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ARTICLE

Rapid Four-Component Reactions in Water: Synthesis of Pyranopyrazoles Catalyzed by Ammonium Chloride and Their Antimicrobial Evaluation

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ABSTRACT

An environmentally benign synthesis of pyranopyrazoles derivatives has been developed by a four-component reaction between aldehyde, malononitrile, hydrazine hydrate and ethyl acetoacetate in the presence of ammonium chloride in water medium. This method follows the principle of green chemistry by using environmentally benign synthetic method along with use of ammonium chloride as a catalyst and green reaction medium. All the derivatives were evaluated for antimicrobial screening against two Gram-negative bacteria, two Gram-positive bacteria and three fungal species. Among all the tested compounds, it was found that compounds **N-3**, **N-4** and **N-9** revealed better activity against the Gram-positive rather than the Gram-negative bacteria.

KEYWORDS

Enviromentally benign, Pyranopyrazoles, Antimicrobial evaluation.

INTRODUCTION

One of the main reasons of environmental pollution which can damage to our planet is the use of conservative energy sources and use of poisonous and hazardous chemicals in manufacture processes. For the well being of human, polluting technologies must be substitute by benign alternatives. Green chemistry offers more eco-friendly and green alternatives to conservative chemistry practices such as energy efficient energy sources, reduction or exclusion of the use of poisonous and hazardous chemicals in manufacturing processes solvents play a key role in chemical reactions to soluble and bring the reactants in homogeneous phase. Instead of using poisonous and hazardous organic solvents, water can be used as green solvent due to it's abundant, non-toxic, non-corrosive and non-flammable nature. Of promising target to set the greener synthetic protocol, we explore the synthesis of pyranopyrazole derivatives employing water as an eco-friendly reaction medium and ammonium chloride use as a catalyst.

Pyranopyrazoles are fundamental scaffold in natural products and also utilize in the synthesis of promising agents *viz.*, antitumor [1], antibacterial [2], anti-inflammatory [3], antifungal [4] and molluscicidal [5]. Pyranopyrazoles are an important class of heterocyclic compounds. They find appli-

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cations as pharmaceutical ingredients and biodegradable agrochemicals [6-10]. The first reported pyranopyrazole was synthesized by the reaction of 3-methyl-1-phenylpyrazolin-5-one and tetracyanoethylene [6]. Various 6-amino-5-cyano-4-aryl-4*H*-pyrazolo[3,4-*b*]pyrans were synthesized by condensation reaction of malanonitrile, arylidiene with 3-methylpyrazoline-5-ones [7,8]. Sharanin *et al.* [10] have developed a three-component reaction between pyrazolone, an aldehyde and malononitrile in ethanol using triethylamine as the catalyst. Shestopalov and co-workers [11,12] reported the synthesis of pyrazolopyran *via* a three-component condensation between N-methylpiperidone, pyrazoline-5-one andmalononitrile in absolute ethanol. Peng and co-workers [13] have developed a two-component reaction between pyran derivatives and hydrazinehydrate to form pyranopyrazoles in aqueous media.

The synthesis of pyranopyrazoles derivatives have been undertaken by involving Biginelli type four components reaction of substituted aldehyde, malononitrile, hydrazine hydrate and ethyl acetoacetate in water or ethanolic media as per **Scheme-I**.

EXPERIMENTAL

The chemicals were purchased from Merck. The reaction monitoring were carried out using thin-layer chromatography (TLC) on precoated silica gel GF254 plates purchased E-Merck Co and visualized by under exposure of UV light. The melting point was examined by open capillary method and are uncorrected. IR spectrums of synthesized compounds were examined using KBr pellet method on SHIMADZU-FTIR-8400 spectrophotometer. ^{1}H NMR spectra were recorded on Bruker 300 MHz NMR spectrometer in $CDCl_3$ and DMSO-d_6 as a solvent. Tetramethylsilane used as a reference slandered for chemical shift values. Mass spectrum was recorded using JOEL SX 102/DA-600-Mass spectrometer and C, H, N analysis was carried out using elemental analyzer Heraus.

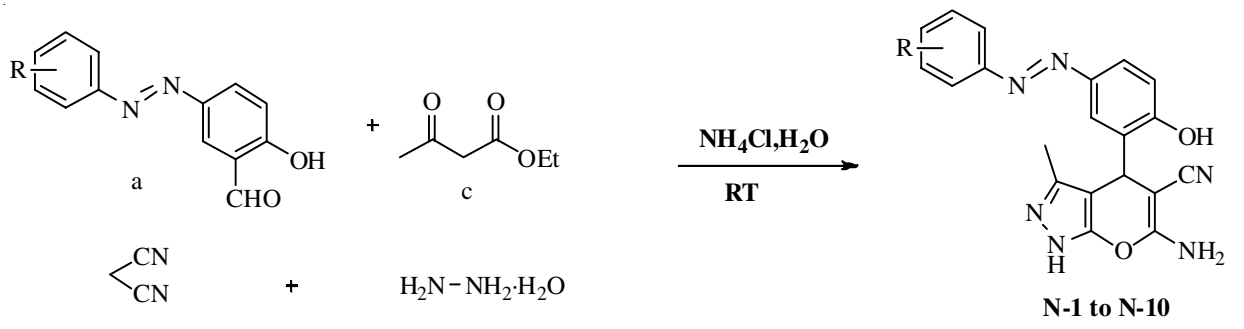
General procedure: The multi component condensation reaction of hydrazine hydrate (1.5 mmol), ethyl acetoacetate (1 mmol), malononitrile (1.2 mmol), substituted salicylaldehyde (1 mmol) and ammonium chloride (0.6 mmol) in the presence of water (5 mL) as a media under stirring at room temperature. The progress of reactions was monitored by TLC, after the completion of reaction; the product worked up in water and filtrated the solid mass, was dried and recrystallized in ethanol. The characterizations of synthesized compounds were carried out using Mass spectral data, IR, ^{1}H NMR and elemental analyses.

Spectral data

(*E*)-6-Amino-4-(2-hydroxy-5-(phenyldiazenyl)phenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (N-1): Yield: 70 %; m.p.: 144 °C; R_f: 0.52. IR (KBr, ν_{max}, cm^{-1}): 3533 (O-H *str.*), 3122 (N-H *str.*), 3022 (C-H *str.* aromatic ring), 2970, 2858 (C-H *str.* alkane), 2275 (C-N *str.*), 1587, 1415 (C=C *str.* aromatic ring), 1363, 1292, 1259 (C-H bending of alkane), 1259 (C-O *str.*), 1068 (C-O-C *str.*), 802 (C-H bending of *p*-di-substituted aromatic ring), 688 (C-H bending of five adjacent hydrogen atom of mono substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.071-2.092 (s, 3H, Ha), 4.777 (s, 1H, Hb), 6.892 (s, 2H, Hcc′), 7.197 (d, 1H, Hd, *J* = 8.1), 7.215 (s, 1H, He, *J* = 8.4 Hz), 7.919-7.938 (m, 3H, Hfff′, *J* = 7.6 Hz), 8.150-8.138 (m, 2H, Hg, *J* = 4.8), 9.557 (s, 1H, Hh), 11.247-11.258 (s, 1H, Hi); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 13.30, 19.41, 59.64, 112.69, 113.21, 114.10, 116.90, 121.33, 122.23, 126.00, 128.66, 131.12, 138.69, 145.12, 152.32, 158.82, 163.22, 176.32; MS: *m/z* 372; Anal. calcd. for $C_{20}H_{16}N_6O_2$: C, 64.51; H, 4.33; N, 22.57 %; Found: C, 61.47; H, 4.36; N, 22.39 %.

(*E*)-6-Amino-4-(5-((4-chlorophenyl)diazenyl)-2-hydroxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (N-2): Yield: 76 %; m.p.: 212 °C; R_f: 0.38. IR (KBr, ν_{max}, cm^{-1}): 3410 (O-H *str.*), 3389 (N-H *str.* 1°), 3230 (N-H *str.* 2°), 3033 (C-H *str.* aromatic ring), 2970, 2858 (C-H *str.* alkane), 2224 (C=N *str.*), 1587, 1415 (C=C *str.* aromatic ring), 1363, 1278, 1259 (C-H bending of alkane), 1259 (C-O *str.*), 1068 (C-O-C *str.*), 832 (C-H bending of *p*-di-substituted aromatic ring), 802 (C-H bending of *p*-di-substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm 2.030-2.041 (s, 3H, Ha), 4.663 (s, 2H, Hb), 6.892-6.991 (d, 2H, Hcc′, *J* = 7.8), 7.091 (s, 1H, Hd), 7.881-7.891 (d, 2H, He, *J* = 4.1 Hz), 8.106-8.124 (d, 2H, Hff′, *J* = 7.8 Hz), 9.551 (s, 1H, Hg), 11.210-11.245 (s, 1H, Hh); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 13.14, 19.31, 58.91, 113.41, 114.22, 117.40, 120.91, 124.03, 124.21,125.88, 129.03, 129.33, 136.53, 138.81, 145.23, 150.71, 158.64, 163.68, 176.18; MS: *m/z* 406.87; Anal. calcd. for $C_{20}H_{15}N_6O_2Cl$: C, 59.05; H, 3.72; N, 20.66 Cl, 8.71 %; Found: C, 59.13; H, 3.99; N, 21.01; Cl, 8.65 %.

(*E*)-6-Amino-4-(2-hydroxy-5-((4-(trifluoromethyl)-phenyl)diazenyl)phenyl)-3-methyl-1,4-dihydropyrano[2,3-



where, R = **N1:** H, **N2:** 4-Cl, **N3:** 4-CF_3, **N4:** 2-Cl, **N5:** 4-OCH_3, **N6:** 4-CH_3, **N7:** 4-Br, **N8:** 4-I, **N9:** 4-NO_2, **N10:** 4-F

Scheme-I

***c*]pyrazole-5-carbonitrile (N-3):** Yield: 78 %; m.p.: 248 °C; R_f: 0.41. IR (KBr, ν_{max}, cm^{-1}): 3410 (O-H *str.*), 3389 (N-H *str.* 1°), 3230 (N-H *str.* 2°), 3033 (C-H *str.* aromatic ring), 2970, 2858 (C-H *str.* alkane), 2224 (C=N *str.*), 1587, 1415 (C=C *str.* aromatic ring), 1363, 1278, 1259 (C-H bending of alkane), 1259 (C-O *str.*), 1068 (C-O-C *str.*), 832 (C-H bending of *p*-di-substituted aromatic ring), 799 (C-F bending), 802 (C-H bending of *p*-di-substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.073-2.078 (s, 3H, Ha), 4.769 (s, 1H, Hb), 6.882-7.195 (s, 2H, Hcc′), 7.198-7.217 (d, 1H, Hd, *J* = 8.6), 7.663-7.7.683 (d, 1H, He, *J* = 8.4 Hz), 7.881-7.893 (s, 1H, Hf, *J* = 3.9 Hz), 7.982-7.021 (d, 2H, Hgg′, *J* = 8.8), 8.105-8.126 (d, 2H, Hhh′, *J* = 8.2 Hz), 9.552-9.568 (s, 1H, Hi), 11.241-11.259 (s, 1H, Hj); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 13.12, 19.23, 58.91, 113.41, 114.81, 117.63, 121.39, 112.98, 123.41,124.01, 125.39, 133.41, 138.81, 138.78, 145.23, 155.81, 158.39, 163.73, 175.98; MS: *m/z* 440.39; Anal. calcd. for $C_{21}H_{15}N_6O_2F_3$: C, 57.27; H, 3.43; N, 19.08; F, 12.94 %; Found: C, 57.49; H, 3.41; N, 19.17 %.

(*E*)-6-Amino-4-(5-((2-chlorophenyl)diazenyl)-2-hydroxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (N-4): Yield: 72 %; m.p.: 200 °C; R_f: 0.37. IR (KBr, ν_{max}, cm^{-1}): 3422 (O-H *str.*), 3379 (N-H *str.* 1°), 3241 (N-H *str.* 2°), 3026 (C-H *str.* aromatic ring), 2956, 2871 (C-H *str.* alkane), 2241 (C=N *str.*), 1585, 1422 (C=C *str.* aromatic ring), 1363, 1278, 1259 (C-H bending of alkane), 1267 (C-O *str.*), 1041 (C-O-C *str.*), 832 (C-H bending of *p*-di-substituted aromatic ring), 766 (C-Cl bending), 741 (C-H bending of *o*-di-substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.066-2.071 (s, 3H, Ha), 4.766 (s, 1H, Hb), 6.886-7.194 (s, 2H, Hcc′), 7.194-7.214(d, 1H, Hd, *J* = 8.6), 7.445-7.465 (m, 1H, He, *J* = 8.4 Hz), 7.458-7.469 (m, 1H, Hf, *J* = 8.2 Hz), 7.982-8.021 (d, 1H, Hg, *J* = 8.8), 8.105-8.126 (d, 1H, Hh, *J* = 8.2 Hz), 9.565-9.579 (s, 1H, Hi), 11.239-11.254 (s, 1H, Hj); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 13.22, 19.28, 59.39, 113.84, 114.86, 117.31, 121.27, 124.36, 127.18, 128.09, 130.02, 132.72, 139.70, 145.31, 153.01, 158.14, 163.76, 176.04, 15; MS: *m/z* 406.83; Anal. calcd. for $C_{20}H_{15}N_6O_2Cl$: C, 59.05; H, 3.72; N, 20.66; Cl, 8.71 %; Found: C, 59.13; H, 3.83; N, 20.55 %.

(*E*)-6-Amino-4-(2-hydroxy-5-((4-methoxyphenyl)-diazenyl)phenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]-pyrazole-5-carbonitrile (N-5): Yield: 69 %; m.p.: 169 °C; R_f: 0.46. IR (KBr, ν_{max}, cm^{-1}): 3417 (O-H *str.*), 3390 (N-H *str.* 1°), 3230 (N-H *str.* 2°), 3041 (C-H *str.* aromatic ring), 2978, 2853 (C-H *str.* alkane), 2232 (C=N *str.*), 1581, 1411 (C=C *str.* aromatic ring), 1355, 1271, 1265 (C-H bending of alkane), 1259 (C-O *str.*), 1068 (C-O-C *str.*), 832 (C-H bending of *p*-di-substituted aromatic ring), 802 (C-H bending of *p*-di-substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.065-2.066 (s, 3H, Ha), 3.842 (s, $1H_b$)4.765 (s, 1H, Hc), 6.852-7.176 (s, 2H, Hdd′), 7.192-7.214(d, 1H, He, *J* = 8.6), 7.668-7.7.686 (d, 1H, Hf, *J* = 8.4 Hz), 7.883-7.896 (s, 1H, Hg, *J* = 3.9 Hz), 7.982-8.021 (d, 2H, Hhh, *J* = 8.8), 8.105-8.126 (d, 2H, Hii′, *J* = 8.2 Hz), 9.555-9.569 (s, 1H, Hj), 11.241-11.259 (s, 1H, Hk); ^{13}C NMR (DMSO-d_6) δ ppm: 13.12, 19.33, 55.81, 59.28, 113.08, 113.98, 114.01, 114.81, 117.39, 121.41, 124.01,124.22, 125.93, 139.42, 145.07, 158.04, 162.87, 163.71, 176.12; MS *m/z* 402.41; Anal. calcd. for $C_{23}H_{22}N_6O_3$: C, 64.18; H, 5.15; N, 19.52 %; Found: C, 64.22; H, 5.09; N, 19.81 %.

(*E*)-6-Amino-4-(2-hydroxy-5-(*p*-tolyldiazenyl)phenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (N-6): Yield: 79 %; m.p.: 151 °C; R_f: 0.54. IR (KBr, ν_{max}, cm^{-1}): 3412 (O-H *str.*), 3387 (N-H *str.* 1°), 3233 (N-H *str.* 2°), 3046 (C-H *str.* aromatic ring), 2975, 2853 (C-H *str.* alkane), 2241 (C=N *str.*), 1581, 1412 (C=C *str.* aromatic ring), 1365, 1255, 1276 (C-H bending of alkane), 1259 (C-O *str.*), 1068 (C-O-C *str.*), 845 (C-H bending of *p*-di-substituted aromatic ring), 882 (C-H bending of *p*-di-substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.055-2.059 (s, 3H, Ha), 3.012 (s, $1H_b$)4.764 (s, 1H, Hc), 6.855-7.152 (s, 2H, Hdd′), 7.191-7.213(d, 1H, He, *J* = 8.6), 7.665-7.7.684 (d, 1H, Hf, *J* = 8.4 Hz), 7.882-7.897 (s, 1H, Hg, *J* = 3.9 Hz), 7.925-7.938 (d, 2H, Hhh, *J* = 8.8), 8.105-8.126 (d, 2H, Hii′, *J* = 8.2 Hz), 9.523-9.539 (s, 1H, Hj), 11.255-11.264 (s, 1H, Hk); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 13.39, 19.23, 21.42, 60.02, 112.91, 114.83, 117.42, 121.39, 122.93,122.81, 126.01, 129.33, 129.41, 139.10, 140.61, 143.23, 149.09, 158.33, 163.72, 176.22; MS: *m/z* 386.42; Anal. calcd. for $C_{21}H_{18}N_6O_2$: C, 65.27; H, 4.70; N, 21.75; O, 8.28 %; Found: C, 65.52; H, 4.69; N, 21.39; O, 8.72 %.

(*E*)-6-Amino-4-(5-((4-bromophenyl)diazenyl)-2-hydroxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (N-7): Yield: 71 %; m.p.: 179 °C; R_f: 0.42. IR (KBr, ν_{max}, cm^{-1}): 3415 (O-H *str.*), 3378 (N-H *str.* 1°), 3265 (N-H *str.* 2°), 3055 (C-H *str.* aromatic ring), 2975, 2853 (C-H *str.* alkane), 2245 (C=N *str.*), 1585, 1425 (C=C *str.* aromatic ring), 1378, 1255, 1282 (C-H bending of alkane), 1259 (C-O *str.*), 1068 (C-O-C *str.*), 810 (C-H bending of *p*-di-substituted aromatic ring), 840 (C-H bending of *p*-di-substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.075-2.081 (s, 3H, Ha), 4.656 (s, 1H, H_b), 6.830-6.872 (s, 2H, Hc), 7.196-7.212 (d, 1H, Hd, *J* = 8.2 Hz)), 7.638-7.657(d, 1H, He, *J* = 8.6), 7.640-7.652 (s, 1H, Hf, *J* = 3.4 Hz), 7.882-7.898 (d, 2H, Hgg′, *J* = 8.2 Hz), 8.110-8.127 (d, 2H, Hhh, *J* = 8.8), 9.510-9.525 (s, 1H, Hj), 11.256-11.275 (s, 1H, Hk); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 14.01, 19.31, 59.24, 113.19, 114.07, 114.21, 117.37, 121.42, 125.33,125.44, 131.90, 132.01, 139.68, 144.98, 151.73, 158.31, 163.73, 176.47; MS: *m/z* 451.28; Anal. calcd. for $C_{20}H_{15}N_6O_2Br$: C, 53.23; H, 3.35; N, 18.62; Br, 17.35; O, 7.09; %; Found: C, 53.62; H, 3.22; N, 18.71 %.

(*E*)-6-Amino-4-(2-hydroxy-5-((4-iodophenyl)diazenyl)-phenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (N-8): Yield: 68 %; m.p.: 158 °C; R_f: 0.47. IR (KBr, ν_{max}, cm^{-1}): 3422 (O-H *str.*), 3371 (N-H *str.* 1°), 3252 (N-H *str.* 2°), 3064 (C-H *str.* aromatic ring), 2875, 2853 (C-H *str.* alkane), 2231 (C=N *str.*), 1578, 1426 (C=C *str.* aromatic ring), 1352, 1268, 1282 (C-H bending of alkane), 1251 (C-O *str.*), 1065 (C-O-C *str.*), 822 (C-H bending of *p*-di-substituted aromatic ring), 836 (C-H bending of *p*-di-substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.066-2.072 (s, 3H, Ha), 4.661 (s, 1H, H_b), 6.831-6.873 (s, 2H, Hc), 7.193-7.209 (d, 1H, Hd, *J* = 8.2 Hz)), 7.636-7.654(d, 1H, He, *J* = 8.6), 7.641-7.649 (s, 1H, Hf, *J* = 3.4 Hz), 7.878-7.896 (d, 2H, Hgg′, *J* = 8.2 Hz), 8.110-8.127 (d, 2H, Hhh, *J* = 8.8), 9.522-9.536 (s, 1H, Hj), 11.253-11.269 (s, 1H, Hk); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 14.21, 19.22, 59.36, 113.14, 114.08,

114.18, 117.46, 121.43, 125.65,125.74, 131.91, 132.03, 139.56, 144.42, 151.65, 158.45, 163.78, 176.56; MS: *m/z* 498.03; Anal. calcd. for $C_{20}H_{15}N_6O_2I$: C, 48.21; H, 3.03; N, 16.87, I, 25.47; O, 16.87; %; Found: C, 48.22; H, 3.09; N, 15.99, I, 24.98 %.

(*E*)-6-Amino-4-(2-hydroxy-5-((4-nitrophenyl)diazenyl)-phenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (N-9): Yield: 77 %; m.p.: 269 °C; R_f: 0.36. IR (KBr, ν_{max}, cm^{-1}): 3431 (O-H *str.*), 3371 (N-H *str.* 1°), 3258 (N-H *str.* 2°), 3065 (C-H *str.* aromatic ring), 2875, 2856 (C-H *str.* alkane), 2247 (C=N *str.*), 1578, 1426 (C=C *str.* aromatic ring), 1352, 1268, 1282 (C-H bending of alkane), 1442 (NO_2 streching), 1263 (C-O *str.*), 1085 (C-O-C *str.*), 818 (C-H bending of *p*-di-substituted aromatic ring), 828 (C-H bending of *p*-di-substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.038-2.042 (s, 3H, Ha), 4.656 (s, 1H, H_b), 6.842-6.856 (s, 2H, Hc), 7.189-7.204 (d, 1H, Hd, *J* = 8.2 Hz)), 7.642-7.658(d, 1H, He, *J* = 8.6), 7.641-7.649 (s, 1H, Hf, *J* = 3.4 Hz), 7.992-8.012 (d, 2H, Hgg′, *J* = 8.2 Hz), 8.114-8.129 (d, 2H, Hhh, *J* = 8.8), 9.518-9.533 (s, 1H, Hj), 11.253-11.269 (s, 1H, Hk); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 14.18, 19.18, 59.42, 113.23, 114.14, 114.63, 117.44, 121.56, 125.53,125.46, 131.91, 132.03, 139.56, 144.42, 151.65, 158.45, 163.78, 176.78; MS: *m/z* 417.39; Anal. calcd. for $C_{20}H_{15}N_7O_4$: C, 57.55; H, 3.62; N, 23.49, O; 15.33 %; Found: C, 57.46; H, 3.61; N, 23.66; O, 14.99 %.

(*E*)-6-Amino-4-(5-((4-fluorophenyl)diazenyl)-2-hydroxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (N-10): Yield: 66 %; m.p.: 242 °C; R_f: 0.32. IR (KBr, ν_{max}, cm^{-1}): 3442 (O-H *str.*), 3344 (N-H *str.* 1°), 3245 (N-H *str.* 2°), 3033 (C-H *str.* aromatic ring), 2869, 2854 (C-H *str.* alkane), 2236 (C=N *str.*), 1554, 1429 (C=C *str.* aromatic ring), 1355, 1263, 1284 (C-H bending of alkane), 1142 (F-C *str.*), 1266 (C-O *str.*), 1069 (C-O-C *str.*), 823 (C-H bending of *p*-di-substituted aromatic ring), 831 (C-H bending of *p*-di-substituted aromatic ring); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.021-2.036 (s, 3H, Ha), 4.656 (s, 1H, H_b), 6.856-6.868 (s, 2H, Hc), 7.187-7.202 (d, 1H, Hd, *J* = 8.2 Hz)), 7.639-7.654 (d, 1H, He, *J* = 8.6), 7.638-7.647 (s, 1H, Hf, *J* = 3.4 Hz), 7.992-8.012 (d, 2H, Hgg′, *J* = 8.2 Hz), 8.243-8.261 (d, 2H, Hhh, *J* = 8.8), 9.524-9.533 (s, 1H, Hj), 11.249-11.261 (s, 1H, Hk); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 13.18, 19.29, 59.39, 113.27, 114.19, 114.36, 117.78, 121.46, 125.39, 126.46, 130.91, 132.03, 139.44, 144.78, 151.86, 158.87, 163.81, 176.56; MS: *m/z* 390.38; Anal. calcd. for $C_{20}H_{15}N_6O_2F$: C, 61.54; H, 3.87; N, 21.53; O, 8.20; F, 4.87 %; Found: C, 61.44; H, 3.79; N, 21.39; O, 8.39; F, 4.78 %.

RESULTS AND DISCUSSION

Many protocol has been developed for the synthesis of pyrano[2,3-*c*]pyrazole derivatives however the crucial things to develop efficient strategies under mild reaction conditions. The present synthesis was carried out under different reaction parameters *viz.* solvent, temperature, catalyst. The better reaction conditions were obtained using ammonium chloride under room temperature in water.

Biological evaluation

Antimicrobial evaluation: All of the synthesized compounds (**N-1** to **N-10**) were evaluated for their antimicrobial evaluation (MIC) *in vitro* by broth dilution method [14,15] under two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441, two Gram-positive bacteria *Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 443 and three fungal strains *Aspergillus Niger* MTCC 282, *Candida albicans* MTCC 227, *Aspergillus clavatus* MTCC 1323 taking external standards chloramphenicol, ciprofloxacin, ampicillin, nystatin, norfloxacin and griseofulvin. The strains of species were acquires from The Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for the synthesized molecules defined as the lowest concentration of the compound preventing the visible growth, were examined by using micro dilution broth method according to NCCLS standards [15]. The preparation of dilution series of the compounds and reference drugs were carried out in Muellere-Hinton agar. DMSO (1 mL) is used as a solvent for the dissolution of standard (10 mg). Further dilutions with melted Muellere-Hinton

TABLE-1
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS **N1-10**

Compd. No.	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*	*A. clavatus*
N-1	500	1000	500	100	1000	>1000	1000
N-2	100	100	500	1000	>1000	500	1000
N-3	25	12.5	50	25	50	25	250
N-4	25	25	50	50	500	500	250
N-5	500	100	500	500	500	500	>1000
N-6	500	1000	250	1000	500	500	>1000
N-7	250	100	500	500	500	250	250
N-8	250	100	100	250	1000	500	250
N-9	12.5	25	25	25	500	250	250
N-10	100	500	250	100	500	1000	>1000
Ampicillin	250	100	100	100	–	–	–
Chloramphenicol	50	50	50	50	–	–	–
Ciprofloxacin	50	50	25	25	–	–	–
Norfloxacin	10	10	10	10	–	–	–
Nystatin	–	–	–	–	100	100	100
Griseofulvin	–	–	–	–	500	100	100

agar were carried out to obtain the series of concentrations of 1.56, 3.12, 6.25, 10, 12.5, 25, 50, 62.5, 100, 125, 250, 500 and 1000 µg mL^{-1}. The tubes were inoculated at 37 °C for 24 h with 10^8 cfu mL^{-1} (colony forming unit/mL). The results obtained from antibacterial and antifungal susceptibility testing are described in Table-1. The compounds **N-3**, **N-4** and **N-9** found to be most promising against broad spectrum antibacterial activity against both Gram-negative bacteria, Gram-positive and as compared with ciprofloxacin. The compound **N-9** was found to be 4-fold more potent against *S. pyogens* (MIC = 12.5 µg/mL) and *S. aureus* and the compound **N-3**, **N-4** exhibited 2-fold more effective against *S. pyogens* (MIC = 25 µg/mL) and *S. aureus* compared to the standard drug. While the compound **N-4** showed comparable activity against 2-fold more activity against *S. pyogens* (MIC = 25 µg/mL) and *P. aeruginosa* and *E. coli*. Moreover, compound **N-4** possessing 2-fold inhibition against *S. aureus* and comparable inhibition against *P. aeruginosa*. As per the results obtained from evaluation, the compounds possessing withdrawing substituents such as flouro, chloro and Nitro at 2nd and 4th position of phenyl ring gives better antimicrobial activity. The compound N-3 with active pharmacophore triflouro at 3rd position gives 4 and 2-fold higher activity against *C. albicans* (MIC = 50 µg/mL) and *A. niger*, *A. clavatus* (MIC = 25 µg/mL), respectively comparison to the standard drug griseofulvin.

Conclusion

We report an atom-economical multi-component reaction at room temperature by stirring in the presence of ammonium chloride as mild, cost effective and green catalyst along with water as the eco-friendly green solvent to synthesize different pyranopyrazoles. The results obtained from evaluation makes them promising tools for additional *in vivo* and *in vitro* evaluations for the development of lead with potential antimicrobial activity in the treatment of numerous dieses. Further research and development are in progress in our laboratory in this area.

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