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Synthesis of furan tethered 2-pyrazolines via 1,3-dipolar cycloaddition reactions: *In vitro* evaluation for their antioxidant and antimicrobial activities, molecular docking and ADMET studies

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ABSTRACT

In the present study, we report the synthesis of series of novel substituted pyrazoles via 1,3-dipolar cycloaddition reaction. Nitrile imines generated by the catalytic dehydrogenation of hydrazones by chloramine-T, were trapped in situ by 4-(furan-2-yl)but-3-en-2-one to obtain an isomeric mixture of furan tethered 2-pyrazolines. The new compounds were characterized by 1H NMR, 13C NMR, MS studies and elemental analyses. The synthesized new 2-pyrazolines have been screened *in vitro* for their antioxidant and antimicrobial activities. Molecular docking and ADMET studies was done to understand the mode of action of synthesized compounds. Among the synthesized series, 1-(3-(4-chlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone 5f, 1-(3-(2,3-dichlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone 5g and 1-(3-(2,4-dichlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone 5hshowed excellent DPPH radical scavenging and antimicrobial potential against the tested bacteria and fungi species.

Keywords: *antimicrobial, antioxidant, cycloaddition, dipolar, inhibition, radical scavenging.*

1. INTRODUCTION

An interest in antioxidant activity of small molecules, to prevent the deleterious effects caused by free radicals in the human body, is attracting the attention of the wider research community. Free radicals are arising due to the oxidative stress resulting from an imbalance between free radical generation and their quenching [1]. Pyrazoles demonstrated utility in quenching of free radicals and, hence, have tremendous potential for exploration as lead candidates for drug discovery against conditions that result from oxidative damage [2]. Since, their discovery by Huisgen [3,4], 1, 3-dipolar cycloaddition reactions were regarded as the power full tool for the construction of five membered heterocycles such as pyrazoles [5,6], pyrrolidines [7], isoxazoles [8], oxadiazoles [9] etc. For instance, Nitrile imines generated by the catalytic dehydrogenation of phenyl hydrazones undergo 1, 3-dipolar cycloaddition with alkenes to produce pyrazoles [10]. Alternatively, pyrazole derivatives are synthesized by the condensation reaction of chalcones with phenylhydrazine hydrochloride in dry acetic acid [11].

Furan is important structural moieties because of its derivatives have wide range of pharmaceutical interest because of biological activities [12]. They have known to exhibit interesting biological activities such as antinociceptive [13], nematocidal [14], anti-inflammatory [15], antimicrobial [16], genotoxic [17], and anticancer activities [18]. On the other hand, pyrazoles are important nitrogen containing heterocycles well known for their broad spectrum of biological activities and recognizing amounts of research work have been directed in this class of compounds. This is because of its ease of preparation and an interesting template in pharmaceutical chemistry. The pyrazole nucleus is a ubiquitous feature of fertile source of medicinal agents such as antitumor [19], antimicrobial and antioxidant [20], anticonvulsant [21], and CNS depressant [22] properties.

The broader synthetic and biological applications of furan and pyrazole derivatives, prompted us to design a small molecules with both furan and pyrazole moieties, which could act as potent bioactive molecules. In search of new antimicrobial and antioxidant molecules, we here in report the synthesis of furan conjugated pyrazoles via 1,3-dipolar cycloaddition reactions, and the results of their *in vitro* antimicrobial and antioxidant activities, and in silico docking studies.

2. EXPERIMENTAL SECTION

2.1.General Methods. Melting points were determined by an open capillary tube method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC). ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 MHz and 100 MHz spectrometer respectively. The solvent $CDCl_3$ with TMS as an internal standard was used to record the spectra. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on ESI/APCI-Hybrid Quadrupole, Synapt G2 HDMS ACQUITY UPLC model spectrometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer.

2.2. Synthesis of hydrazones,3(a–i). A solution of colorless phenylhydrazine hydrochloride **2** (5 mmol) and crystallized sodium acetate (5 mmol) in distilled water (10 mL) was mixed with solution of aldehydes **1(a–i)** (5 mmol) in ethyl alcohol. The mixture was then warmed for 5-10 minutes and cooled in ice water. The crystals formed were filtered, washed with a little cold water and recrystallized from ethanol.

2.3. General procedure for the synthesis of pyrazoles, 5(a–i), 6(a–i). A mixture of hydrazones **3(a–i)** (5 mmol), 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and chloramine-T trihydrate (7.5 mmol) was refluxed on a water bath for 3-4 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the salts formed were filtered off; the solvent was evaporated in vacuum. The residual mass was extracted into ether (1 x 25 mL), washed successively with water (3 x 20 mL), 5% sodium hydroxide (2 x 10 mL), brine solution (1 x 15 mL) and dried over anhydrous sodium sulphate. Evaporation of the solvent gave isomeric mixture of the products **5(a–i)** and **6(a–i)**. The products were separated by HPLC.

***2.3.1. 1-(4-(Furan-2-yl)-1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone* 5a**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-benzylidene-2-phenylhydrazine **3a** (5 mmol), as gummy mass in 78% yield.^{1}H NMR ($CDCl_3$; δ ppm): 2.230 (s, 3H, CH_3), 3.606 (d, 1H, *J=7.1Hz*, 4-H), 3.883 (d, 1H, *J=7.3Hz*, 5-H), 6.881-7.233 (m, 3H, Ar-H), 6.599-7.878 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.31 (1C, CH_3), 38.64 (1C, 4-C), 71.25 (1C, 5-C), 106.16 (1C), 109.43(1C), 111.68 (1C), 114.32 (1C), 114.65 (1C), 116.52 (1C), 116.73 (1C), 119.38 (1C), 128.31 (1C), 128.48 (1C), 129.23 (1C), 129.64 (1C), 130.34 (1C), 140.76 (1C), 142.24 (1C, 3-C), 144.54 (1C), 162.43 (1C), 205.76 (1C, C=O). MS (*m/z*): 330.09 (M+, 100); Anal. Calcd. for $C_{21}H_{18}N_2O_2$ (%): C, 76.34; H, 5.49; N, 8.48; Found: C, 76.48; H, 5.55; N, 8.32.

***2.3.2. 1-(4-(Furan-2-yl)-3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone* 5b**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(4-methoxybenzylidene)-2-phenylhydrazine **3b** (5 mmol) as semisolid in 71% yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.019 (s, 3H, CH_3), 3.098 (s, 3H, OCH_3), 3.453 (d, 1H, *J=7.3Hz*, 4-H), 3.875 (d, 1H, *J=7.5Hz*, 5-H), 6.453-6.789 (m, 2H, Ar-H), 6.983-7.765 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.23 (1C, CH_3), 38.78 (1C, 4-C), 54.68 (1C, OCH_3), 71.03 (1C, 5-C), 106.19 (1C), 109.23(1C), 111.09 (1C), 114.45 (1C), 114.65 (1C), 116.32 (1C), 116.46 (1C), 119.64 (1C), 128.65 (1C), 128.83 (1C), 129.23 (1C), 129.49 (1C), 130.66 (1C), 140.45 (1C), 142.65 (1C, 3-C), 144.32 (1C), 162.72 (1C), 205.58 (1C, C=O). MS (*m/z*): 360.12 (M+, 100); Anal. Calcd. for $C_{22}H_{20}N_2O_3$ (%): C, 73.32; H, 5.59; N, 7.77; Found: C, 73.24; H, 5.38; N,7.60.

***2.3.3. 1-(3-(3,4-Dimethoxyphenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone* 5c**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(3,4-dimethoxybenzylidene)-2-phenylhydrazine **3c** (5 mmol) as viscous mass in 66% yield.^{1}H NMR ($CDCl_3$; δ ppm): 2.367 (s, 3H, CH_3), 3.673 (s, 3H, OCH_3), 3.789 (s, 3H, OCH_3), 3.762 (d, 1H, *J=7.6Hz*, 4-H), 3.812 (d, 1H, *J=7.1Hz*, 5-H), 6.815 (m, 1H, Ar-H), 7.052-7.561 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.56 (1C, CH_3), 38.55 (1C, 4-C), 55.11(1C, OCH_3), 55.54(1C, OCH_3), 71.46 (1C, 5-C), 106.15 (1C), 109.01(1C), 111.10 (1C), 114.64 (1C), 114.70 (1C), 116.12 (1C), 116.20 (1C), 119.54 (1C), 128.72 (1C), 128.79 (1C), 129.66 (1C), 129.74 (1C), 130.30 (1C), 140.36 (1C), 142.12 (1C, 3-C), 144.86 (1C), 162.56 (1C), 205.80 (1C, C=O). MS (*m/z*): 390.08 (M+, 100); Anal. Calcd. for $C_{23}H_{22}N_2O_4$ (%): C, 70.75; H, 5.68; N, 7.17; Found: C, 70.61; H, 5.52; N, 7.05.

***2.3.4. 1-(4-(Furan-2-yl)-1-phenyl-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)ethanone* 5d**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(4-methylbenzylidene)-2-phenylhydrazine **3d** (5 mmol) as gummy mass in 67% yield.^{1}H NMR ($CDCl_3$; δ ppm): 2.001 (s, 3H, CH_3), 2.231 (s, 3H, CH_3), 3.423 (d, 1H, *J=7.4Hz*, 4-H), 3.812 (d, 1H, *J=6.9Hz*, 5-H), 6.499-6.981 (m, 2H, Ar-H), 6.530-7.608 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.44 (1C, CH_3), 38.36 (1C, 4-C), 71.28 (1C, 5-C), 106.23 (1C), 109.57(1C), 111.45 (1C), 114.78 (1C), 114.98 (1C), 116.46 (1C), 116.87 (1C), 119.14 (1C), 128.65 (1C), 128.09 (1C), 129.77 (1C), 129.84 (1C), 130.54 (1C), 140.23 (1C), 142.47 (1C, 3-C), 144.69 (1C), 162.38 (1C), 205.76 (1C, C=O). MS (*m/z*): 344.11 (M+, 100); Anal. Calcd. for $C_{22}H_{20}N_2O_2$ (%): C, 76.72; H, 5.85; N, 8.13; Found: C, 74.61; H, 5.72; N, 7.98.

***2.3.5. 1-(3-(4-Fluorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone* 5e**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(4-fluorobenzylidene)-2-phenylhydrazine **3e** (5 mmol) as gummy solid in 73% yield.^{1}H NMR ($CDCl_3$; δ ppm): 2.180 (s, 3H, CH_3), 3.690 (d, 1H, *J=7.6Hz*, 4-H), 4.010 (d, 1H, *J=7.1Hz*, 5-H), 6.126-6.390 (m, 2H, Ar-H), 7.281-7.802 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.56 (1C, CH_3), 38.55 (1C, 4-C), 71.46 (1C, 5-C), 106.15 (1C), 111.10 (1C), 114.64 (1C), 114.70 (1C), 116.12 (1C), 116.20 (1C), 119.54 (1C), 128.72 (1C), 128.79 (1C), 129.66 (1C), 129.74 (1C), 130.30 (1C), 140.36 (1C), 142.12 (1C, 3-C), 144.86 (1C), 162.56 (1C), 205.80 (1C, C=O). MS (*m/z*): 348.13 (M+, 100); Anal. Calcd. for $C_{21}H_{17}FN_2O_2$ (%): C, 72.40; H, 4.92; N, 8.04; Found: C, 72.26; H, 4.71; N, 7.88.

***2.3.6. 1-(3-(4-Chlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone* 5f**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(4-chlorobenzylidene)-2-phenylhydrazine **3f** (5 mmol) as gummy mass in 80% yield.^{1}H NMR ($CDCl_3$; δ ppm): 2.099 (s, 3H, CH_3), 3.542 (d, 1H, *J=7.3Hz*, 4-H), 3.810 (d, 1H, *J=7.0Hz*, 5-H), 7.326-7.892 (m, 2H, Ar-H), 7.153-7.655 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.17 (1C, CH_3), 38.65 (1C, 4-C), 71.32 (1C, 5-C), 106.24 (1C), 109.24(1C), 111.65 (1C), 114.37 (1C), 114.54 (1C), 116.39 (1C), 116.86 (1C), 119.48 (1C), 128.61 (1C), 128.81 (1C), 129.45 (1C), 129.86 (1C), 130.13 (1C), 140.25 (1C), 142.09 (1C, 3-C), 144.67 (1C), 162.47 (1C), 205.55 (1C, C=O). MS (*m/z*): 366.05 (M+2, 33), 364.06 (M+, 100); Anal. Calcd. for $C_{21}H_{17}ClN_2O_2$ (%): C, 69.14; H, 4.70; N, 7.68; Found: C, 69.02; H, 4.58; N, 7.49.

***2.3.7. 1-(3-(2,3-Dichlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone* 5g**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(2,3-dichlorobenzylidene)-2-phenylhydrazine **3g** (5 mmol) as gummy mass in 65% yield.^{1}H NMR ($CDCl_3$; δ ppm): 2.063 (s, 3H, CH_3), 3.654 (d, 1H, *J=7.2Hz*, 4-H), 4.230 (d, 1H, *J=7.6Hz*, 5-H), 7.301 (m, 1H, Ar-H), 6.659-7.631 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.38 (1C, CH_3), 38.43 (1C, 4-C), 71.76 (1C, 5-C), 106.29 (1C), 109.22(1C), 111.21 (1C), 114.54 (1C), 114.65 (1C), 116.15 (1C), 116.54 (1C), 119.38 (1C), 128.33 (1C), 128.49 (1C), 129.68 (1C), 129.88 (1C), 130.44 (1C), 140.45 (1C), 142.43 (1C, 3-C), 144.65 (1C), 162.39 (1C), 205.76 (1C, C=O). MS (m/z): 400.08(M+2, 33), 398.03

(M+, 100); Anal. Calcd. for $C_{21}H_{16}Cl_2N_2O_2$ (%): C, 63.17; H, 4.04; N, 7.02; Found: C, 63.06; H, 3.88; N, 6.90.

***2.3.8. 1-(3-(2,4-Dichlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone* 5h**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(2,4-dichlorobenzylidene)-2-phenylhydrazine **3h** (5 mmol) as gummy mass in 69% yield.^{1}H NMR ($CDCl_3$; δ ppm): 2.076 (s, 3H, CH_3), 3.427 (d, 1H, *J=7.2Hz*, 4-H), 3.620 (d, 1H, *J=7.5Hz*, 5-H), 7.645 (m, 1H, Ar-H), 6.123-7.466 (m, 10H, Ar-H);^{13}C NMR ($CDCl_3$; δ ppm): 24.48 (1C, CH_3), 38.66 (1C, 4-C), 71.64 (1C, 5-C), 106.31 (1C), 109.09(1C), 111.17 (1C), 114.48 (1C), 114.68 (1C), 116.16 (1C), 116.32 (1C), 119.75 (1C), 128.65 (1C), 128.87 (1C), 129.56 (1C), 129.88 (1C), 130.26 (1C), 140.54 (1C), 142.23 (1C, 3-C), 144.65(1C), 162.48 (1C), 205.75 (1C, C=O).MS (m/z): 402.03 (M+4, 64.5); 400.04 (M+2, 13); 398.03 (M+, 100); Anal. Calcd. for $C_{21}H_{16}Cl_2N_2O_2$ (%): C, 63.17; H, 4.04; N, 7.02; Found: C, 63.11; H, 3.87; N, 6.88.

***2.3.9. 1-(4-(Furan-2-yl)-3-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone* 5i**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(4-nitrobenzylidene)-2-phenylhydrazine **3i** (5 mmol) as gummy mass in 80% yield.^{1}H NMR ($CDCl_3$; δ ppm): 2.023 (s, 3H, CH_3), 3.764 (d, 1H, *J=6.9Hz*, 4-H), 3.450 (d, 1H, *J=7.1Hz*, 5-H), 7.843-7.203 (m, 2H, Ar-H), 6.992-7.956 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 24.38 (1C, CH_3), 38.47 (1C, 4-C), 71.07 (1C, 5-C), 106.01 (1C), 109.11(1C), 111.14 (1C), 114.54 (1C), 114.65 (1C), 116.09 (1C), 116.32 (1C), 119.67 (1C), 128.48 (1C), 128.88 (1C), 129.75 (1C), 129.86 (1C), 130.28 (1C), 140.26 (1C), 142.43 (1C, 3-C), 144.78 (1C), 162.47 (1C), 205.98 (1C, C=O). MS (m/z): 375.09 (M+, 100); Anal. Calcd. for $C_{21}H_{17}N_3O_4$ (%): C, 67.19; H, 4.56; N, 11.19; Found: C, 67.08; H, 4.37; N, 10.98.

***2.3.10. 1-(5-(Furan-2-yl)-3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)ethanone* 6b**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(4-methoxybenzylidene)-2-phenylhydrazine **3b** (5 mmol) as gummy mass in 16% yield. ^{1}H NMR ($CDCl_3$; δ ppm): 2.364 (s, 3H, CH_3), 2.887 (d, 1H, *J=6.8Hz*, 4-H), 3.53 (s, 3H, OCH_3), 4.310 (d, 1H, *J=7.0Hz*, 5-H), 6.846-7.812 (m, 2H, Ar-H), 6.245-7.723 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 27.28 (1C, CH_3), 53.67 (1C, 5-C), 56.76 (1C, 4-C), 107.17 (1C), 111.31 (1C), 114.58 (1C), 114.76 (1C), 116.18 (1C), 116.43(1C), 119.45 (1C), 128.43 (1C), 128.59 (1C), 129.44 (1C), 129.57 (1C), 130.67 (1C), 140.43 (1C), 144.65 (1C), 147.23 (1C, 3-C), 158.67 (1C), 162.54 (1C), 201.76 (1C, C=O). MS (*m/z*): 360.08 (M+, 100); Anal. Calcd. for $C_{22}H_{20}N_2O_3$ (%): C, 73.32; H, 5.59; N, 7.77; Found: C, 73.16; H, 5.42; N, 7.60.

***2.3.11. 1-(3-(4-Fluorophenyl)-5-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)ethanone* 6e**: Obtained from 4-(furan-2-yl)but-3-en-2-one **4** (5 mmol) and 1-(4-fluorobenzylidene)-2-phenylhydrazine **3b** (5 mmol) as oily mass in 20% yield.^{1}H NMR ($CDCl_3$; δ ppm): 2.286 (s, 3H, CH_3), 2.996 (d, 1H, *J=7.0Hz*, 4-H), 4.230 (d, 1H, *J=7.2Hz*, 5-H), 6.456-6.602 (m, 2H, Ar-H), 7.324-7.816 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$; δ ppm): 27.33 (1C, CH_3), 53.40 (1C, 5-C), 56.51 (1C, 4-C), 107.10 (1C), 111.26 (1C), 114.60 (1C), 114.65 (1C), 116.15 (1C), 116.22 (1C), 119.55 (1C), 128.56 (1C), 128.64 (1C), 129.33 (1C), 129.40 (1C), 130.35 (1C), 140.53 (1C), 144.90 (1C), 147.10 (1C, 3-C), 162.30 (1C), 201.86 (1C, C=O). MS (*m/z*): 348.06 (M+, 100); Anal. Calcd. for $C_{21}H_{17}FN_2O_2$ (%): C, 72.40; H, 4.92; N, 8.04; Found: C, 72.20; H, 4.75; N, 7.94.

2.4. Biological evaluation of 2-pyrazoline derivatives 5(a-i)

2.4.1. Antioxidant activity. The antioxidant potential of the synthesized 2-pyrazolines **5(a-i)** was performed by 1,1-Diphenylpicrylhydrazyl (DPPH) radical scavenging assay [23]. 1 mL of DPPH solution (0.1mM in 95% methanol) was mixed with different aliquots of test samples (25, 50, 75 and 100 µg/mL) in methanol. The mixture was shaken vigorously, and allowed to stand for 20 min at room temperature. The absorbance was read against blank at 517nm in an ELICO SL-159 UV-Vis spectrophotometer. Radical scavenging potential was calculated as a percentage (I %) of DPPH decoloration using the equation; I% = $(A_0-A_1/A_0) \times 100$

A_0 = absorbance of the control without test compounds; A_1 = absorbance of test compounds.

2.4.2. Antimicrobial activity. Antimicrobial activities of the synthesized compounds were determined as minimum inhibitory concentrations (MIC's) by serial dilution method [24]. The nutrient broth, which contains logarithmic serially two-fold diluted amount of compounds **5(a–i)**, and control was inoculated with approximately 5 x 10^5 c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 h at 37ºC for bacterial, and 72 h at 37 ºC for fungal stains and the growth was monitored visually. The tests were conducted in triplicates against bacterial pathogens *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*; and against fungal stains *Aspergillus niger*, *Aspergillus flavus* and *Candila albicans.* Ciprofloxacin and nystatin were used as positive controls against bacterial and fungal species, respectively; methanol was used as solvent control.

2.5. Molecular docking and ADMET studies

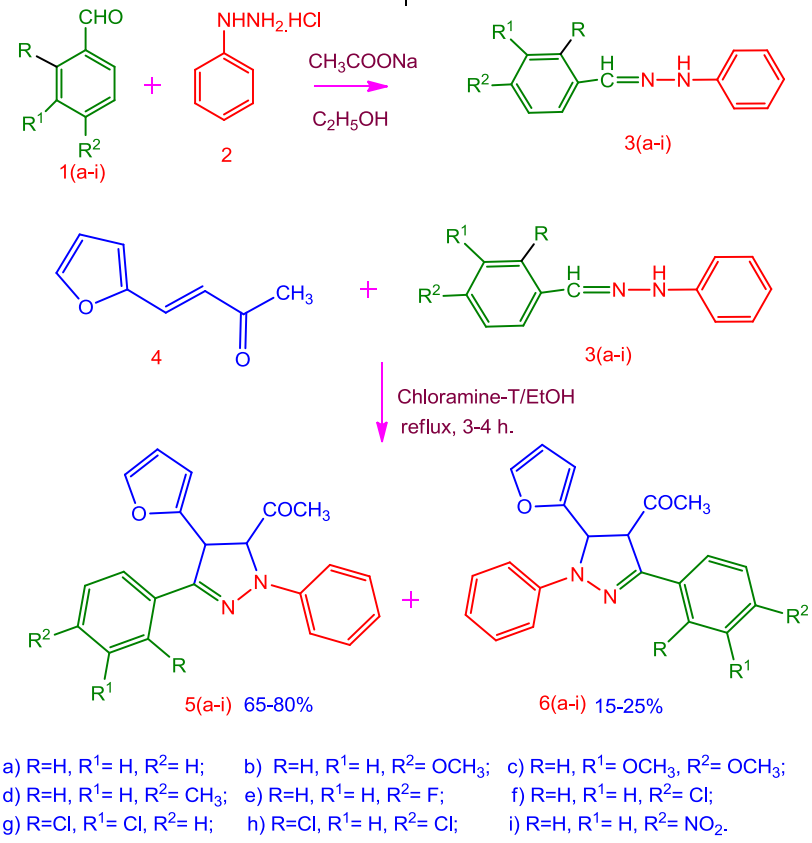
The structural drawing and geometry cleaning of novel potential leads **5(a-i)** were performed in Maestro 9.3 of Schrödinger suite 2012 platform and then subjected to other parameters *via* energy minimization by using OPLS 2005 force field, addition of hydrogen atoms, neutralization of charged groups, generation of ionization states and set pH 7.5 using Epik. Generation of tautomers and stereoisomers of 32 per ligand and low-energy ring conformations and optimize the geometries followed by generating low energy ring conformation per ligand were computed, optimized by LigPrep and used for molecular docking.

The co-ordinates of Sec3p - Rho1p complex from *S. cerevisiae*, Mevalonate 5-diphosphate decarboxylase from *E. coli*, and Copper, Zinc Superoxide dismutase were obtained from the Brookhaven Protein Data Bank, whose PDB ids are 3A58, 1FI4 and 1CB4 respectively. Crystal structure was imported and refined by a multistep process through the protein preparation wizard of Maestro 9.3, which includes energy minimization using OPLS-2005 force field, correct bond orders were assigned, hydrogen atoms were added and the water molecules where removed beyond 5Å from hetero atom, formal charges, amide groups of Asn and Gln were optimized. All amino acid flips were assigned to correct geometry and hydrogen bonds were optimized. Using PROPKA,

pH was fixed and optimized to 7.5. Non-hydrogen atoms were minimized by restrained minimization to default RMSD to 0.3Å. Using Extra-precision (XP) docking and scoring each compound were docking into the receptor grid of radii 20Å × 20Å × 20Å and the docking calculation were judge based on the Glide score. ADMET, the prediction program was used to calculate ADMET properties using Discovery 2.5v and molecular visualization was done under Maestro [23].

3. RESULTS SECTION

3.1. Chemistry. Chloramine-T served as an effective catalytic dehydrogenating agent for the conversion of hydrazones to nitrile imines, and also effective aziridination agent for alkenes [25]. Initially, the precursor aldehyde hydrazones **3(a–i)** were prepared by reacting aromatic aldehydes **1(a–i)** with phenylhydrazine hydrochloride **2** and sodium acetate in ethanol. Then, the oxidative dehydrogenation of reaction of hydrazones **3(a–i)** by chloramine-T afforded nitrile imines, which were *in situ* trapped by 4-(furan-2-yl)but-3-en-2-one **4** to obtain isormeric mixture of 1,3-diaryl-1-(4-(furan-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5(a–i)** (major) and 1,3-diaryl-1-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-4-yl)ethanone **6(a–i)** (minor) (**Fig. 1**).



a) R=H, R^1= H, R^2= H; b) R=H, R^1= H, R^2= OCH_3; c) R=H, R^1= OCH_3, R^2= OCH_3;
d) R=H, R^1= H, R^2= CH_3; e) R=H, R^1= H, R^2= F; f) R=H, R^1= H, R^2= Cl;
g) R=Cl, R^1= Cl, R^2= H; h) R=Cl, R^1= H, R^2= Cl; i) R=H, R^1= H, R^2= NO_2.

Figure 1. Schematic diagram for the synthesis of 2-pyrazolines by 1,3-dipolar cycloaddition.

In search of new potent antioxidant and antimicrobial agents, we successfully carried out 1,3-dipolar cycloaddition reaction of 4-(furan-2-yl)but-3-en-2-one **4** with aldehyde hydrazones, **3(a-i)** using chloramine-T as catalytic dehydrogenating agent. Nitrile imines generated *in situ* by the dehydrogenation of aldehyde hydrazones **3(a-i)** were trapped *in situ* by 4-(furan-2-yl)but-3-en-2-one **4** to obtain a enantiomeric mixture of 2-pyrazolines **5(a-i)** and **6(a-i)**.

^{1}H NMR, ^{13}C NMR, Mass spectra and elemental analysis provided the structural proof of the compounds **5(a-i)** and **6(a-i)**. In ^{1}H NMR spectrum, compound 1-(3-(4-fluorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5e** showed a singlet at δ 2.180 ppm due to CH_3 protons. 4-H coupled with 5-H proton and appears as doublet at δ 3.690 (*J=7.6Hz*) ppm; while 5-H appears as doublet at δ 4.010 (*J=7.1Hz*) ppm. Multiplets appear at δ 6.126-6.390 ppm for two protons, and at δ 7.281-7.802 ppm for ten protons were unambiguously due to aromatic protons. In ^{13}C NMR spectrum, the carbons of newly formed pyrazole ring 4-C, 5-C, and 3-C coupled at δ 38.55, 71.46 and 142.12 ppm, respectively. Signals appeared at δ 24.56, and 205.80 ppm were due to CH_3, and C=O carbons. Aromatic carbons showed their signals in the aromatic carbons absorption region. Compound, **5e** showed a base peak at *m/z*: 348.13 correspond to its molecular mass. Synthesized compounds **5(a-g)** showed similar and consistent pattern signals in their respective spectra and gave satisfactorily elemental analyses data with theoretically calculated values confirms their structures. Coupling constants (*J*) of 4-H and 5-H (*J= 6.7-7.9Hz*) suggests that cycloaddition took place in *cis* fashion.

As the isomeric compounds **6(a-i)** were obtained as minor products, the characterization was done only for few compounds. Compound 1-(3-(4-fluorophenyl)-5-(furan-2-yl)-1-phenyl-4,5-

dihydro-1H-pyrazol-4-yl)ethanone**6e** showed a singlet at δ 2.286 and δ 3.840 ppm due to CH_3 protons. The 4-H resonates as doublet at δ 2.996 (*J=7.0Hz*) ppm; while the 5-H appears as doublet at δ 4.230 (*J=7.2Hz*) ppm. Multiplets appear at δ 6.456-6.602 ppm and at δ 7.324-7.816 ppm for two and five protons each were due to aromatic protons.The carbons of newly formed pyrazoline ring 5-C, 4-C, and 3-C coupled at δ 53.40, 56.51, and 147.10 ppm, respectively. Signals appeared at δ 27.33 and 201.86 ppm were due to CH_3 and C=O carbons. All other carbons absorbed in the aromatic region. Compound, **6e** showed a base peak at *m/z*: 348.06 correspond to its molecular mass.

3.2. Biological activity

3.2.1. Antioxidant activity. The DPPH radical scavenging abilities of 2-pyrazolines **5**(**a-i**) were performed in triplicate; the results were expressed as a mean ± standard deviation (SD) and were summarized in **Table 1**.

Table 1.DPPH radical scavenging activity of the synthesized 2-pyrazolines **5**(**a-i**).

Compounds	% Radical Scavenging*			
	25 (µg/mL)	50 (µg/mL)	75 (µg/mL)	100 (µg/mL)
5a	15.86±0.55	17.75±0.55	21.74±0.57	24.06±0.48
5b	15.26±0.48	17.22±0.55	21.30±0.40	24.05±0.60
5c	12.34±0.51	12.85±0.60	18.20±0.70	20.30±0.52
5d	13.55±0.85	14.30±0.66	16.70±0.66	21.84±0.95
5e	13.70±0.44	14.40±0.36	17.50±0.70	20.22±0.64
5f	20.15±0.66	25.45±0.80	29.10±0.61	39.00±0.46
5g	19.98±0.85	24.80±0.60	29.95±0.65	39.60±0.76
5h	20.42±0.64	25.36±0.51	33.80±0.41	38.40±0.55
5i	11.85±0.65	14.92±0.75	17.86±0.68	18.90±0.75
AA	15.08±0.89	17.87±0.89	21.98±0.31	24.25±0.22

* Values are mean ± SD of three replicates; AA=Ascorbic acid (positive control).

The results shows that, amongst the series, compounds 1-(3-(4-chlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5f**, 1-(3-(2,3-dichlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5g** and 1-(3-(2,4-dichlorophenyl) – 4-(furan-2-yl) – 1 – phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5h**showed the activity which is incidentally greater than the standard ascorbic acid. Compound 1-(4-(furan-2-yl)-3-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5i** having strong electron withdrawing nitro substitution showed lesser radical scavenging abilities amongst the series. Compounds **5a** and **5e** having no and fluoro substitutions shows good activities; while the compounds **5b, 5c** and **5d** having electron donating methoxy substitutions in the aromatic ring exhibited moderate radical scavenging potentials.

3.2.2 ***Antimicrobialactivity.*** The antimicrobial screening tests of synthesized pyrazoles **5**(**a-i**) were performed in triplicate and the results were taken as a mean ± standard deviation (SD) and are summarized in **Table 2**.

Table 2. Minimum inhibitory concentration (MIC) of compounds **5**(**a**–**i**) (in µg/mL*).

Compound	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*A. flavus*	*C. albicans*
5a	87.5±0.35	50.0±0.70	25.0±0.62	67.5±0.46	75.0±0.55	67.5±0.35
5b	87.5±0.35	87.5±0.80	67.5±0.65	67.5±0.50	87.5±0.45	87.5±0.45
5c	75.0±0.45	75.5±0.50	37.5±0.45	75.5±0.70	75.0±0.65	75.0±0.40
5d	50.0±0.66	67.5±0.40	50.0±0.50	67.5±0.55	87.5±0.56	87.5±0.55
5e	37.5±0.50	37.5±0.56	50.0±0.51	87.5±0.30	37.5±0.90	67.5±0.75
5f	12.5±0.55	25.0±0.45	12.5±0.38	12.5±0.65	25.0±0.86	50.0±0.50
5g	25.0±0.60	12.5±0.40	12.5±0.30	25.0±0.52	25.0±0.45	37.5±0.46
5h	12.5±0.56	12.5±0.76	12.5±0.70	12.5±0.55	25.0±0.40	25.0±0.75
5i	75.0±0.56	87.5±0.45	37.5±0.35	75.0±0.55	87.5±0.45	87.5±0.60
Cipro[a]	25.0±0.40	25.0±0.60	12.5±0.50	----	----	----
Nyst[b]	----	----	----	25.0±0.45	25.0±0.35	50.0±0.90

*Values are mean ± SD of three replicates; [a]Ciproafloxacin-positive control against bacteria species; [b]Nystatin-positive control against fungi species.

From the preliminary studies, it was observed that, compounds 1-(3-(4-chlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5f**,1-(3-(2,3-dichlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone**5g**and 1-(3-(2,4-dichlorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5h** with chloro substitutions in the aromatic ring showed excellent inhibition potential against all the tested organisms. Compound 1-(3-(4-fluorophenyl)-4-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5e** having fluro substitution shows moderate inhibition against *S. aureus, E. coli, A. flavus, C. albicans* less active against *P. aeruginosa* and *A. niger*; while 1-(4-(furan-2-yl)-1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone **5a** found moderately active against *E. coli, P. aeruginosa* and less active against *S. aureus, A. flavus*and *A. niger* organisms. Compound **5i** having electron withdrawing nitro substitution in the aromatic ring showed poorer activities against the tested organisms. In comparison with the standards; compounds **5f**, **5g** and **5h** having chloro substitutions excellent inhibition abilities against all tested organisms. While compounds, **5b, 5c** and **5d** having electron donating methoxy substitutions exhibited moderate to lesser inhibitory effects.

3.2.3. Molecular docking and ADMET. Molecular docking has carried out for novel potential leads **5(a-i)**. Docking results induced comparative investigation based on docking score of the hits with that of the reported inhibitors. Oxidative stress induced by ROS brings about various enzymatic activities to scavenge the free radicals. Molecular docking studies showed an interacting map of Sec3p - Rho1p complex from *S. cerevisiae* (**Fig. 2A**), Mevalonate 5-diphosphate decarboxylase from *E. coli*) (**Fig. 2B**) and Copper, Zinc Superoxide dismutase (**Fig. 2C**) with compound **5h, 5h** and **5b** respectively.Compound **5h** showed π-π stacking with Phe35 and salt bridge along with hydrogen bond with Lys167 and Lys123 with Sec3p - Rho1p complex from *S. cerevisiae*, whereas withCopper, Zinc Superoxide dismutase, 5b interacted with Gly106 via hydrogen bond and π-π stacking with Arg113. Based on XP glide score, **5h** and **5b** showed a promising scoring function, when compared to other structurally related compounds as tabulated in **Table 3**. Pharmacodynamics parameters play an important role to determine the success of drug for therapeutic use. ADMET descriptors simulation for all the compounds showed promising values which follows within the range as indicated in **Fig. 3** [20].

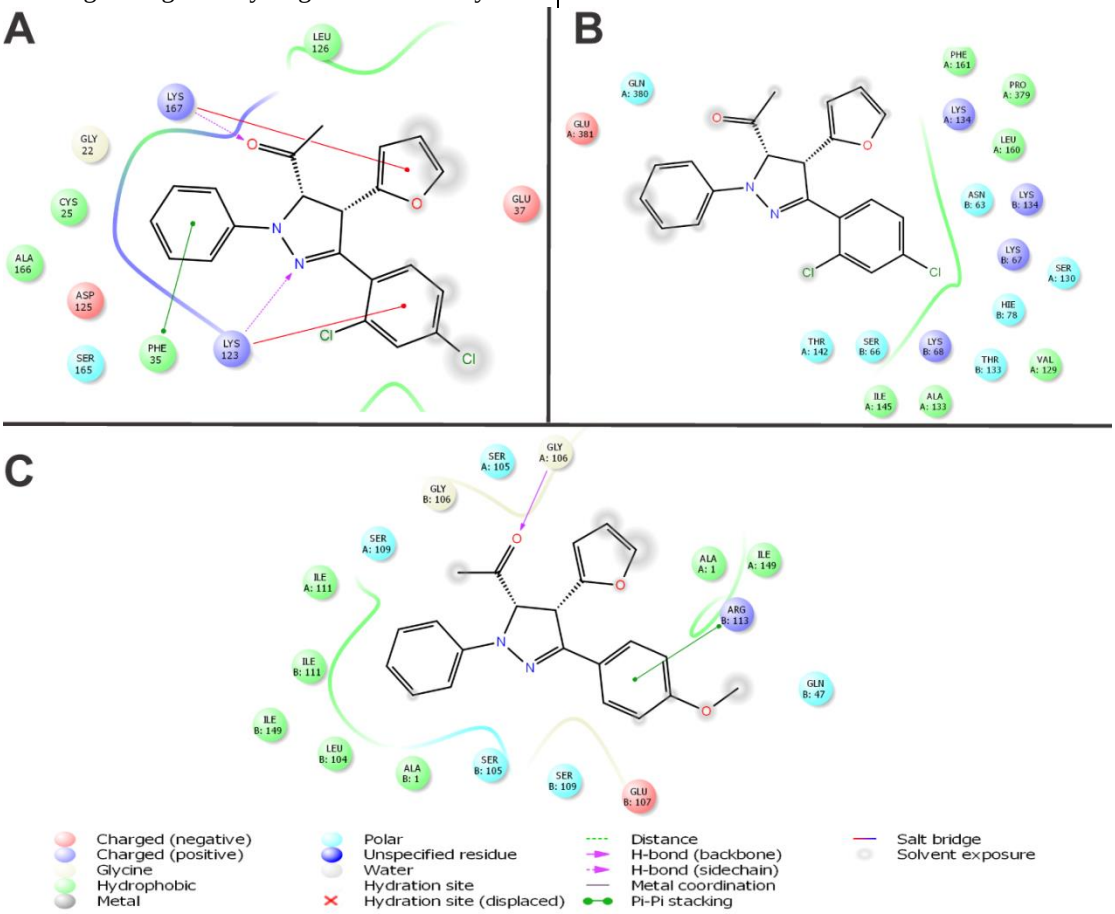


Figure 2. Molecular docking interactive map of compound **5h** into the Sec3p - Rho1p complex from *S. cerevisiae* (A), Mevalonate 5-diphosphate decarboxylase from *E. coli* (B) and compound **5b** with Copper, Zinc Superoxide dismutase binding pocket.

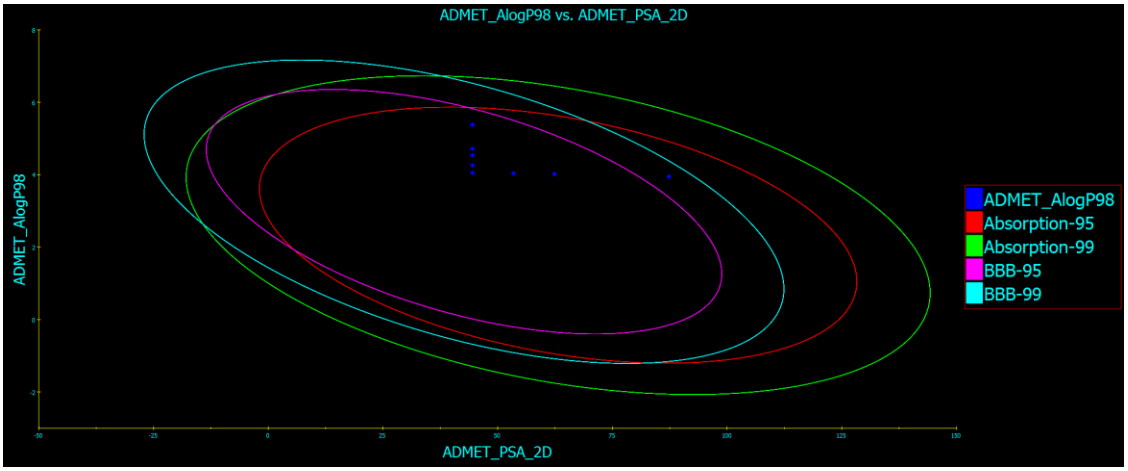


Figure 3. ADMET interactive descriptors evaluation of novel compounds **5(a-i)**. Blue dots represent compounds.

Table 3. Molecular docking scores of all the synthesized compounds against Sec3p - Rho1p complex from *S. cerevisiae*, Mevalonate 5-diphosphate decarboxylase and Copper, Zinc Superoxide dismutase as obtained through Glide docking.

Compounds	3A58	1FI4	1CB4

	glide gscore	glide emodel	glide gscore	glide emodel	glide gscore	glide emodel
5a	-3.04	-32.92	-2.96	-32.76	-2.06	-46.87
5b	-2.02	-32.58	-3.34	-45.70	**-3.00**	**-41.43**
5c	-3.02	-30.63	-4.24	-42.21	-2.11	-44.88
5d	-2.45	-36.58	-2.14	-46.91	-1.97	-45.26
5e	-2.96	-37.65	-2.04	-47.01	-1.97	-40.61
5f	-2.62	-48.76	-1.77	-48.57	-1.77	-45.69
5g	-2.98	-35.18	-2.11	-48.00	-1.63	-41.53
5h	**-3.08**	**-39.50**	**-4.32**	**-40.79**	-1.02	-41.63
5i	0.91	-56.55	-2.01	-45.07	1.43	-38.54
Ciprofloxacin	-5.79	-46.88	-3.02	-42.67	--	--
Ascorbic acid	--	--	--	--	-4.21	-45.23

4. CONCLUSIONS

In the present study, we report the synthesis of isomeric mixture of pyrazoles via 1,3-dipolar cycloaddition reactions. The presence of chloro substitutions in the aromatic ring of the substituted pyrazoles was anticipated to key function for their biological potency. *In vitro* pharmacological evaluation results showed that, among the synthesized series, compounds **5f**, **5g**, and **5h** showed excellent DPPH radical scavenging potential, and antimicrobial potential against the tested *S. aureus*, *E. coli*, *P. aeruginosa*, *A. niger*, *A. flavus* and *C. albicans* organisms.

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