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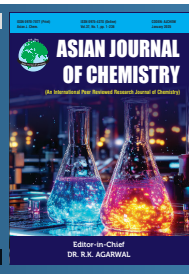

Synthesis and Biological Assessment of Novel Pyridine-Pyrazole Hybrids: Molecular Docking and Antioxidant Studies

SAI PRASAD D. PHAYDE and C.B. VAGISH*

Department of Physical and Chemical Sciences, Sri Sathya Sai University for Human Excellence, Kalaburagi-585313, India

*Corresponding author: E-mail: vagish.cb@sssuhe.ac.in

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In this study, pyridine-appended pyrazole derivatives were synthesized through chalcone formation using 3-acetylpyridine and 3-pyridinecarboxaldehyde, followed by reaction with substituted phenyl hydrazines under mild conditions. The methodology offered good yields and easy purification *via* column chromatography. The structural characterization was performed using ^{1}H NMR, ^{13}C NMR and mass spectrometry. Density functional theory (DFT) studies at the B3LYP/6-31G level provided insights into electronic properties, including HOMO-LUMO energy gaps and molecular electrostatic potential (MEP), predicting the biological interactions. Biological evaluation using the DPPH radical scavenging assay confirmed significant antioxidant activity, highlighting the potential of these compounds in the medicinal chemistry.

Keywords: Pyrazole-pyridine hybrids, Chalcone, Molecular docking studies, Antioxidant assay.

INTRODUCTION

Chalcones are one of the unique chemical structures found in many natural products which belong to the family of flavonoids and are of considerable interest in medicinal chemistry [1]. Recently, natural chalcones and synthetic derivatives have been reported with various biological and pharmacological activities [2-4]. Chalcone derivatives with heterocycles in particular are gaining more prominence as potential drug candidates owing to their comparable or superior activities with the existing drugs [5,6]. For this reason, a lot of research is now focused on planning to design and develop new chalcones incorporating heterocyclic scaffolds.

Hybridization of various heterocyclic compounds represents a deliberate approach in the synthesis of novel bioactive molecules by fusing two or more pharmacologically active compounds. This process enables the resultant hybrid molecules to act on multiple targets simultaneously, thereby harnessing the synergistic effects from each pharmacophore [7-9]. Pyrazole, a heterocyclic molecule having two nitrogens in its structure, has gathered considerable interest because of its diverse biological activities like antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anti-tubercular and anticancer properties [10-15]. Similarly, pyridine also displays a variety of biological applications and is the second most prevalent heterocycle in the drugs approved by FDA [16-19]. Because of their extensive chemical properties, which allow them to engage in several kind of biochemical interactions, pyridine derivatives are extensively used as ligands in the formation of coordination compounds which find use as medicinal agents like anti-tubercular, anti-tumor, antiviral, antioxidant, anti-inflammatory, antimicrobial activity, *etc*. [20-25].

In a quest to seek novel antioxidant candidates, we were encouraged to synthesize a series of pyridine-pyrazole hybrids and evaluated them for their molecular reactivity relationship using *in silico* docking. Furthermore, the synthesized compounds were also evaluated for antioxidant activity using DPPH scavenging method.

EXPERIMENTAL

All the solvents, reagents and chemicals were of AR grade and purchased from Sigma-Aldrich, India. TLC with silica gel 60 F_{254} aluminium sheets were used to monitor the progress of the reaction and the spots were visualized with UV light. ^{1}H NMR spectra were recorded on Agilent-NMR 400 MHz

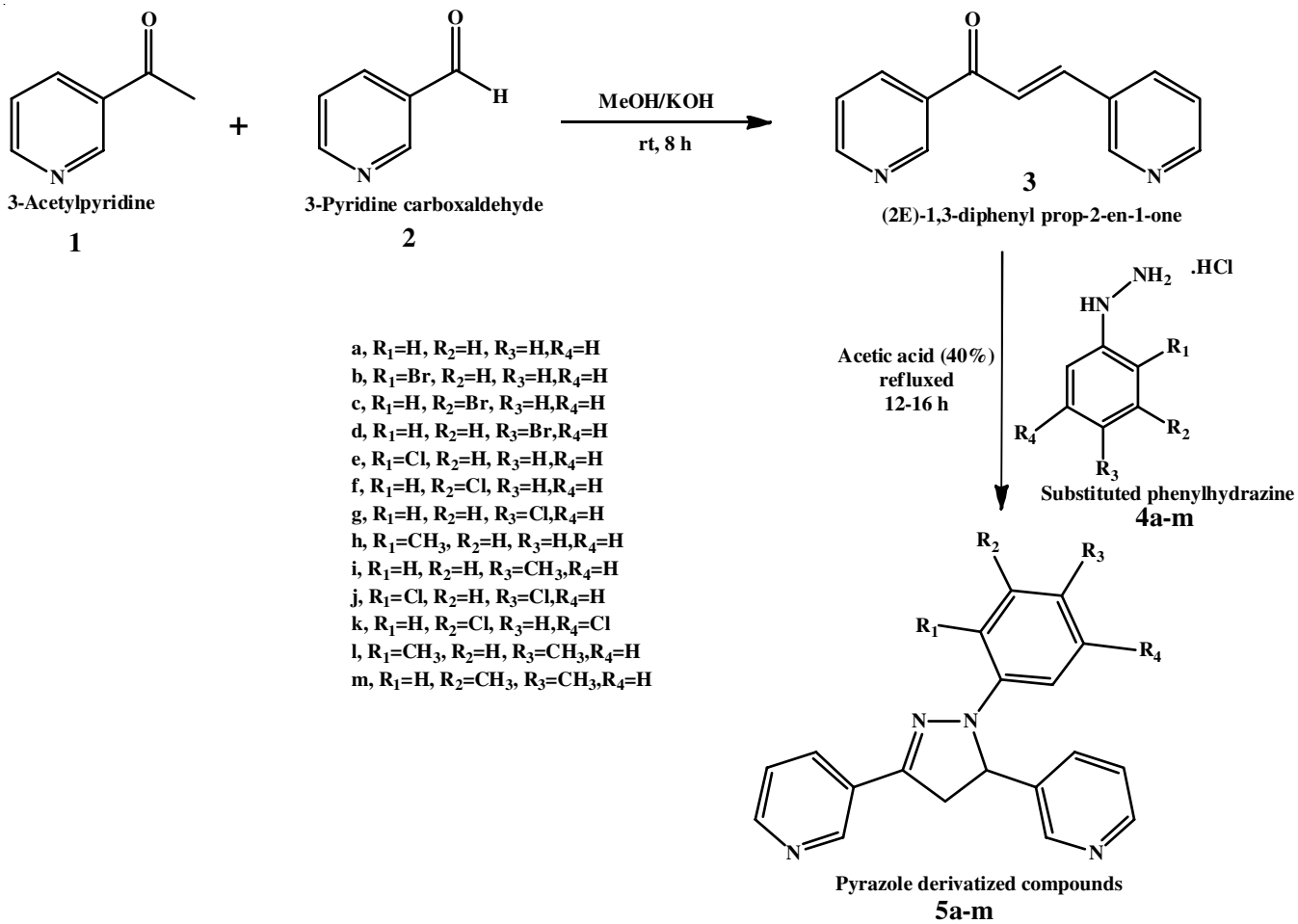
spectrophotometer. $CDCl_3$ and/or DMSO-d_6 was used as solvent with tetramethylsilane (TMS) as an internal standard. ^{13}C NMR spectra were recorded on Agilent-NMR 100 MHz spectrophotometer and $CDCl_3$ and/or DMSO-d_6 was used as solvent with tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on Mass Lynx SCN781 spectrophotometer TOF mode. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane:ethyl acetate and/or chloroform: methyl alcohol as eluents at different ratio.

Synthesis of pyrazole-based Compounds: In first step, chalcone was synthesized using Claisen-Schmidt reaction of 3-acetylpyridine (1.10 g, 10 mmol) and 3-pyridinecarboxaldehyde (1.25 g, 10 mmol) in methyl alcohol at room temperature, with agitation maintained for 8 h. Then, chalcone (**3**, 2.10 g, 10 mmol) was reacted with subsituted phenyl hydrazine hydrochloride mixed thoroughly in a round bottom flask containing 40% acetic acid and refluxed for 12-16 h [26]. TLC was utilized to monitor the progress of the reaction. The reaction mixture was cooled and poured into a crushed ice. The obtained solid were filtered, washed with water and recrystallized from ethyl alcohol to obtain the pyridine-substituted pyrazoles (**Scheme-I**).

(*E*)-1,3-Di(pyridin-3-yl)prop-2-en-1-one (3): Yield: 68%; ^1H NMR ($CDCl_3$, δ ppm): 6.892-6.904 (s, 1H, CH=), 7.103-7.124 (s, 1H, Ar-H), 7.239-7.303 (s, 1H, Ar-H), 7.378-7.381 (s, 2H, Ar-H), 7.398-7.400 (s, 2H, Ar-H), 7.418-7.420 (m, 2H, Ar-H), 7.536 (s, 2H, Ar-H), 7.578-7.596 (m, 1H, Ar-H), 8.017-8.054 (d, 1H =CH); ^{13}C NMR ($CDCl_3$, δ ppm): 113.64 (1C), 113.78 (1C), 119.57 (1C), 126.98 (1C), 130.61 (1C), 131.10 (1C), 131.39 (1C), 134.64 (1C), 134.74 (1C), 142.35 (1C), 163.32 (1C), 187.64 (1C, C=O). MS *m/z*: 211.40 (M+1, 100), 212.40 (M+2, 30); Elemental analysis of $C_{13}H_{10}N_2O$; calcd. (found) (%): C, 74.27 (74.10); H, 4.79 (4.56); N, 13.33 (13.12); O, 7.61 (7.50).

3,3′-(1-Phenyl-4,5-dihydro-1*H*-pyrazole-3,5-diyl)-dipyridine (5a): Yield: 70%. ^1H NMR ($CDCl_3$, δ ppm): 3.152-3.213 (dd, 1H, C_4-H_a), 3.768-3.841 (dd, 1H, C_4-H_b), 5.389-5.438 (dd, 1H, C_5-H), 6.744-6.841 (m, 3H, Ar-H), 7.047-7.086 (m, 2H, Ar-H), 7.187-7.247 (m, 4H, Ar-H), 7.609-7.629 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 43.14 (1C, C-4), 59.10 (1C, C-5), 108.29 (1C), 110.62 (1C), 111.33 (1C), 113.85 (1C), 119.33 (1C), 123.61 (1C), 125.20 (1C), 129.81 (1C), 130.34 (1C), 132.79 (1C), 134.66 (1C), 139.10 (1C), 146.32 (1C), 148.29 (1C), 149.18 (1C), 150.26 (1C, C-3). MS *m/z*: 300.03 (M+, 100); Elemental analysis of $C_{19}H_{16}N_4$; calcd. (found) (%): C, 75.98 (75.66); H, 5.37 (5.09); N, 18.65 (18.48).

3,3′-(1-(2-Bromophenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5b): Yield: 64% yield. ^1H NMR ($CDCl_3$, δ ppm): 3.10 (2H, dd), 5.87 (1H, d), 6.98 (1H, d), 7.19 (1H, s), 7.32 (1H, d), 7.37 (1H, d), 7.40 (1H, d), 7.49 (1H, d), 7.64 (2H, d), 7.77 (1H, d), 8.46 (1H, s), 8.48 (1H, s), 8.52 (1H, d),



Scheme-I: Synthesis of pyridine appended pyrazoles

8.76 (1H, dd). ^{13}C NMR ($CDCl_3$, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 113.6 (1C, s), 117.7 (1C, s), 123.4 (1C, s), 123.5 (1C, s), 127.8 (1C, s), 128.2 (1C, s), 128.2 (1C, s), 133.0 (1C, s), 134.3 (1C, s), 135.3 (1C, s), 139.5 (1C, s), 139.5 (1C, s), 148.6 (1C, s), 148.6 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS *m/z*: 378 (M+, 100), 380 (M+2, 99). Elemental analysis of $C_{19}H_{15}N_4Br$; calcd. (found) (%): C, 60.15 (59.87); H, 3.95 (3.85); Br, 21.10 (20.82); N, 14.80 (14.65).

3,3′-(1-(3-Bromophenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5c): Yield: 58%, ^{1}H NMR ($CDCl_3$, δ ppm): 3.09 (2H, dd), 5.84 (1H, d), 6.92 (1H, d), 7.22 (1H, s), 7.35 (1H, s), 7.36 (1H, d), 7.40 (1H, d), 7.64 (1H, d), 7.75 (1H, s), 7.77 (1H, d), 8.46 (1H, s), 8.48 (1H, s), 8.52 (1H, d), 8.75 (1H, dd). ^{13}C NMR ($CDCl_3$, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 116.4 (1C, s), 120.0 (1C, s), 122.8 (1C, s), 123.4 (1C, s), 123.4 (1C, s), 127.8 (1C, s), 130.1 (1C, s), 130.4 (1C, s), 134.3 (1C, s), 135.3 (1C, s), 138.1 (1C, s), 139.5 (1C, s), 148.6 (1C, s), 148.6 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 379 (M+, 100), 381 (M+2, 99). Elemental analysis of $C_{19}H_{15}N_4Br$; calcd. (found) (%): C, 60.15 (59.94); H, 3.95 (3.82); Br, 21.10 (20.80); N, 14.80 (14.71).

3,3′-(1-(4-Bromophenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5d): Yield: 62%, ^{1}H NMR ($CDCl_3$, δ ppm): 3.17 (2H, dd), 5.75 (1H, d), 7.37 (1H, d), 7.41 (1H, s), 7.66 (1H, d), 7.78 (1H, d), 8.48 (1H, s), 8.48 (1H, s), 8.49 (1H, d), 8.77 (1H, dd); ^{13}C NMR ($CDCl_3$, δ ppm): 46.1 (1C, s), 56.6 (1C, s), 123.4 (1C, s), 123.4 (1C, s), 127.8 (1C, s), 134.3 (1C, s), 135.3 (1C, s), 139.5 (1C, s), 148.6 (1C, s), 148.6 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 377 (M+, 100), 379 (M+2, 99). Elemental analysis of $C_{19}H_{15}N_4Br$; calcd. (found) (%): C, 60.15 (60.05); H, 3.95 (3.80); Br, 21.10 (20.90); N, 14.80 (14.75).

3,3′-(1-(2-Chlorophenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5e): Yield: 55%, ^{1}H NMR ($CDCl_3$, δ ppm): 3.10 (2H, dd), 5.87 (1H, d), 7.24 (1H, d), 7.32 (1H, s), 7.36 (1H, d), 7.40 (1H, d), 7.42 (1H, s), 7.47 (1H, d), 7.64 (1H, d), 7.77 (1H, d), 8.46 (1H, s), 8.48 (1H, s), 8.52 (1H, d), 8.76 (1H, dd); ^{13}C NMR ($CDCl_3$, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 117.7 (1C, s), 122.1 (1C, s), 123.4 (1C, s), 123.4 (1C, s), 127.8 (1C, s), 128.2 (1C, s), 128.3 (1C, s), 129.2 (1C, s), 133.6 (1C, s), 134.3 (1C, s), 135.3 (1C, s), 139.5 (1C, s), 148.6 (1C, s), 148.6 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 334 (M+, 100), 336 (M+2, 33). Elemental analysis of $C_{19}H_{15}N_4Cl$; calcd. (found) (%): C, 68.10 (67.72); H, 4.47 (4.22); Cl, 10.63 (10.13); N, 16.80 (16.45).

3,3′-(1-(3-Chlorophenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5f): Yield: 61%, ^{1}H NMR ($CDCl_3$, δ ppm): 3.10 (2H, dd), 5.76 (1H, d), 7.00 (1H, d), 7.17 (1H, d), 7.36 (1H, d), 7.37 (1H, s), 7.40 (1H, d), 7.77 (1H, d), 7.81 (1H, s), 8.02 (1H, d), 8.46 (1H, s), 8.48 (1H, s), 8.52 (1H, d), 8.75 (1H, dd); ^{13}C NMR ($CDCl_3$, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 117.6 (1C, s), 122.8 (1C, s), 123.4 (1C, s), 123.4 (1C, s), 127.0 (1C, s), 127.8 (1C, s), 130.0 (1C, s), 132.3 (1C, s), 134.3 (1C, s), 135.3 (1C, s), 138.1 (1C, s), 139.5 (1C, s), 148.6 (1C, s), 148.6 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 333 (M+, 100), 335 (M+2, 34). Elemental analysis of $C_{19}H_{15}N_4Cl$; calcd. (found) (%): C, 68.10 (67.60); H, 4.47 (4.29); Cl, 10.63 (10.20); N, 16.80 (16.37).

3,3′-(1-(4-Chlorophenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5g): Yield: 60%, ^{1}H NMR ($CDCl_3$, δ ppm): 3.11 (2H, dd), 5.83 (1H, d), 7.36 (1H, d), 7.40 (1H, d), 7.47 (1H, s), 7.65 (1H, d), 7.77 (1H, d), 8.02 (1H, d), 8.46 (1H, s), 8.48 (1H, s), 8.52 (1H, d), 8.75 (1H, d); ^{13}C NMR ($CDCl_3$, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 123.4 (1C, s), 123.4 (1C, s), 126.0 (2C, s), 127.8 (1C, s), 128.9 (2C, s), 133.7 (1C, s), 134.3 (1C, s), 135.3 (1C, s), 139.5 (1C, s), 140.4 (1C, s), 148.6 (1C, s), 148.6 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 335 (M+, 100), 337 (M+2, 34). Elemental analysis of $C_{19}H_{15}N_4Cl$; calcd. (found) (%): C, 68.10 (67.67); H, 4.47 (4.31); Cl, 10.63 (10.05); N, 16.80 (16.13).

3,3′-(1-(*o*-Tolyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)-dipyridine (5h): Yield: 66%, ^{1}H NMR ($CDCl_3$, δ ppm): 2.25 (3H, s), 3.10 (2H, dd), 5.85 (1H, d), 6.95 (1H, d), 7.04 (1H, s), 7.12 (1H, d), 7.24 (1H, d), 7.36 (1H, d), 7.40 (1H, d), 7.77 (1H, s), 8.02 (1H, d), 8.46 (1H, s), 8.48 (1H, s), 8.52 (1H, d), 8.75 (1H, dd); ^{13}C NMR ($CDCl_3$, δ ppm): 17.7 (1C, s), 46.1 (1C, s), 59.2 (1C, s), 115.6 (1C, s), 123.4 (1C, s), 123.4 (1C, s), 127.8 (1C, s), 128.2 (1C, s), 128.4 (1C, s), 129.0 (1C, s), 134.3 (1C, s), 135.3 (1C, s), 136.0 (1C, s), 139.5 (1C, s), 140.4 (1C, s), 148.6 (1C, s), 148.6 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 314 (M+, 100). Elemental analysis of $C_{20}H_{18}N_4$; calcd. (found) (%): C, 76.30 (75.77); H, 5.84 (5.46); N, 17.88 (17.43).

3,3′-(1-(*p*-Tolyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)-dipyridine (5i): Yield: 68%, ^{1}H NMR ($CDCl_3$, δ ppm): 2.20 (3H, s), 3.11 (2H, dd), 5.84 (1H, d), 7.04 (1H, d), 7.11 (1H, s), 7.36 (1H, d), 7.40 (1H, d), 7.77 (1H, d), 8.02 (1H, d), 8.46 (1H, s), 8.48 (1H, s), 8.52 (1H, d), 8.75 (1H, dd); ^{13}C NMR ($CDCl_3$, δ ppm): 21.3 (1C, s), 46.1 (1C, s), 59.2 (1C, s), 123.2 (2C, s), 123.4 (1C, s), 123.4 (1C, s), 127.8 (1C, s), 129.6 (2C, s), 134.3 (1C, s), 135.3 (1C, s), 139.5 (1C, s), 140.4 (1C, s), 141.5 (1C, s), 148.6 (1C, s), 148.6 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 315 (M+, 100). Elemental analysis of $C_{20}H_{18}N_4$; calcd. (found) (%): C, 76.30 (75.81); H, 5.84 (5.35); N, 17.88 (17.52).

3,3′-(1-(2,4-Dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5j): Yield: 71%, ^{1}H NMR ($CDCl_3$, δ ppm): 3.10 (2H, dd), 5.85 (1H, d), 7.37 (1H, d), 7.40 (1H, d), 7.41 (1H, d), 7.42 (1H, d), 7.65 (1H, d), 7.67 (1H, d), 7.77 (1H, d), 8.46 (1H, d), 8.48 (1H, d), 8.49 (1H, d), 8.76 (1H, d); ^{13}C NMR ($CDCl_3$, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 123.4 (1C, s), 123.5 (1C, s), 126.2 (1C, s), 127.8 (1C, s), 128.6 (1C, s), 128.8 (1C, s), 128.9 (1C, s), 133.2 (1C, s), 133.6 (1C, s), 134.3 (1C, s), 135.3 (1C, s), 139.5 (1C, s), 148.6 (1C, s), 148.7 (1C, (s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS *m/z*: 368.06 (M+, 100), 370.06 (M+2, 63.9%), 372.05 (M+4, 10.2%). Elemental analysis of $C_{19}H_{14}N_4Cl_2$; calcd. (found) (%): C, 61.78 (61.23); H, 3.79 (3.62); Cl, 19.24 (18.96); N, 15.17 (15.02).

3,3′-(1-(3,5-Dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5k): Yield: 62%, ^{1}H NMR ($CDCl_3$, δ ppm): 3.10 (2H, dd), 5.77 (1H, d), 7.11 (1H, t), 7.23 (2H, dd), 7.36 (1H, d), 7.40 (1H, d), 7.64 (1H, d), 7.77 (1H, d), 8.46 (1H, d), 8.48 (1H, d), 8.52 (1H, d), 8.76 (1H, d); ^{13}C NMR ($CDCl_3$, δ ppm): 46.1 (1C, s), 59.2 (1C, s), 117.6 (2C, s), 123.4 (1C, s), 123.5 (1C, s), 127.8 (1C, s), 127.9 (1C, s), 134.3 (1C, s), 134.4

(1C, s), 135.3 (1C, s), 135.4 (1C, s), 138.1 (1C, s), 139.5 (1C, s), 148.6 (1C, s), 148.7 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 368.23 (M+, 100), 370.40 (M+2, 62.8%), 372.44 (M+4, 10.6%). Elemental analysis of $C_{19}H_{14}N_4Cl_2$; calcd. (found) (%): C, 61.78 (61.43); H, 3.79 (3.51); Cl, 19.24 (19.07); N, 15.17 (14.98).

3,3′-(1-(2,4-Dimethylphenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5l): Yield: 64%, ^{1}H NMR ($CDCl_3$, δ ppm): 2.19 (3H, s), 2.23 (3H, s), 3.10 (2H, d), 5.77 (1H, d), 7.00 (1H, d), 7.02 (1H, d), 7.05 (1H, d), 7.36 (1H, d), 7.40 (1H, d), 7.77 (1H, d), 8.02 (1H, d), 8.46 (1H, d), 8.48 (1H, d), 8.52 (1H, d), 8.75 (1H, d); ^{13}C NMR ($CDCl_3$, δ ppm): 17.7 (1C, s), 21.3 (1C, s), 46.1 (1C, s), 59.2 (1C, s), 117.7 (1C, s), 123.4 (1C, s), 123.5 (1C, s), 125.8 (1C, s), 127.8 (1C, s), 129.4 (1C, s), 129.6 (1C, s), 134.3 (1C, s), 134.8 (1C, s), 135.3 (1C, s), 139.5 (1C, s), 140.4 (1C, s), 148.6 (1C, s), 148.7 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 328.17 (M+, 100.0%). Elemental analysis of $C_{21}H_{20}N_4$; calcd. (found) (%): C, 76.82 (76.12); H, 6.09 (6.01); N, 17.07 (16.92).

3,3′-(1-(3,4-Dimethylphenyl)-4,5-dihydro-1*H*-pyrazole-3,5-diyl)dipyridine (5m): Yield: 64%, ^{1}H NMR ($CDCl_3$, δ ppm): 2.18 (3H, s), 2.23 (3H, s), 3.11 (2H, dd), 5.83 (1H, d), 6.47 (1H, d), 6.62 (1H, dd), 6.83 (1H, d), 7.36 (1H, d), 7.40 (1H, d), 7.77 (1H, d), 8.02 (1H, d), 8.46 (1H, d), 8.48 (1H, d), 8.52 (1H, d), 8.75 (1H, d); ^{13}C NMR ($CDCl_3$, δ ppm): 19.9 (1C, s), 20.1 (1C, s), 46.1 (1C, s), 59.2 (1C, s), 119.3 (1C, s), 123.2 (1C, s), 123.4 (1C, s), 123.5 (1C, s), 127.8 (1C, s), 130.0 (1C, s), 130.9 (1C, s), 133.4 (1C, s), 134.3 (1C, s), 135.3 (1C, s), 138.1 (1C, s), 139.5 (1C, s), 148.6 (1C, s), 148.7 (1C, s), 149.2 (1C, s), 149.6 (1C, s), 152.4 (1C, s). MS (*m/z*): 327.97 (M+, 100.0%). Elemental analysis of $C_{21}H_{20}N_4$; calcd. (found) (%): C, 76.82 (76.24); H, 6.09 (5.97); N, 17.07 (16.97).

Molecular docking studies: A docking simulation using the AutodockVina v1.5.6 was conducted to examine potential binding mechanisms of specific chemical entities [27]. Both protein and ligand in .pdbqt file were provided as input for the receptor and ligand, respectively, prior to the docking process beginning. The selected compounds were individually docked with the receptor (PDB ID: 4NER) to carry out the protein-ligand docking. Compound structures were created using the Chem Sketch tool and saved in the mol format and then by using OpenBabel version 2.4.1 mol format converted into .pdbqt and protein also converted into .pdbqt to perform molecular docking. The RCSB Protein Data Bank was used to obtain the target proteins for docking. Understanding the interactions between ligand-5e and the amino acids of human 15-lipoxygenase-2 with a substrate mimic (PDB ID: 4NRE) is the goal of investigating this docked complex. To visualize the docking molecule, we used BIOVIA Discovery Studio Visualizer version 21.1.1.0.20298.

Computational details: The computation was performed using the Gaussian 09W version-7.0 program and the graphical depiction of the molecular structure was developed using the Gauss View 5.0 tool. Geometry optimization was carried out on the surveyed compound at the level of B3LYP/6-31 g using the unconstrained DFT technique. Following the synthesis of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) molecular orbitals, the molecular electrostatic potential (MEP) was computed. The band gap value extracted from the HOMO-LUMO and the electron density determined using the DFT method with the basis sets (B3LYP/6-31 g) can be utilized as predictors to evaluate the sensitivity of compound towards biological targets [28,29].

Antioxidant activity

DPPH assay: The antioxidant activity of the synthesized pyridine appended pyrazole derivatives (**5a-m**) were evaluated through free radical scavenging (DPPH) assay [30,31]. A 0.4 mM of DPPH in ethanol was mixed with the test compound at different concentrations (0.15 mM, 0.30 mM and 0.45 mM) and kept at 37 ºC for 30 min. The absorbance of standard ascorbic acid and tested compounds were assessed using Elico SL 159 UV-Vis spectrophotometer at 517 nm. The assessments were conducted in triplicate.

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the standard and A_1 is the absorbance of the test samples at different concentrations. The % inhibition was plotted against concentration and from the graph, the IC_{50} was calculated.

Hydroxyl radical scavenging activity: A reported method was used to carry out the hydroxyl radical scavenging assay [32]. Test compounds (25, 50, 75, and 100 µg/mL in methanol), 0.1 mL of phosphate buffer, 0.2 mL of 2-deoxyribose, 0.1 mL of H_2O_2 (10 mM), 0.1 mL of ascorbic acid (1 mM), 0.1 mL of EDTA and 0.01 mL of $FeCl_3$ (100 mM) were incubated at 37 ºC for 60 min. After that, 1 mL of cold 2.8% trichloroacetic acid was added to stop the reaction, and 1 mL of 1% thiobarbituric acid was added to boiling water for 15 min to measure the reaction product. At 535 nm, the absorbance was measured. As a positive control, butylated hydroxyanisole (BHA) was used. A decrease in the reaction mixture's absorbance suggests the hydroxyl radical scavenging activity.

RESULTS AND DISCUSSION

A series of pyridine appended pyrazole derivatives (**5a-m**) was synthesized, as illustrated in **Scheme-I**, *via* chalcone formation using 3-acetylpyridine and 3-pyridine-carboxaldehyde, followed by reaction with substituted phenyl hydrazines under mild conditions. The ^{1}H NMR spectra for compounds **5a-m** lacked the doublet signals characteristic of the alkenyl protons found in chalcones **3**. This absence confirms the [3+2] cycloaddition reaction between chalcones and phenylhydrazine hydrochlorides, resulting in the formation of pyridine-pyrazole derivatives (**5a-m**). Additionally, the methylene protons on the C-4 atom of the newly synthesized pyrazole ring in compounds **5a-m** displayed a typical ABX spin pattern, indicating their diastereotopic nature.

^{1}H NMR of compound **5a** showed that the methylene protons of C-4 iota of recently shaped pyrazole ring displayed ordinary ABX turn and are of diastereotopic nature. For instance, the C_4-H_a proton appears as doublet of doublet at δ 3.152-3.213

ppm; whereas, C_4-H_b proton appears as doublet of doublet at δ 3.768-3.841 ppm, respectively. Instead of appearing as a triplet, C_5-H resonates with both C_4-H_a and C_4-H_b and appears as doublet of doublet at δ 5.389-5.438 ppm. An array of signals appeared as multiplet in the region δ 6.744-6.795 ppm for two protons, δ 6.818-6.841 ppm for one proton, δ 7.047-7.086 ppm for two protons, δ 7.187-7.247 ppm for four protons and δ 7.609-7.629 ppm for the protons were unambiguously assigned to the pyridine and aromatic ring protons. In ^{13}C NMR, compound **5a** shows a signals due to carbon-4, carbon-5 and carbon-3, carbons of synthesized novel pyrazole ring corresponds at δ 43.14, 59.10 and 150.26 ppm. A peak for C-4 at δ 43.14 ppm and C-5 at δ 59.10 ppm confirms that the pyrazole ring is not aromatic, but is of partially reduced dihydropyrazole form. Signals appeared at δ 108.29-149.10 ppm are unambiguously due to aromatic carbons.

Molecular docking studies: The synthesized compounds **5a-m** exhibited a binding affinity in the range of -7.7 to -9.2 kcal/mol for human 15-lipoxygenase-2 with a substrate mimic (PDB ID: 4NRE) protein (Table-1). Ligand **5e** demonstrated the strongest binding affinity of -9.2 kcal/mol and demonstrated hydrogen bonding with the Phe88, Trp109, Ile403 Thr406 and Trg407 residues (Fig. 1). This compound may be considered for *in vitro* and *in vivo* inquiry towards the construction of a potential antioxidant inhibitor.

TABLE-1
DOCKING SCORE OF COMPOUNDS
WITH RESPECTIVE 2D STRUCTURE

Compd.	Binding score/Affinity (kcal/mol)	Compd.	Binding score/Affinity (kcal/mol)
5a	-8.2	**5h**	-8.5
5b	-8.1	**5i**	-8.2
5c	-8.2	**5j**	-8.4
5d	-7.7	**5k**	-8.4
5e	-9.2	**5l**	-8.4
5f	-8.2	**5m**	-8.4
5g	-8.1		

Computational results: Ligand **5e** has dipole moment of 2.6781 Debye and a molecular energy of -1411.94635630 a.u. A more stable optimal form is indicated by a lower molecular fundamental energy value. According to DFT, the total energy of a molecule is determined by the electron density surrounding its nucleus. Although the LUMO is a partially filled orbital that prefers to absorb electrons, which are the HOMO is situated in an orbital that tends to emit electrons. The HOMO-LUMO spectrum decrease has been shown to be strongly polarizable, which could lead to promising therapeutic candidates. Ligand **5e** has the lowest energy band gap value of 0.14722 a.u. in Fig. 2, demonstrating greater antioxidant activity.

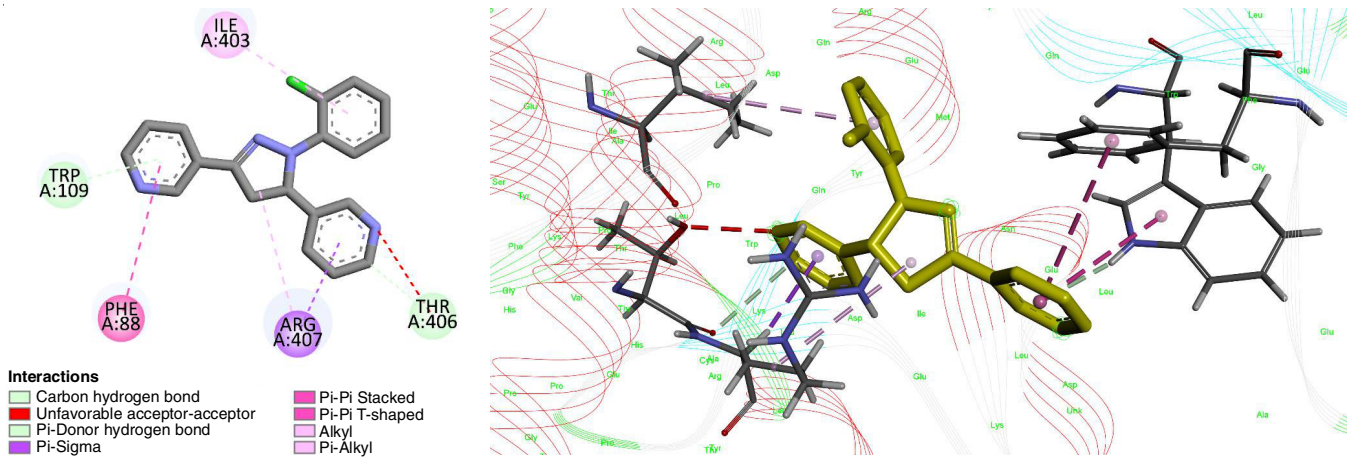


Fig. 1. 2D and 3D molecular docking interaction analysis of compound **5e**

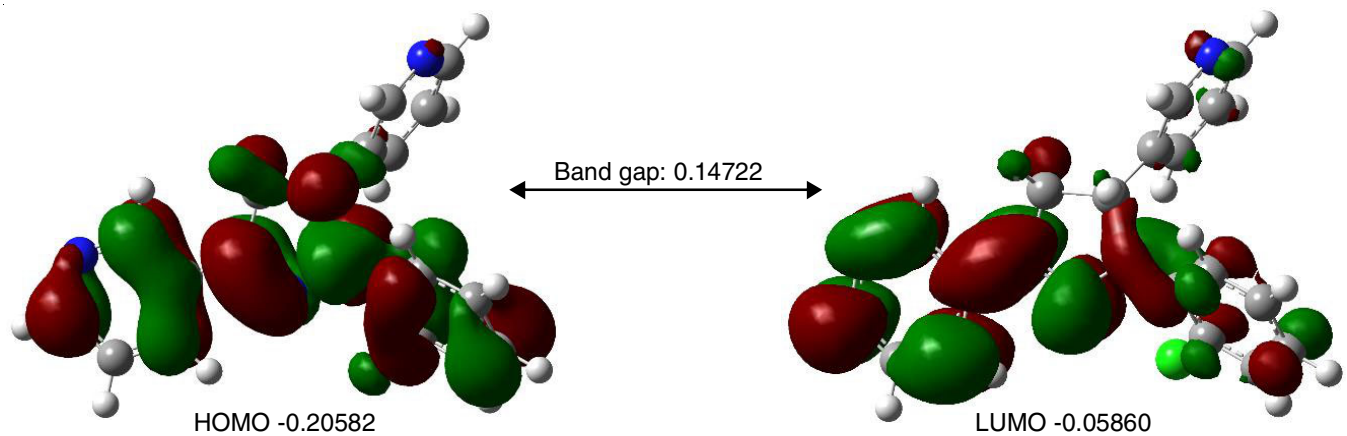


Fig. 2. Molecular orbital of the optimized compound **5e**

Antioxidant activity: A colour change from purple to yellow signifies a drop in absorbance, attributable to the decrease of the DPPH free radical through the donation of a hydrogen atom by an antioxidant molecule. In this work, compound **5e** having *ortho*-Cl group in the benzene exhibited exceptional DPPH radical capabilities when compared to standard ascorbic acid (Table-2). Whereas compounds **5a**, **5b**, **5d** and **5h** exhibited moderate to antioxidant activities.

The hydroxyl radical is a highly reactive free radical generated in the biological processes and possesses the ability to interfere with the biomolecules found in live cells. The obtained results of hydroxyl radical scavenging activity of the synthesized pyridine appended pyrazole compounds (**5a-m**) showed modest to significant cellular defense efforts. The results indicated that compound **5e** exhibit exceptional efficacy in hydroxyl radical scavenging comparable to standard BHA (Table-3). Compounds **5a**, **5b**, **5d** and **5h** exhibit good activity whereas the remaining compounds have demonstrated either moderate or poor activity.

Conclusion

Pyridine-appended pyrazoles (**5a-m**) were synthesized by (3+2) cycloaddition reaction with substituted phenyl hydrazine and pyridine chalcones. This approach demonstrated the effectiveness of phenyl hydrazine in pyrazole formation as well as the versatility of chalcones as intermediates. Strong enzyme-binding potential was revealed by sophisticated computational investigations, such as molecular docking and DFT computations, which shed light on molecular interactions and stability. The strongest effects were shown by compounds **5e**, **5a**, **5b**, **5d** and **5h**. Significant antioxidant activity was confirmed by biological evaluation using DPPH and hydroxyl radical scavenging assays. This research offers a promising basis for developing new antioxidant and therapeutic agents by combining synthetic chemistry, computational modeling and biological screening.

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TABLE-2
DPPH RADICAL SCAVENGING ACTIVITY OF THE COMPOUNDS **5a-m**

Compounds	% Radical scavenging activity*			
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
5a	27.20 ± 0.50	31.10 ± 0.65	36.35 ± 0.45	39.50 ± 0.45
5b	28.40 ± 0.45	31.40 ± 0.80	34.11 ± 0.90	37.60 ± 0.52
5c	32.90 ± 0.40	35.80 ± 0.55	38.90 ± 0.23	41.20 ± 0.72
5d	29.40 ± 0.53	32.40 ± 0.70	35.11 ± 0.83	39.60 ± 0.88
5e	21.25 ± 0.88	24.55 ± 0.35	28.20 ± 0.28	32.45 ± 0.70
5f	33.71 ± 0.98	35.70 ± 0.78	37.40 ± 0.60	40.65 ± 0.90
5g	36.13 ± 0.24	40.90 ± 0.40	44.35 ± 0.55	48.20 ± 0.18
5h	25.20 ± 0.44	28.10 ± 0.65	32.35 ± 0.45	35.50 ± 0.45
5i	34.65 ± 0.80	37.84 ± 0.45	31.89 ± 0.70	34.10 ± 0.30
5j	43.71 ± 0.46	45.70 ± 0.92	47.40 ± 0.60	50.65 ± 0.90
5k	46.13 ± 0.87	50.90 ± 0.40	54.35 ± 0.55	58.20 ± 0.10
5l	35.20 ± 0.46	38.10 ± 0.65	32.35 ± 0.45	35.50 ± 0.45
5m	44.65 ± 0.80	47.84 ± 0.45	51.89 ± 0.70	54.10 ± 0.35
AA[a]	15.10 ± 0.22	17.85 ± 0.84	21.90 ± 0.55	24.50 ± 0.30

*Values are mean ± SD of three replicates; [a]Ascorbic acid used as a standard antioxidant.

TABLE-3
HYDROXYL RADICAL SCAVENGING ACTIVITY OF THE COMPOUNDS **5a-m**

Compounds	% Radical scavenging activity* (% I) (µg/mL)			
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
5a	29.45 ± 0.23	33.25 ± 0.12	38.22 ± 0.65	40.24 ± 0.42
5b	30.25 ± 0.52	33.02 ± 0.18	36.35 ± 0.22	39.22 ± 0.23
5c	33.78 ± 0.23	37.47 ± 0.59	40.30 ± 0.28	43.50 ± 0.12
5d	31.22 ± 0.32	33.02 ± 0.07	36.41 ± 0.32	40.55 ± 0.29
5e	20.34 ± 0.48	25.36 ± 0.52	29.75 ± 0.82	35.12 ± 0.32
5f	35.29 ± 0.22	37.40 ± 0.58	39.87 ± 0.55	42.14 ± 0.25
5g	38.18 ± 0.26	41.96 ± 0.85	45.55 ± 0.35	49.45 ± 0.82
5h	28.02 ± 0.49	29.18 ± 0.59	34.45 ± 0.65	37.22 ± 0.85
5i	36.25 ± 0.54	39.41 ± 0.89	41.92 ± 0.45	44.89 ± 0.12
5j	46.45 ± 0.65	49.45 ± 0.56	51.25 ± 0.78	53.78 ± 0.56
5k	42.23 ± 0.45	44.02 ± 0.85	46.25 ± 0.87	48.45 ± 0.36
5l	38.56 ± 0.87	41.25 ± 0.78	44.47 ± 0.58	48.12 ± 0.68
5m	40.45 ± 0.40	44.65 ± 0.78	49.25 ± 0.45	58.02 ± 0.25
[a]BHA	12.10 ± 0.06	17.60 ± 0.11	25.62 ± 0.19	32.12 ± 0.31

*Mean values ± Standard Deviation of three replicates (n = 3); [a]BHA was used as a reference.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interests regarding the publication of this article.

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