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Synthesis of pyranopyrazoles by using eco-friendly montmorillonite K-10 catalyst-their antioxidant and antimicrobial assays

G. Raveendra Reddy[a,b], V. Hanuman Reddy[b], T. Veera Reddy*[a] and K. Naresh[c]

[a]*Department of Chemistry, Vikrama Simhapuri University, Nellore, India*
[b]*CSC Division, Indian Institute of Chemical Technology, Hyderabad, India*
[c]*Department of Bio-chemistry Sri Venkateswara University, Tirupat, India*

ABSTRACT

*Eco-friendly montmorillonite K-10 reusable catalyst mediated three-component reaction in aqueous and organic medium at room temperature has been developed for the synthesis of dihydropyrano[2,3-c]pyrazole derivatives and studied their biological properties. The compounds **4d**, **4c** and **4h** showed excellent antioxidant activity. All the compounds exhibited higher antibacterial activity towards Gram-negative Escherichia coli bacteria. The compound **4f** exhibited antifungal activity almost equal to the standard drug.*

Key words: Pyranopyrazoles; Montmorillonite K-10; Antimicrobial activity; Antioxidant activity

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INTRODUCTION

Designing organic reactions in aqueous media is another attractive area in green chemistry [1]. Water is an abundant and environmentally benign solvent. A number of classic reactions which were carried out strictly under anhydrous conditions, in hazardous and toxic organic solvents, can also be carried out in water with proper use of catalysts and reaction conditions. Multi-component reactions (MCRs) are those reactions in which three or more reactants react together to give the product in a single step under suitable reaction conditions [2]. MCRs offer the advantage of simplicity and synthetic efficiency over conventional reactions. The MCRs have the additional advantages of selectivity, synthetic convergency and atom economy [3]. MCRs which are carried out in water as a medium offer better environmental protection, hence, are considered as clean and green reactions. 2-Pyrones and their fused derivatives have attracted a great deal of interest due to their wide range of biological activities [4–9]. In addition, pyrazoles act as core nucleus in various drugs due to their activities such as antidiabetic anti-tumour, antipyretic, anti-inflammatory, anti-hypertensive and antidepressant agents [10–15]. It was thought worthwhile to combine both pharmacophoric groups in one molecular frame [16–23]. Pyranopyrazoles are an important class of heterocyclic compounds. They find applications as pharmaceutical ingredients and biodegradable agrochemicals [24–26]. The first reported pyranopyrazole was synthesized from the reaction between 3-methyl-1-phenylpyrazolin-5-one and tetracyanoethylene [24]. Various 6-amino-5-cyano-4-aryl-4H-pyrazolo[3,4-b]pyrans were synthesized by the reaction of arylidienemalononitrile with 3-methylpyrazoline-5-ones or by the condensation of 4-arylidienepyrazoline-5-one with malononitrile [25]. Dihydropyrano[2,3-c]pyrazole derivatives have very important biological activities such as antimicrobial [18], insecticidal [19] and anti-inflammatory [20]. Furthermore dihydropyrano[2,3-c]pyrazoles showed molluscicidal activity [27,28]. We report herein, an alternative protocol for

the three-component synthesis of dihydropyrano[2,3-c]pyrazoles in the presence of montmorillonite K-10 as a catalyst in water & ethanol and studied their antioxidant and antimicrobial biological assays.

MATERIALS AND METHODS

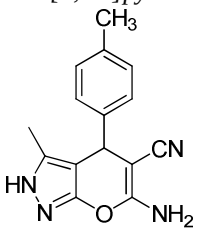
1. General

Melting points were measured on an Electrothermal 9100 apparatus and were uncorrected. IR spectra were recorded on an FTIR spectrometer (KBr) and reported in reciprocal centimeters (cm-1). NMR spectra were recorded for 1H NMR at 300MHz, 500MHZ and for 13C NMR at 75MHz. For 1H NMR, tetramethylsilane (TMS) served as internal standard (δ = 0) and data were reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t= triplet, q =quartet, m = multiplet, br = broad), and coupling constant in Hz. For 13C NMR, CDCl3 (δ = 77.27) was used as internal standard and spectra were obtained with complete proton decoupling. HRMS data were obtained using Electrospray ionization (ESI) ionization. The compound 3 was prepared as per literature procedure [29].

1.1. Typical procedure for the preparation of 2,4-dihydropyrano[2,3-c]pyrazole derivatives (4a-n)

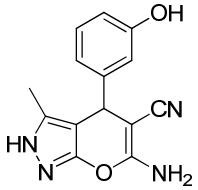
Phenyl-1*H*-pyrazol-5(4*H*)-one (1.0 mmol) (**3**) was added to a magnetically stirred mixture of montmorillonite K 10 (2.0 mmol), substituted aromatic aldehyde (**1a**) (1.0 mmol) and malononitrile (**2**) (1.0 mmol) in water-ethanol (2:1) solvent at room temperature. The mixture was stirred until completion of reaction (5-9 h), diluted with ethyl acetate (EtOAc) and filtered through a plug of cotton. To separate EtOAc solvent and was concentrated under vacuum to afford pyranopyrazole derivative. The cotton plug containing the catalyst was dipped into EtOAc (10 mL) in a beaker when the montmorillonite K 10 settled down to the bottom of the beaker. The cotton was removed and the EtOAc decanted off. The recovered catalyst after being air dried and treated at 100 ◦C for 2 h under reduced pressure. Then again reused this catalyst.

*1.1.1. 6-amino-3-methyl-4-p-tolyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**4a**).*



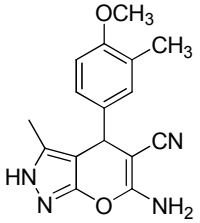
Off white solid. **Mp**: 212**–**215 °C; **IR ν_{max} (KBr)**: 1033, 1129, 1218, 1252, 1384, 1494, 1592, 1634, 2186, 3106, 3258 cm^{-1} ; **^{1}H NMR** (300 MHz, $CDCl_3$ + DMSO-d_6: δ_H (ppm) 2.02 (s, 3H, CH_3), 2.47 (s, 3H, Ar-CH_3), 4.73 (s, 1H, *4H*), 5.53 (s, 1H, NH_2), 7.24-7.60 (d, 4H, Ar-H), 11.66 (s, 1H, NH); **^{13}C NMR** (75 MHz, $CDCl_3$+DMSO-d_6): δ_C (ppm) 9.6, 20.5, 35.7, 57.3, 97.6, 117.1, 120.6, 127.2, 128.8, 135.4, 141.3, 154.6, 160.6**;** **HRMS** (ES^+) calcd for $C_{15}H_{15}N_4O$ $(M + H)^+$267.12404 found *m/z* 267.12351.

1.1.2. 6-amino-4-(3-hydroxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile **(4b).**



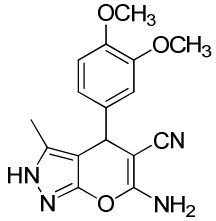
Yellow solid. **Mp**: 256**–**258 °C; **IR ν_{max} (KBr)**: 1046, 1400, 1481, 1588, 1646, 2174, 3157, 3356 cm^{-1}; **^{1}H NMR** (300 MHz, CD_3OD-d_4: δ_H (ppm) 1.88 (s, 3H, CH_3), 4.5 (s, 1H, 4*H*), 6.59-6.71 (m, 3H, Ar-H), 7.09-7.15 (t, 1H, Ar-H), **^{13}C NMR** (75 MHz, CD_3OD-d_4): δ_C (ppm) 10.0, 38.0, 82.3, 110.8, 115.1, 115.6, 120.1, 121.9, 130.5, 138.3, 146.9, 148.4, 158.8, 160.4**;** **HRMS** (ES^+) calcd for $C_{14}H_{13}O_2N_4$ $(M + H)^+$269.10330 found *m/z* 269.10296.

1.1.3. 6-amino-4-(4-methoxy-3-methylphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile **(4c).**



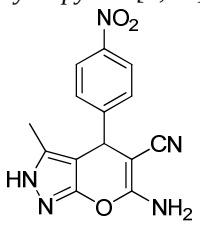
Yellow solid. **Mp**: 212**–**215 °C; **IR ν_{max} (KBr)**: 1056, 1414, 1493, 1606, 1653, 2190, 3164, 3390 cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 1.21 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 3.16 (s, 3H, -OCH_3), 3.83 (s, 1H, 4*H*), 5.32 (s, 2H, NH_2), 6.10-6.12 (d, 1H, Ar-H), 6.26 (s, 1H, Ar-H), 6.32-6.36 (d, 1H, Ar-H), 11.11(s, 1H, NH), **^{13}C NMR** (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C (ppm) 9.6, 15.9, 38.5, 54.8, 58.3, 97.5, 109.2, 120.7, 125.6, 129.2, 135.3, 135.5, 155.9, 160.4**; HRMS** (ES^+) calcd $C_{16}H_{17}O_2N_4$ $(M + H)^+$297.13460 found *m/z* 297.13396.

1.1.4. 6-amino-4-(3,4-dimethoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***(4d).***



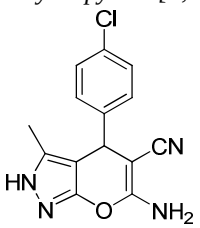
White solid. **Mp**: 235**–**238 °C; **IR ν_{max} (KBr)**: 1024, 1134, 1218, 1395, 1498, 1590, 1642, 2177, 2312, 3122, 3737cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 1.37 (s, 3H, CH_3), 3.32-3.35 (s, 6H, -2OCH_3), 4.04 (s, 1H, 4*H*), 5.27 (s, 2H, NH_2), 6.26-6.34 (t, 2H, Ar-H), 7.11 (s, 1H, Ar-H), 11.22 (s, 1H, NH), **^{13}C NMR** (75MHz, $CDCl_3$ + DMSO-d_6): δ_C (ppm) 9.5, 35.8, 55.2, 58.8, 96.9, 110.2, 110.4, 119.2, 120.3, 135.7, 147.2, 148.2, 154.5, 160.0**; HRMS** (ES^+) calcd for $C_{16}H_{17}O_3N_4$ $(M + H)^+$313.12952 found *m/z* 313.12901.

1.1.5. 6-amino-3-methyl-4-(4-nitrophenyl)-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***(4e).***



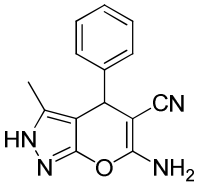
white solid. **Mp**: 263**–**265 °C; **IR ν_{max} (KBr)**: 1048, 1350, 1401, 1515, 1592, 1647, 2194, 3088, 3221, 3474cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 1.42 (s, 3H, CH_3), 4.30 (s, 1H, 4*H*), 5.47-5.53 (s, 2H, NH_2), 6.92-7.00 (d, 2H, Ar-H), 7.73-7.78 (d, 2H, Ar-H), 11.44 (s, 1H, NH), **^{13}C NMR** (75MHz, DMSO-d_6): δ_C (ppm) 9.6, 35.7, 55.8, 96.4, 120.3, 123.7, 128.7, 135.7, 146.2, 151.9, 154.5, 161.0**; HRMS** (ES^+) calcd for $C_{14}H_{12}O_3N_5$ $(M + H)^+$298.09347 found *m/z* 298.09296.

1.1.6. 6-amino-4-(4-chlorophenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile **(4f).**



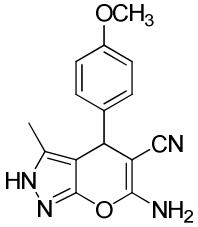
Yellow solid. Yellowish solid. **Mp**: 160**–**164 °C; **IR ν_{max} (KBr)**: 1089,1149, 1214, 1296, 1518, 1656, 2310, 3737cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 1.90 (s, 3H, CH_3), 3.88-3.94 (d, 1H, 4*H*), 4.71-4.75 (d, 1H, NH), 6.62-6.72 (m, 4H, Ar-H), **^{13}C NMR** (75 MHz, DMSO-d_6): δ_C (ppm) 9.5, 16.9, 48.7, 97.4, 112.5, 128.3, 128.5, 133.0, 137.1, 138.7, 159.4**; HRMS** (ES^+) calcd for $C_{14}H_{12}ON_4Cl$ $(M + H)^+$287.06942 found *m/z* 287.06885.

1.1.7. 6-amino-3-methyl-4-phenyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile **(4g).**



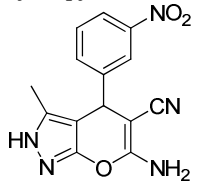
white solid. **Mp**: 174**–**178 °C; **IR ν_{max} (KBr)**: 1063, 1160, 1216, 1293, 1519, 1656, 2311, 3365, 3736cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 1.78 (s, 3H, CH_3), 3.96-4.01 (d, 1H, NH), 4.93-4.96 (s, 1H, 4*H*), 6.98-7.02 (m, 2H, Ar-H), 7.16-7.23 (m, 3H, Ar-H); **^{13}C NMR** (75MHz, $CDCl_3$ + DMSO-d_6): δ_C (ppm) 9.5, 26.8, 42.4, 48.6, 97.9, 112.7, 127.2, 127.4, 128.3, 138.5, 159.4, 174.0**; HRMS** (ES$^+$) calcd for $C_{14}H_{13}N_4O$ (M + H)$^+$ 253.10839 found *m/z* 253.10792 (M + H)$^+$.

1.1.8. 6-amino-4-(4-methoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile **(4h).**



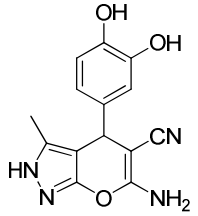
Pale Yellowish solid. **Mp**: 170**–**175 °C; **IR ν_{max} (KBr)**: 1013, 1175, 1270, 1504, 1553, 2214, 2309, 3737cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 2.00 (s, 3H, CH_3), 3.69 (s, 3H,Ar-OCH_3), 4.15-4.20 (d, 1H, 4*H*), 5.12-5.21 (t, 1H, NH), 6.74-6.78 (d, 2H, Ar-H), 7.32-7.35 (d, 2H, Ar-H), **^{13}C NMR** (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C (ppm) 9.5, 27.1, 41.8, 55.3, 98.2, 113.5, 114.5, 123.6, 128.4, 130.8, 133.0, 138.3, 159.3, 164.1**; HRMS** (ES$^+$) calcd for $C_{15}H_{15}N_2O_4$ (M + H)$^+$283.11895 found *m/z* 283.11851.

1.1.9. 6-amino-3-methyl-4-(3-nitrophenyl)-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***(4i).***



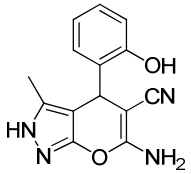
white solid. **Mp**: 248**–**250 °C; **IR ν_{max} (KBr)**: 1045, 1167, 1349, 1401, 1519, 1597, 1651, 2194, 3109, 3216, 3742 cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 1.27 (s, 3H, CH_3), 4.17 (s, 1H, 4*H*), 5.55-5.63 (s, 2H, NH_2), 6.96-7.10 (m, 2H, Ar-H), 7.44-7.57 (t, 2H, Ar-H), **^{13}C NMR** (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C (ppm) 9.5, 36.0, 57.1, 95.8, 119.9, 121.4, 121.7, 129.0, 133.5, 135.5, 145.7, 147.7, 154.5, 160.6**; HRMS** (ES$^+$) calcd for $C_{14}H_{12}O_3N_5$ (M + H)$^+$298.09347 found *m/z* 298.09279.

1.1.10. 6-amino-4-(3,4-dihydroxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***(4j).***



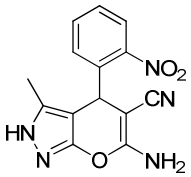
Pale yellow solid. **Mp**: 184**–**187 °C; **IR ν_{max} (KBr)**: 1049, 1108, 1215, 1275, 1404, 1489, 1592, 1642, 2174, 3162, 3738cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 1.82 (s, 3H, CH_3), 3.62 (s, 1H, 4*H*), 5.25 (s, 2H, NH_2), 6.73-6.93 (m, 3H, Ar-H), **^{13}C NMR** (75 MHz, DMSO-d_6): δ_C (ppm) 9.6, 35.5, 45.6, 57.8, 98.0, 114.4, 115.0, 118.1, 120.7, 135.3, 143.9, 145.0, 154.5, 160.4**; HRMS** (ES$^+$) calcd for $C_{14}H_{13}O_3N_4$ (M + H)$^+$285.09822 found *m/z* 285.09790.

1.1.11. 6-amino-4-(2-hydroxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***(4k).***



white solid. **Mp**: 228**–**230 °C; **IR ν_{max} (KBr)**: 1041, 1177, 1401, 1490, 1601, 1647, 2193, 3176, 3354, 3394cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 2.00 (s, 3H, CH_3), 4.76 (s, 1H, 4*H*), 5.62 (s, 2H, NH_2), 6.90-7.18 (m, 4H, Ar-H), 7.59 (s, 1H, NH), **^{13}C NMR** (75 MHz, DMSO-d_6): δ_C (ppm) 9.7, 28.5, 54.9, 104.8, 115.4, 120.7, 123.4, 124.1, 127.4, 128.8, 136.4, 148.3, 158.9, 160.0**;** **HRMS** (ES$^+$) calcd for $C_{14}H_{13}O_2N_4$ (M + H)$^+$269.10330 found *m/z* 269.10260.

1.1.12. 6-amino-3-methyl-4-(2-nitrophenyl)-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile **(4l).**

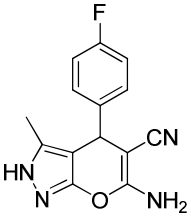


Pale yellow solid. **Mp**: 238**–**240 °C; **IR ν_{max} (KBr)**: 1124, 1215, 1304, 1356, 1519, 1653, 2311, 2906, 3737cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$ + DMSO-d_6: δ_H (ppm) 2.02 (s, 3H, CH_3), 5.15-5.30 (t, 3H, 4*H* + NH_2), 7.26-7.30 (m, 1H, Ar-H), 7.47-7.98 (m, 3H, Ar-H), **^{13}C NMR** (75 MHz, DMSO-d_6): δ_C (ppm) 9.6, 27.2, 48.3, 96.6, 113.3, 124.4, 129.0, 129.5, 132.0, 133.1, 138.5, 148.8, 159.1, 173.6**;** **HRMS** (ES$^+$) calcd for $C_{14}H_{12}N_5O_3$ (M + H)$^+$298.09347 found *m/z* 298.09309.

1.1.13. 6-amino-3-methyl-4-(pyridin-3-yl)-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***(4m).***

white solid. **Mp**: 246**–**248 °C; **IR ν_{max} (KBr)**: 1053, 1152, 1184, 1224, 1259, 1404, 1534, 1659, 2186, 3349, 3414, 3445cm^{-1}; **^{1}H NMR** (300 MHz, CD_3OD-d_6: δ_H (ppm) 1.87 (s, 3H, CH_3), 4.74 (s, 1H, 4*H),* 7.41-7.46 (t, 1H, Ar-H), 7.64-7.70 (d, 1H, Ar-H), 8.43-8.46 (d, 2H, Ar-H), **^{13}C NMR** (75 MHz, CD_3OD-d_4): δ_C (ppm) 10.0, 35.3, 58.2, 79.5, 98.2, 121.4, 125.6, 137.8, 138.2, 141.8, 148.9, 149.5, 156.7, 163.0**;** **HRMS** (ES$^+$) calcd for $C_{13}H_{12}ON_5$ (M + H)$^+$254.10364 found *m/z* 254.10311.

5.1.1.14. 6-amino-4-(4-fluorophenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***(4n).***



white solid. **Mp**: 242-244 °C; **IR ν_{max} (KBr)**: 1050, 1157, 1223, 1397, 1494, 1592, 1643, 2193, 3096, 3225, 3477cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6: δ_H (ppm) 1.43 (s, 3H, CH_3), 4.18 (s, 1H, 4*H*), 5.30-5.32 (s, 2H, NH_2), 6.54-6.61 (q, 2H, Ar-H), 6.73-6.79 (t, 2H, Ar-H), **^{13}C NMR** (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C (ppm) 9.4, 26.3, 35.5, 59.0, 96.7, 114.4, 114.6, 119.9, 128.5, 135.5, 138.8, 160.0**;** **HRMS** (ES$^+$) calcd for $C_{14}H_{12}ON_4F$(M + H)$^+$271.09897 found *m/z* 271.09844.

2. Biological assays

2.1. DPPH radical scavenging activity. The hydrogen atom or electron donation ability of the compound was measured from the bleaching of the purple coloured methanol solution of 2, 2- diphenyl-1-picrylhydrazyl (DPPH)

[30,31]. This spectrophotometric assay uses the stable radical DPPH as a reagent. 1mL of various concentrations of the compound (25, 50, 75 and 100µg/mL) in methanol were added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30 minutes incubation period at room temperature, the absorbance was read against blank at 517 nm. Along with test compounds, a standard ascorbic acid was analyzed in the same concentration. The percent of inhibition (I %) of free radical production from DPPH was calculated by using the following equation.

$$I\% = [(A \text{ control} - A \text{ of sample} / A \text{ control})] \times 100.$$

where Acontrol is the absorbance of the control reaction (containing all reagents and Ascorbic acid), Asample is the absorbance of the test compound (containing all reagents and test compound) and Ablank is the absorbance of the blank (containing only reagents).

2.2. Nitric oxide (NO) scavenging activity. Nitric oxide scavenging activity was measured by slightlymodified methods of Green et al. [32] and Marcocci et al. [33]. Nitric oxide radicals (NO) were generated from sodium nitroprusside. One milliliter of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75, and 100 mg/mL) of the test compounds and incubated at 25 ^{o}C for 150 min. After incubation, 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1%sulfanilamide, 2% H_3PO_4, and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Ascorbic acid was used as standard. Nitric oxide scavenging activity was calculated by the following equation

$$I\% = [(A \text{ control} - A \text{ of sample} / A \text{ control})] \times 100.$$

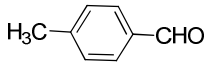
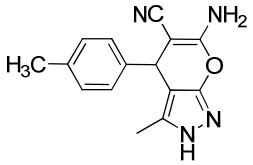
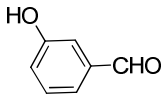
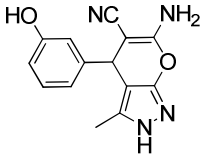
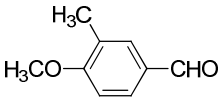
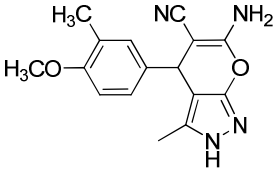
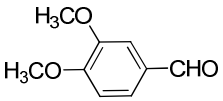
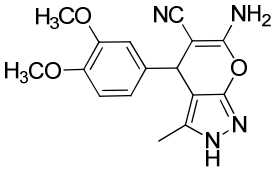
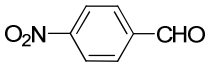
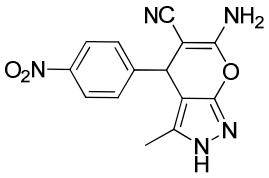
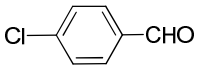
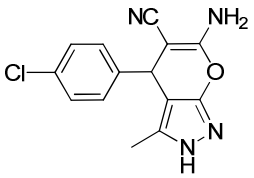
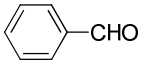
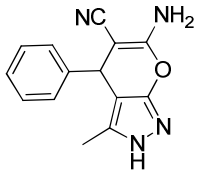
where Acontrol is the absorbance of the control reaction (containing all reagents and Ascorbic acid), Asample is the absorbance of the test compound (containing all reagents and test compound) and Ablank is the absorbance of the blank (containing only reagents).

2.3. Hydrogen peroxide (H_2O_2) scavenging activity. The H_2O_2 scavenging ability of the test compound was determined according to the method of Ruch et al. [34]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 25, 50, 75, and 100 mg/mL concentrations of the test compounds in 3.4 mL phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percent of scavenging of H_2O_2 was calculated by the following equation

$$I\% = [(A \text{ control} - A \text{ of sample} / A \text{ control})] \times 100$$

where Acontrol is the absorbance of the control reaction (containing all reagents and ascorbic acid), Asample is the absorbance of the test compound (containing all reagents and test compound) and Ablank is the absorbance of the blank (containing only reagents).

Table. 1 Substitutions, time and yields of the products 4a–n

S.No	Aromatic aldehydes (1)	Products (4a-n)	Time(h)	Yield(%)
a			8	75
b			7	78
c			7	80
d			6	86
e			6	78
f			7	79
g			6	64

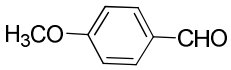
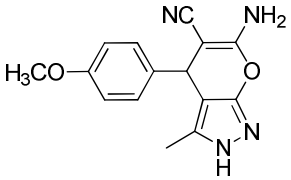
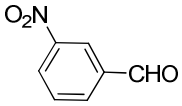
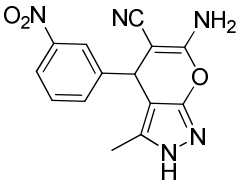
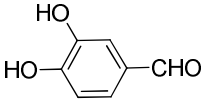
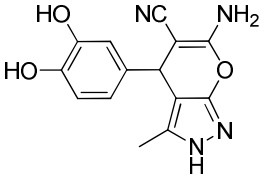
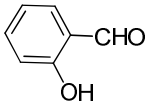
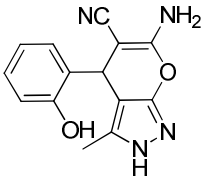
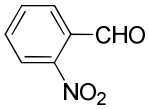
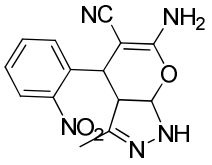
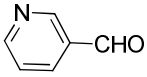
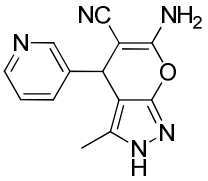
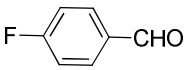
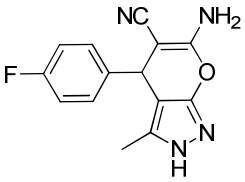
h			7	75
i			6	77
j			6	85
k			7	76
l			5	80
m			8	74
n			7	82

Table 2. *In vitro* antioxidant activity of compounds 4a–n by DPPH method

Compound	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
4a	36.05	40.11	58.41	64.09
4b	24.19	34.70	48.12	52.44
4c	40.71	46.24	65.71	70.05
4d	41.23	49.57	67.21	73.15
4e	6.17	9.51	11.02	14.11
4f	14.13	21.24	29.70	31.65
4g	20.91	29.32	36.11	47.20
4h	38.61	42.54	60.92	67.15
4i	04.91	07.24	10.62	12.87
4j	35.43	40.01	56.24	60.72
4k	30.27	36.72	51.08	57.04
4l	3.28	5.08	8.46	10.19
4m	19.15	24.20	30.15	41.23
4n	08.10	11.34	15.71	18.11
Ascorbic Acid	42.0	54.0	68.0	76.0

Table 3. *In vitro* antioxidant activity of compounds 4a–n by NO method

Compound	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
4a	40.53	43.19	60.22	63.01
4b	29.50	36.75	50.97	55.41
4c	45.22	52.34	65.92	72.54
4d	48.75	59.21	69.04	76.18
4e	10.18	13.29	21.45	29.77
4f	18.67	24.13	31.28	40.91
4g	24.91	30.18	43.56	51.28
4h	42.08	47.28	61.70	69.25
4i	07.11	11.84	15.48	22.47
4j	38.45	41.12	58.47	60.12
4k	33.39	38.05	53.16	58.23
4l	04.48	08.57	15.42	21.10
4m	20.06	26.41	34.78	45.11
4n	14.54	19.71	26.40	34.18
Ascorbic Acid	51.15	62.43	71.62	83.08

Table 4. *In vitro* antioxidant activity of compounds 4a–n by H_2O_2 method

Compound	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
4a	21.82	31.56	45.03	64.17
4b	18.56	29.53	43.10	59.25
4c	23.93	34.41	52.08	69.46
4d	24.76	36.35	58.95	70.68
4e	07.64	11.45	17.94	25.24
4f	13.45	19.5	25.15	38.47
4g	16.14	26.04	38.69	44.05
4h	22.45	32.80	49.26	68.24
4i	05.25	09.01	14.79	21.46
4j	20.63	31.46	47.92	65.09
4k	19.64	29.95	43.51	58.93
4l	02.22	08.41	11.37	17.84
4m	15.1	22.35	34.07	42.86
4n	10.87	16.70	21.38	32.51
Ascorbic Acid	46.39	65.36	77.34	85.21

Table 5. *In vitro* antibacterial activity of compounds 4a–n

Compound code	Zone of inhibition (in mm) at conc. 200 µg/mL after 24 h			
	B. subtilis (ATCC-6633)	*S. aureus* (ATCC-19433)	*E. coli* (ATCC-8739)	*P. vulgaris* (ATCC-29213)
4a	15.94	17.28	19.37	18.15
4b	03.36	06.50	7.45	5.22
4c	09.53	11.95	12.42	10.29
4d	11.37	12.86	14.70	10.91
4e	14.22	16.04	18.65	15.47
4f	24.13	26.57	28.00	22.45
4g	20.03	21.56	23.78	17.64
4h	06.88	09.67	10.34	09.10
4i	22.52	25.14	26.47	21.78
4j	05.46	07.19	08.28	06.30
4k	19.24	20.37	20.01	16.57
4l	12.08	14.89	16.45	15.09
4m	21.49	23.85	24.18	19.04
4n	22.01	24.11	25.64	20.95
Ciprofloxacin	25.52	27.04	29.75	23.19

Table 6. *In vitro* antifungal activity of compounds 4a–n

Compound code	Zone of inhibition (in mm) at conc. 200 µg/mL after 72 h	
	A. niger (MTCC-1881)	*A. flavus* (MTCC-1884)
4a	15.01	18.24
4b	10.54	11.07
4c	03.92	06.29
4d	06.32	09.11
4e	19.71	20.11
4f	25.15	27.91
4g	17.74	19.63
4h	13.63	15.32
4i	22.54	22.16
4j	23.42	25.75
4k	14.52	17.09
4l	19.71	21.34
4m	08.00	09.57
4n	22.00	23.14
Ketoconazole	25.49	28.05

2.3. Antimicrobial activity

Preparation of test samples. The test solutions of the samples were prepared in dimethylformamide (DMF). The antibiotics: Ciprofloxacin was used as standard for antibacterial screening and Ketoconazole was used as a standard for antifungal screening. The antibacterial standard was dissolved in sterile distilled water. The antifungal standard was dissolved in buffered 70% propanol.

2.3.1. Antibacterial screening test. The antibacterial activity of the synthesized compounds **4a–n** were studied against different Gram-positive [*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 19433)] and Gram-negative [*Escherichia coli* (ATCC 8739), *Proteus vulgaris* (ATCC 29213)]. For detection of antibacterial activity the filter paper disc diffusion method [35] was employed. Nutrient agar (NA) was used as the basal medium for test bacteria. These agar media were inoculated with 0.5 mL of the 24 h liquid cultures containing 1×10^7 cells/mL. The diffusion time was 24 h at 5 ºC and the incubation time was 12 hr at 37 ºC for bacteria. Discs with only DMSO were used as control. The diameter (in mm) of the observed inhibition zones were taken as a measure of inhibitory activity.

*2.3.2. Antifungal screening test***.** The antifungal activity of the synthesized compounds **4a-n** were evaluated towards two plant pathogenic and mold fungi, *viz*., *Aspergillus niger* (MTCC 1881) and *Aspergillus flavus* (MTCC 1884). The antifungal activity was assessed by poisoned food technique [36] with some modifications. Potato Dextrose

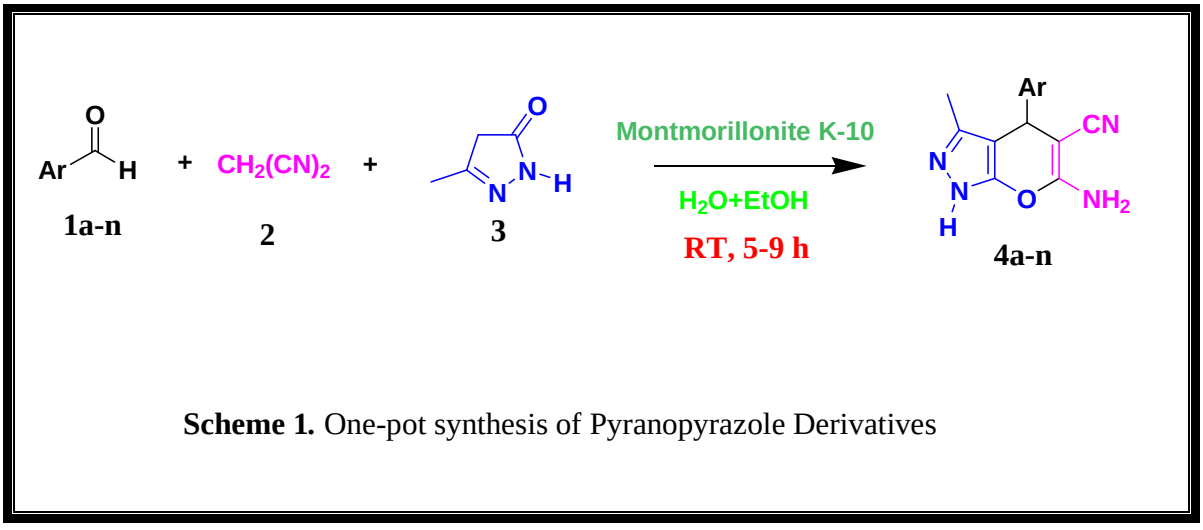
agar (PDA) was used as basal medium for test fungi. Glass petri dishes were sterilized and 15 mL of sterilized melted PDA medium (~ 45 ºC) was poured into each petri dish (90 mm). After solidification of the medium small portions of mycelium of each fungus were spread carefully over the center of each PDA plate with the help of sterilized needles. Thus each fungus was transferred to a number of PDA plates. The PDA plates were then incubated at (25 ± 2 ºC) and after five days of incubation they were ready for use. The prepared discs of test samples were placed gently on the solidified agar plates freshly seeded with the test organisms with sterile forceps. Control discs were also placed on the test plates to compare the effect of solvents, respectively. The plates were then kept in a refrigerator at 4 ºC for 24 h in order that the materials had sufficient time to diffuse to a considerable area of the plates. Afterwards the plates were incubated at 37.5 ºC for 72 h.

RESULTS AND DISCUSSION

Chemistry

In our interest towards the exploitation of montmorillonite K-10 as a catalyst in organic synthesis and developed new synthetic rout. Herein, we wish to report a one-pot multicomponent synthesis of highly substituted pyridines. Experimental protocol was simple and has been achieved by the reaction of electron donating or electron withdrawing, mono and di functional groups at different positions in aromatic aldehydes (**1a**–**n**), malononitrile (**2**), and 3-methyl-1*H*-pyrazol-5(4*H*)-one (**3**) using montmorillonite K-10 in aqueous and ethanol medium at room temperature (Scheme 1). It was found that, various aromatic aldehydes containing electron donating or electron withdrawing functional groups at different positions did show a difference in the reaction time but the yields of products were almost same (Table 1, **4a**–**n**). But, the presence of electron with drawing nitro group in aromatic ring at Ortho, Para and Meta shows variations slightly in yields which was negligible. Ortho nitro substituted aldehyde shows negligible higher yield than the corresponding Meta and Para substituted aldehydes, even reaction time also less. This may be due to presence of nitro group at Ortho easily condensed with malononitrile compare to other. Thus, we have demonstrated a simple, efficient, and a novel one-pot three-component protocol for the synthesis of some new pyranopyrazole derivatives in water and trace ethanol using montmorillonite K-10 as a readily available, inexpensive, and efficient catalyst involves environmentally friendly procedure. The advantages offered by this method were simple reaction condition, short reaction time, ease of product isolation, and excellent yields without using chromatography techniques. All the synthesized compounds were characterized by IR, NMR and HRMS analysis.

Scheme 1. One-pot synthesis of pyranopyrazole derivatives



Scheme 1. One-pot synthesis of Pyranopyrazole Derivatives

Biological results

1. Antioxidant activity

The compounds **4a**–**n** were tested for antioxidant property by 2,2',-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) and hydrogen peroxide (H_2O_2) methods. The observed data on the antioxidant activity of the compounds and control drug were shown in Tables **2-4**. The aim of this study was to identify the potential heterocyclic compound

for antioxidant activity. We observed that substitution effect means electron donating and with drawing groups present on the moiety influences the antioxidant activity. The compounds **4d**, **4c** and **4h** showed excellent radical scavenging activity in all the three methods. This may be due to more electron donating groups that were two methoxy groups in **4d,** methyl & methoxy groups in **4c** and methoxy group in **4h** leads to higher antioxidant property than the other compounds when compared with the standard drug ascorbic acid. While the compounds **4a**, **4j** and **4k** exhibited good activity, whereas the compounds **4b**, **4g**, **4m** and **4f** displayed low activity. However, the other compounds which contain electron withdrawing groups present on the aromatic ring showed negligible activity. Thus the compounds **4n, 4e**, **4i** and **4l** exhibited least antioxidant property. Besides, the perusal of Tables indicated that radical scavenging activity in DPPH, NO and H_2O_2 methods increases with increase in concentration.

2. Antimicrobial activity

The compounds **4a**–**n** was evaluated for antibacterial and antifungal activities at concentration 200 µg/mL. The results of antibacterial activity data presented in Table 5 revealed that the compounds **4f**, **4i** and **4n** displayed higher antibacterial activity than the other compounds in all the Gram-positive and Gram-negative bacterias mentioned in the table 5. On the other hand, the compounds **4g**, **4m** and **4k** exhibited good antibacterial activity, while the other compounds were moderate to low activity. In addition to this all the compounds exhibited higher antibacterial activity towards Gram-negative *Escherichia coli* bacteria. Ciprofloxacin used as a standred drug. All the tested compounds inhibited the spore germination against the fungi Aspergillus niger and *Aspergillus flavus*. In fact all the compounds displayed slightly higher antifungal activity towards *A. flavus* than A. niger. The compound **4f** exhibited greater antifungal activity particularly toward *A. flavus* at 200 µg/mL than the rest of the compounds. When compared to the standard drug *Ketoconazole* (Table 6) it almost equal activity.

CONCLUSION

We have developed montmorillonite K 10 reusable catalyst mediated three-component reaction in aqueous and organic medium at room temperature for the synthesis of dihydropyrano[2,3-c]pyrazole derivatives and studied their antioxidant and antimicrobial properties. The compounds **4d**, **4c** and **4h** showed excellent antioxidant activity. All the compounds exhibited higher antibacterial activity towards Gram-negative *Escherichia coli* bacteria. The compound **4f** exhibited antifungal activity almost equal to the standard drug.

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