found: C, 62.81; H, 3.81; N, 17.49.

RESULTS AND DISCUSSION

Initially, focus was to develop an efficient protocol for the reaction using dimethyl amino pyridine (DMAP) catalyst in the presence of suitable solvent. The solvents were selected based on their feasibility, environmental safety, and ease of handling. Based on that ethanol, water, PEG-400 and glycerol were selected for the reaction (*Table* 1).

***Table* 1.** Selection of solvent for the synthesis of dihydropyrano [2, 3-*c*]pyrazole

Entry	Solvent (5 mL)	Temperature	Time (min.)	Yield (%)*
1	Ethanol	80 °C	90	90
2	Water	100 °C	180	72
3	PEG-400	100 °C	35	92
4	Glycerol	80 °C	60	88

Reaction condition: Malononitrile (1 mmol), 4-Chlorobenzaldehyde (1 mmol) and 3-Methyl-1-phenyl-2-pyrazolin-5-one (1 mmol) with DMAP catalyst (10 mol %).
*Isolated yield.

***Table* 2.** Optimization of catalyst concentration for the synthesis of dihydropyrano[2, 3-*c*]pyrazole

Entry	DMAP Conc. (mol %)	Time (min.)	Yield (%)*
1	0	45	94
2	10	45	92
3	20	50	93
4	25	35	91

Reaction condition: Malononitrile (1 mmol), 4-Chlorobenzaldehyde (1 mmol) and 3-Methyl-1-phenyl-2-pyrazolin-5-one (1 mmol) with PEG-400 as solvent (5 mL) at 100 °C.
*Isolated yield.

Hence, the reaction conditions were optimized by taking model reaction between equimolar mixture of 4-chloro benzaldehyde, malononitrile and 3-methyl-1-phenyl-2-pyrazolin-5-one in the presence of DMAP (10 mol %) catalyst in different testing solvents under reflux condition. The reaction gave the product in good yield in all the cases and it was found to be highest in the presence of PEG-400 as a solvent (92% yield in 35 min.). Further investigation was done on the optimization of catalyst concentration in PEG-400 (5 mL) by varying the catalyst concentration from 0 to 25 mol % (*Table* 2). Surprisingly, the same reaction carried out in the absence of catalyst with PEG-400 as a solvent gave highest yield of 94%, in 45 min. and was better than that with 20 mol % of DMAP in 40 min.

Encouraged with this fascinating result, further investigation was done on optimizing the temperature by varying it from 90 to 110 °C considering five different temperatures (*Table* 3) in the presence of PEG-400 (5 mL) as a solvent. The highest yield was observed, when reactions were carried out at 100 °C for 45 min. Considering the reaction time and yield both catalysed and uncatalysed reaction gave the similar results. Therefore, the reactions carried out without a catalyst with the temperature of 100 °C found to be the best condition for the reaction in the presence of PEG-400 as reaction medium. Finally, PEG-400 mediated multicomponent synthesis of dihydro pyrano[2, 3-*c*]

***Table* 3.** Optimization of reaction temperature for the synthesis of dihydropyrano[2, 3-*c*]pyrazole

Entry	Temperature	Time (min.)	Yield (%)*
1	90 °C	60	87
2	95 °C	50	89
3	100 °C	45	94
4	105 °C	30	90
5	110 °C	30	82

Reaction condition: Malononitrile (1 mmol), 4-Chlorobenzaldehyde (1 mmol) and 3-Methyl-1-phenyl-2-pyrazolin-5-one (1 mmol) with PEG-400 as solvent (5 mL).
*Isolated yield.

pyrazole derivatives considered to be an efficient green method under catalyst free condition.

Formation of products are confirmed by FTIR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. FTIR spectrum showed the characteristic peaks in the region of 2116 and 3460 cm^{-1} for –CN and $-NH_2$ respectively. It is further confirmed by the presence of characteristic peaks around δ 5.17 and 7.5 ppm corresponds to C-H proton at 4th position of pyran ring and NH_2 proton respectively. All the spectral data are in good agreement with the reported values.[32]

The formed pyrano[2, 3-*c*]pyrazole derivatives were further derivatised by reacting with formic acid to form pyrimidine fused pyrano[2, 3-*c*]pyrazoles. Product formation was confirmed by FTIR and proton NMR analysis. The FT-IR spectra of final products shown the characteristic peaks at 3464, 1681 and 1724, 1600, 756 cm^{-1} for NH str, C=O of amide, C=N and C-Cl respectively. It was further confirmed by NMR spectroscopy, which shows the characteristic peaks at δ 10.90, 7.17, and 4.23 ppm which corresponds to NH proton, imine CH proton and CH proton respectively.

Antimicrobial Activity

Compounds were screened for their activity as antimicrobial agents against non-virulent *M. smegmatis*, *E. coli and S. aureus.* In-vitro preliminary screening was done using agar diffusion method for all the molecules (**5a-h**, 10 mM concentration in DMSO). Inhibition zone was recorded in millimeter and readings were taken in triplicates (*Table* 4). Penicillin (for antituberrcular activity) and ciprofloxacin (for antibacterial activity) was used as standard drug for comparison.

The preliminary in vitro antimicrobial screening results shows that the novel pyrimidine annulated dihydropyrano[2, 3-*c*]pyrazole derivatives reported here have the less potential to act as antitubercular, agents against *M. smegmatis* but have shown moderate to good activity against both *S. aureaus* and *E. Coli*. Compound **5b** and **5f** have shown good activity against *E. coli* (gram negative) and moderate activity against *S. aureus* (gram positive). Compound **5h** having $-NO_2$ substitution was best among all the molecules against *S. aureaus* and *M. smegmatis.* Minimum inhibitory concentration was not determined as there no potent activity on comparing with standards.

The possible improvements in the antitubercular activity can be further achieved by slight modifications in the substituents on the basic dihydropyrano[2, 3-*c*]pyrazole nucleus. In view of the above findings, and to identify new pharmacophores that may be of value in designing new, selective, less toxic antimicrobial agents which might serve as new templates.

***Table* 4.** Inhibitory zone (diameter) in mm of synthesized compounds against tested microbial strains by agar diffusion method

Compound	*E. coli*	*S. aureus*	*M. smegmatis*
5a	25	23	9
5b	30	28	12
5c	20	22	8
5d	19	26	10
5f	31	27	9
5g	21	23	8
5h	28	32	13
Ciprofloxacin	41	44	-
Penicillin			24

CONCLUSION

It has been found that, PEG-400 mediated protocol is an efficient and green method for the synthesis of the precursor, dihydropyrano[2, 3-*c*]pyrazole derivative owing to their non-toxic and eco-friendly nature. These molecules were further reacted with formic acid to get pyrimidine fused dihydropyrano[2, 3-*c*]pyrazole. The antitubercular activity studies of the pyrimidine annulated dihydropyrano[2, 3-*c*]pyrazole derivatives have shown moderate activity. Further structural modification might improve the activity against *Mycobacterium smegmatis.* Most of the molecules have shown better antibacterial activity against *E. coli* and *S. aureus.*

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Supporting Information. Additional supporting information is available in the online version of this article.

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