Research article

Design, synthesis, characterization, and antioxidant activity studies of novel thienyl-pyrazoles

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ABSTRACT

In a sustained search for novel and effective antioxidants, a potential therapeutic leads against renal, and neurological disorders. Amongst the heterocycles, pyrazole and their derivatives have been extensively studied for their biological potencies, particularly to a larger extent for their antioxidant properties. Although many of pyrazole derivatives displayed antioxidant activities, still there is a need of developing efficient protocol for their synthesis, involving ecofriendly conditions, molecules of greater antioxidant efficacy and lesser toxicity, etc. In this context, the current study presents an amberlyst-15 catalysed efficient synthesis of 2-pyrazoline derivatives, 5(a–g) via (3 + 2) annulation of chalcones with phenylhydrazines. Structure proofs of new pyrazoles offered by spectral studies, and the molecular structure of compound 5d of the series by crystallographic studies, which revealed an intra molecular hydrogen bond interactions (C–H⋯N type), and stabilization by C–H⋯π and π⋯π molecular interactions. Of the series, compounds 5g and 5h show excellent DPPH (IC50 = 0.245 ± 0.01, and 0.284 ± 0.02 μM), and hydroxyl (IC50 = 0.905 ± 0.01, and 0.892 ± 0.01μM) radical scavenging activities comparable with respective controls, ascorbic acid (IC50 = 0.483 ± 0.01μM) and BHA (IC50 = 1.739 ± 0.01μM). The molecular docking and ADME/Tox studies indicate that, these compounds have good antioxidant activity through π–π stacking with Catalase via Try337 and Phe140, and therefore, might be lead antioxidants for further study.

1. Introduction

Oxidative stress induced by the free radical damage cell membranes, and nucleic acids, which results in aging, cancer, atherosclerosis, and Alzheimer's disease [1]. The studies on small-molecules with antioxidant efficacy to prevent the deleterious effects is important field of research. The drug molecules that contains a pyrazole core remain as choice in Alzheimer's disease [1]. The studies on small-molecules with antioxidant and nucleic acids, which results in aging, cancer, atherosclerosis, and neurological disorders. Amongst the heterocycles, pyrazole and their derivatives have been extensively studied for their biological potencies, particularly to a larger extent for their antioxidant properties. Although many of pyrazole derivatives displayed antioxidant activities, still there is a need of developing efficient protocol for their synthesis, involving ecofriendly conditions, molecules of greater antioxidant efficacy and lesser toxicity, etc. In this context, the current study presents an amberlyst-15 catalysed efficient synthesis of 2-pyrazoline derivatives, 5(a–g) via (3 + 2) annulation of chalcones with phenylhydrazines. Structure proofs of new pyrazoles offered by spectral studies, and the molecular structure of compound 5d of the series by crystallographic studies, which revealed an intra molecular hydrogen bond interactions (C–H⋯N type), and stabilization by C–H⋯π and π⋯π molecular interactions. Of the series, compounds 5g and 5h show excellent DPPH (IC50 = 0.245 ± 0.01, and 0.284 ± 0.02 μM), and hydroxyl (IC50 = 0.905 ± 0.01, and 0.892 ± 0.01μM) radical scavenging activities comparable with respective controls, ascorbic acid (IC50 = 0.483 ± 0.01μM) and BHA (IC50 = 1.739 ± 0.01μM). The molecular docking and ADME/Tox studies indicate that, these compounds have good antioxidant activity through π–π stacking with Catalase via Try337 and Phe140, and therefore, might be lead antioxidants for further study.

Several natural and synthetic pyrazole derivatives possess wide spectrum of pharmacological potentials with druggable properties [9]. For instance, the pyrazole analogs exhibit cytotoxicity against DLA cells, reduces tumour loads, and downregulates tumour progression proliferation [10]. The pyrazole derivatives have anti-diabetic and anti-inflammatory [11], antimicrobial [12], and acetylcholinesterase inhibitor [13] properties. The Salvia officinalis L belongs to Lamiaceae family is known for its antioxidant and many pharmaceutical potencies.
like anti-spasmodic, astringent, sedative, anti-hyperglycemic, and anti-inflammatory [14]. The complexes isolated from Black Sea marine invertebrate tissues have displayed antioxidant, antimicrobial and mitogenic activities [15]. Main protease (Mpro) in the life cycle of SARS-CoV-2 mediate viral replication, transcription, and is a drug target for the virus, the computational results reveal a hypothesis for experimental validation [16].

The pyrazole motif is an important core in the development of antioxidants, that alone or combined with other pharmacophore shows high degree of antioxidant activity [17]. For instance, the N-formyl pyrazolines synthesized via Michael addition displayed promising anticancer and antioxidant [18], and pyrazolo-pyrimidines possess cytotoxic and radical scavenging properties [19]. The reports on the curcumin pyrazole analogs indicated that these alleviate oxidative stress-induced PC12 neuronal damage abilities [20], and pyrazole-thiazoles show antimicrobial and antioxidant activities [21]. The prepared pyrazole-triazole hybrids displayed citotoxic and antioxidant [22], and pyrazole aldehydes have xanthine oxidase inhibitory [23] properties.

Although, a plenty of research on pyrazoles and their biological activities has been reported. Many of the methods adopted for the synthesis requires drastic reaction conditions, and produces less yields, and more toxic by-products, etc. Furthermore, the most of the pyrazole derivatives exhibited lesser antioxidant properties comparable with the standards employed. In this context, in an attempt towards new therapeutics with greater antioxidant potentials and less toxicity, and to overcome drawbacks of synthetic procedure, the present work has been undertaken. The current study demonstrates the Amberlyst-15 catalyzed reaction of...
chalcones, and phenyldiazine hydrochlorides at room temperature to get thiienyl-pyrazoles in good yields. The structures of synthesized compounds were characterized through spectral analysis, and one compound by crystallographic studies. After the structural characterization, the new compounds were assessed for free radical scavenging activities. Furthermore, an in silico molecular docking and ADMET analysis was carried out to validate the results and their mode of action.

2. Experimental

2.1. Materials and methods

All the chemicals and reagents procured from Sigma Aldrich were used as received. Pre-coated silica gel-aluminum plates (Merck, F-254) used for thin-layer chromatography (TLC). IR Spectra were obtained on Elico SCN781 spectrometer (TOF mode). Absorbance was recorded on ELICO SI 159 UV-Vis spectrophotometer. The un-corrected melting points were with Centex apparatus.

2.2. Synthesis of thiienyl-pyrazoles, 5(a-i)

A mixture of chalcones, 3(a-f) (5 mmol), phenyldiazine hydrochlorides, 4(a-b) (5 mmol), and Amberlyst-15 (10%, w/w) in acetonitrile (25 ml) was stirred at room temperature for 30-60 min. After the completion, the separated solid was filtered, washed with diethyl ether (2 × 20 ml), then treated with ethyl acetate (20 ml), and stirred for 10 min and again filtered. The filtrate was concentrated under vacuum, the solid formed was triturated in diethyl ether, filtered and dried to get the products 5(a-i). Alternatively, the reaction was carried out in 30% acetic acid under boiling conditions.

2.2.1. 5-(3-Methylthiophen-2-yl)-1,3-diphenyl-4,5-dihydro-1H-pyrazole, 5a

Obtained from 3-(3-methylthiophen-2-yl)-1-phenylprop-2-en-1-one, 3a (1.14g, 5 mmol) and phenyldiazine hydrochloride, 4a (0.72g, 5 mmol); 1H NMR (CDCl3, δ ppm): 2.665 (s, 3H, CH3), 3.003-3.064 (dd, 1H, J = 6.8, 16.8 Hz, C4-H), 3.731-3.803 (dd, 1H, J = 12.4, 17.2 Hz, C5-H), 5.190-5.237 (dd, 1H, J = 6.4, 12.4 Hz, C6-H), 6.617–6.818 (m, 5H, Ar-H), 6.998–7.063 (m, 4H, Ar-H), 7.208–7.248 (m, 4H, Ar-H); 13C NMR (CDCl3, δ ppm): 129.50 (1C, C-6), 131.13 (1C, C-7), 133.54 (1C, C-8), 143.16 (1C, C-3), 163.51 (1C, C-3). MS (m/z): 318.0 (M+, 100); Anal. Calcd. (found) for C20H18N2S (%): C, 75.44 (75.32); H, 5.70 (5.67); N, 8.80 (8.75).

2.2.2. 3-(4-Fluorophenyl)-5-(3-methylthiophen-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazole, 5b

Obtained from 3-(4-fluorophenophen-2-yl)-1-phenylprop-2-en-1-one, 3b (1.23g, 5 mmol) and phenyldiazine hydrochloride, 4a (0.72g, 5 mmol); 1H NMR (CDCl3, δ ppm): 2.626 (s, 3H, CH3), 3.013-3.069 (dd, 1H, J = 6.8, 16.8 Hz, C4-H), 3.737–3.810 (dd, 1H, J = 12.4, 17.2 Hz, C5-H), 5.197–5.245 (dd, 1H, J = 6.8, 12.4 Hz, C6-H), 6.591–6.890 (m, 2H, Ar-H), 7.011–7.068 (m, 5H, Ar-H), 7.152–7.248 (m, 4H, Ar-H); 13C NMR (CDCl3, δ ppm): 136.68 (1C, C16), 146.42 (1C, C24), 63.72 (1C, C5), 111.29 (1C), 135.56 (1C), 154.15 (1C), 166.52 (1C), 172.6 (1C), 125.44 (1C), 126.08 (1C), 126.46 (1C), 127.47 (1C), 128.97 (1C), 129.87 (1C), 130.29 (1C), 130.46 (1C), 131.31 (1C), 131.80 (1C), 143.16 (1C), 163.51 (1C, C-3). MS (m/z): 338.0 (M+, 100); Anal. Calcd. (found) for C20H18N2S (%): C, 74.44 (75.32); H, 5.70 (5.67); N, 8.80 (8.75).

2.2.3. 1-(3-Chlorophenyl)-3-(3-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazole, 5c

Obtained from 1-(4-fluorophenophen-2-yl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, 3b (1.23g, 5 mmol) and 3-chlorophenylhydrazine (100 MHz) spectra were obtained on Agilent NMR spectrometer. Mass spectra were obtained on Lynx SCN781 spectrometer (TOF mode). Absorbance was recorded on ELICO SI 159 UV-Vis spectrophotometer. The un-corrected melting points were with Centex apparatus.
hydrochloride, 4b (0.89g, 5 mmol); $^1$H NMR (CDCl$_3$, δ ppm): 2.310 (s, 3H, CH$_3$), 3.014–3.073 (dd, 1H, $J = 6.8, 11.6$Hz, C$_4$-H$_a$), 3.713–3.786 (dd, 1H, $J = 12.4, 17.6$Hz, C$_5$-H$_a$), 5.168–5.216 (dd, 1H, $J = 6.8, 12.0$Hz, C$_5$-H$_b$), 6.699–6.809 (m, 4H, Ar–H), 6.995–7.249 (m, 6H, Ar–H); $^{13}$C NMR (CDCl$_3$, δ ppm): 14.06 (1C, CH$_3$), 43.72 (1C, C-4), 64.18 (1C, C-5), 111.26 (1C), 113.51 (1C), 119.07 (1C), 125.27 (1C), 125.62 (1C), 126.43 (2C), 127.37 (1C), 128.55 (1C), 129.02 (1C), 129.41 (1C), 131.54 (1C), 134.87 (1C), 137.67 (1C), 138.45 (1C), 142.95 (1C), 145.28 (1C, C-3). MS (m/z): 370.1 (M+, 100), 372.14 (M+2, 33); Anal. Calcd. (found) for C$_{20}$H$_{14}$ClF$_2$N$_2$S (%): C, 64.77 (64.74); H, 4.35 (4.32); N, 7.55 (7.51).

2.2.4. 1-(3-Chlorophenyl)-3-(4-chlorophenyl)-5-(3-methylthiophen-2-yl)-4,5-dihydro-1H-pyrazole, 5d

Obtained from 1-(4-chlorophenyl)-2-(3-methylthiophen-2-yl)prop-2-en-1-one, 3c (1.31g, 5 mmol) and 3-chlorophenyl hydrochloride, 4b (0.89g, 5 mmol); $^1$H NMR (CDCl$_3$, δ ppm): 2.249 (s, 3H, CH$_3$), 2.940–2.988 (dd, 1H, $J = 17.2, 6.4$Hz, C$_5$-H$_a$), 3.864–3.937 (dd, 1H, $J = 12.0, 17.2$Hz, C$_4$-H$_a$), 5.497–5.543 (dd, 1H, $J = 6.4, 12.4$Hz, C$_5$-H$_b$), 6.592–6.617 (m, 1H, Ar–H), 6.742–6.800 (m, 2H, Ar–H), 7.003–7.154 (m, 5H, Ar–H), 7.384–7.467 (m, 2H, Ar–H); $^{13}$C NMR (CDCl$_3$, δ ppm): 13.78 (1C, CH$_3$), 42.18 (1C, C-4), 60.68 (1C, C-5), 101.35 (1C), 105.84 (1C), 108.14 (1C), 110.73 (1C), 113.20 (1C), 119.08 (1C), 120.52 (1C), 126.43 (1C), 128.08 (1C), 129.90 (1C), 132.44 (1C), 134.13 (1C), 134.99 (1C), 137.30 (1C), 145.27 (1C), 148.05 (1C), 148.73 (1C, C-3). MS (m/z): 390.0 (M$^+$, 10), 388.0 (M$^+$-2, 63), 386.0 (M$^+$+2, 100); Anal. Calcd. (found) for C$_{20}$H$_{14}$ClF$_2$N$_2$S (%): C, 62.02 (61.95); H, 4.16 (4.12); N, 7.23 (7.19).

2.2.5. 5-(3-Methoxyphenyl)-5-(3-methylthiophen-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazole, 5e

Obtained from 1-(3-methoxyphenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one, 3d (1.27g, 5 mmol) and phenylhydrazine hydrochloride, 4a (0.72g, 5 mmol); $^1$H NMR (CDCl$_3$, δ ppm): 2.289 (s, 3H, CH$_3$), 3.323–3.368 (dd, 1H, $J = 4.4, 5.2$Hz, C$_4$-H$_a$), 3.816 (s, 3H, OCH$_3$), 3.877–3.923 (dd, 1H, $J = 11.6, 17.2$Hz, C$_4$-H$_b$), 5.696–5.736 (dd, 1H, $J = 4.8, 12.0$Hz, C$_5$-H), 6.731 (s, 2H, Ar–H), 6.907–7.023 (m, 5H, Ar–H), 7.003–7.154 (m, 5H, Ar–H), 7.384–7.467 (m, 2H, Ar–H); $^{13}$C NMR (CDCl$_3$, δ ppm): 13.78 (1C, CH$_3$), 42.18 (1C, C-4), 60.68 (1C, C-5), 101.35 (1C), 105.84 (1C), 108.14 (1C), 110.73 (1C), 113.20 (1C), 119.08 (1C), 120.52 (1C), 126.43 (1C), 128.08 (1C), 129.90 (1C), 132.44 (1C), 134.13 (1C), 134.99 (1C), 137.30 (1C), 145.27 (1C), 148.05 (1C), 148.73 (1C, C-3). MS (m/z): 390.0 (M$^+$, 10), 388.0 (M$^+$-2, 63), 386.0 (M$^+$+2, 100); Anal. Calcd. (found) for C$_{20}$H$_{14}$ClF$_2$N$_2$S (%): C, 62.02 (61.95); H, 4.16 (4.12); N, 7.23 (7.19).

| Atoms | XRD | DFT |
|-------|-----|-----|
| C18-C14 | 1.743(6) | 1.764 |
| C25-C22 | 1.727(6) | 1.757 |
| S1-C2 | 1.701(6) | 1.733 |
| S1-C6 | 1.723(5) | 1.751 |
| N10-N11 | 1.414(5) | 1.371 |
| N10-C9 | 1.295(7) | 1.292 |
| N11-C7 | 1.477(7) | 1.488 |
| N11-C12 | 1.390(7) | 1.401 |
| C2-C3 | 1.370(8) | 1.364 |
| C3-C4 | 1.413(8) | 1.436 |
| C4-C5 | 1.503(8) | 1.507 |
| C4-C6 | 1.371(8) | 1.377 |
| C6-C7 | 1.497(8) | 1.505 |
| C7-C8 | 1.553(8) | 1.556 |

Correlation Coefficient (CC) 0.9929
### Table 4. Bond angles (°) of compound 5d.

| Atoms       | XRD       | DFT       | Atoms       | XRD       | DFT       |
|-------------|-----------|-----------|-------------|-----------|-----------|
| C2-S1-C6    | 91.8(3)   | 91.4      | N11-C12-C13 | 121.0(5)  | 120.2     |
| N11-N10-C9  | 108.4(4)  | 110.3     | N11-C12-C17 | 120.1(5)  | 120.6     |
| N10-N11-C7  | 112.0(4)  | 112.3     | C13-C12-C17 | 118.8(5)  | 119.3     |
| N10-N11-C12 | 119.2(4)  | 118.6     | C12-C13-C14 | 119.9(5)  | 119.1     |
| C7-N11-C12  | 123.8(4)  | 124.1     | C18-C14-C13 | 118.6(4)  | 118.3     |
| S1-C2-C3    | 111.5(4)  | 111.7     | C18-C14-C15 | 119.2(4)  | 119.2     |
| C2-C5-C4    | 113.3(5)  | 113.6     | C13-C14-C15 | 122.2(5)  | 122.4     |
| C3-C4-C5    | 123.5(5)  | 122.2     | C14-C15-C16 | 118.3(5)  | 117.7     |
| C5-C4-C6    | 111.5(5)  | 111.8     | C15-C16-C17 | 121.3(5)  | 121.6     |
| C5-C4-C6    | 124.5(5)  | 126       | C12-C17-C16 | 119.7(5)  | 119.9     |
| S1-C6-C4    | 111.8(4)  | 111.6     | C9-C19-C20  | 122.2(5)  | 121       |
| S1-C6-C7    | 119.7(4)  | 119.9     | C9-C19-C24  | 120.7(5)  | 120.7     |
| C4-C6-C7    | 128.4(5)  | 128.5     | C20-C19-C24 | 117.1(5)  | 118.3     |
| N11-C7-C6   | 113.3(4)  | 113.5     | C19-C20-C21 | 121.7(5)  | 121       |
| N11-C7-C8   | 101.5(4)  | 101.6     | C20-C21-C22 | 119.2(5)  | 119.4     |
| C6-C7-C8    | 114.4(5)  | 114.3     | C25-C22-C21 | 119.6(4)  | 119.5     |
| C7-C8-C9    | 102.5(4)  | 102.4     | C25-C22-C23 | 120.0(5)  | 119.6     |
| N10-C9-C8   | 113.7(5)  | 112.9     | C21-C22-C23 | 120.5(6)  | 120.9     |
| N10-C9-C19  | 122.4(5)  | 122.1     | C22-C23-C24 | 120.3(6)  | 119.2     |
| C8-C9-C19   | 123.6(5)  | 125       | C19-C24-C23 | 121.3(5)  | 121.2     |
| Correlation Coefficient (CC) |           |           |             |           | 0.9954    |

### Table 5. Torsion angles (°) of compound 5d.

| Atoms       | XRD       | DFT       | Atoms       | XRD       | DFT       |
|-------------|-----------|-----------|-------------|-----------|-----------|
| C6-S1-C2-C3 | -0.5      | -0.2      | N11-C7-C8-C9 | -12.8(5)  | -6        |
| C2-S1-C6-C4 | 0.5(4)    | 0.1       | C7-C8-C9-C19 | -176.0(5) | -177.9    |
| C2-S1-C6-C7 | -176.2(5) | -176.5    | C7-C8-C9-N10 | 9.7(6)    | 3.5       |
| C9-N10-N11-C7 | -7.9(5) | -5.4 | N10-C9-C19-C24 | -177.7(5) | -180     |
| C9-N10-N11-C12 | -161.6(5) | -161.6 | C8-C9-C19-C20 | -170.8(5) | -178.6    |
| N11-N10-C9-C8 | -1.7(6) | 0.9 | C8-C9-C19-C24 | 8.5(8) | 1.6       |
| N11-N10-C9-C19 | -176.1(5) | -177.7 | N10-C9-C19-C20 | 3.1(8) | -0.2      |
| C12-N11-C7-C8 | 165.2(4) | 161.8 | N11-C12-C13-C14 | 174.5(5) | 179.5     |
| N10-N11-C12-C13 | 160.8(5) | 167.8 | C17-C12-C13-C14 | -1.5(8) | 0.3       |
| C7-N11-C12-C17 | -173.7(5) | -166.1 | N11-C12-C17-C16 | -173.7(5) | -179.4    |
| N10-N11-C7-C6 | 136.4(4) | 130.4 | C13-C12-C17-C16 | 2.4(8) | -0.1      |
| N10-N11-C7-C8 | 13.2(5) | 7.2 | C12-C13-C14-C18 | 176.9(4) | 179.8     |
| N10-N11-C12-C17 | -23.3(7) | -12.9 | C12-C13-C14-C15 | 0.6(8) | -0.2      |
| C12-N11-C7-C6 | -71.6(6) | -74.9 | C18-C14-C15-C16 | -178.6(4) | -180       |
| C7-N11-C12-C13 | 10.3(7) | 14.6 | C13-C14-C15-C16 | -0.6(8) | -0.1      |
| S1-C2-C3-C4 | 1.1(6) | 0.3 | C14-C15-C16-C17 | 1.5(8) | 0.2       |
| C2-C5-C4-C6 | -0.8(7) | -0.2 | C15-C16-C17-C12 | -2.4(8) | -0.1      |
| C2-C5-C4-C5 | 179.0(5) | 178.8 | C9-C19-C20-C21 | 178.6(6) | 179.6     |
| C5-C4-C6-S1 | -179.7(4) | -178.9 | C24-C19-C20-C21 | -0.7(9) | 0.2       |
| C5-C4-C6-C7 | -3.4(9) | -2.6 | C9-C19-C24-C23 | -179.3(5) | -179.7    |
| C5-C4-C6-S1 | 0.1(6) | 0.1 | C20-C19-C24-C23 | -0.1(8) | -0.1      |
| C5-C4-C6-C7 | 176.4(5) | 176.3 | C19-C20-C21-C22 | 1.5(9) | 0.2       |
| C4-C6-C7-N11 | 145.2(5) | 145.9 | C20-C21-C22-C25 | -179.7(5) | -180     |
| C4-C6-C7-C8 | -99.1(7) | -98.2 | C20-C21-C22-C23 | -1.6(10) | -0.1      |
| S1-C6-C7-C8 | 77.0(5) | 77.8 | C25-C22-C23-C24 | 179.0(5) | 180       |
| S1-C6-C7-N11 | -38.8(6) | -38.2 | C21-C22-C23-C24 | 0.9(10) | 0.1       |
| C6-C7-C8-C9 | -135.2(5) | -128.6 | C22-C23-C24-C19 | -0.1(9) | 0.1       |
| Correlation Coefficient (CC) |           |           |             |           | 0.9995    |
2.2.8. 3-(3,4-Dimethoxyphenyl)-5-(3-methyliphen-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazole, 5h

Obtained from 1-(3,4-dimethoxyphenyl)-3-(3-methyliphen-2-yl)prop-2-en-1-one, 3f (1.44g, 5 mmol) and phenylhydrazine hydrochloride, 4a (0.72g, 5 mmol); ¹H NMR (CDCl₃, δ ppm): 2.304 (s, 3H, CH₃), 3.142–3.204 (dd, 1H, J = 8.0, 17.2Hz, C₄-H₄), 3.722–3.762 (dd, 1H, J = 4.4, 12.9Hz, C₅-H₅), 3.864 (s, 3H, OCH₃), 5.425–5.475 (dd, 1H, J = 8.0, 12.0Hz, C₆-H₆), 6.799–6.912 (m, 4H, Ar–H), 7.042–7.153 (m, 2H, Ar–H), 7.207–7.410 (d, 4H, Ar–H); ¹³C NMR (CDCl₃, δ ppm): 13.95 (1C, CH₃), 42.94 (1C, C-4), 55.44 (1C, OCH₃), 55.47 (1C, OCH₃), 59.47 (1C, C-5), 110.64 (1C), 112.82 (1C), 113.83 (1C), 114.20 (1C), 124.32 (1C), 126.27 (1C), 127.44 (1C), 129.81 (1C), 129.57 (1C), 129.10 (1C), 130.12 (1C), 131.43 (1C), 135.66 (1C), 145.27 (1C), 150.19 (1C, C-3), MS (m/z): 378.1 (100); Anal. Calcd. (found) for C_{21}H_{20}N₂O₈ (%): C, 75.87 (75.82); H, 6.06 (6.03); N, 8.43 (8.40).

2.2.9. 1-(3-Chlorophenyl)-3-(3,4-dimethoxyphenyl)-5-(3-methyliphen-2-y1)4,5-dihydro-1H-pyrazole, 5i

Obtained from 1-(3,4-dimethoxyphenyl)-3-(3-methyliphen-2-yl)prop-2-en-1-one, 3f (1.44g, 5 mmol) and 3-chlorophenylhydrazine hydrochloride, 4b (0.89g, 5 mmol); ¹H NMR (CDCl₃, δ ppm): 2.304 (s, 3H, CH₃), 3.146–3.207 (dd, 1H, J = 7.6, 17.2Hz, C₄-H₄), 3.767–3.839 (dd, 1H, J = 12.0, 16.8Hz, C₅-H₅), 3.903 (s, 3H, OCH₃), 3.977 (s, 3H, OCH₃), 5.379–5.428 (dd, 1H, J = 7.6, 12.0Hz, C₆-H₆), 6.75–6.851 (m, 4H, Ar–H), 7.039–7.089 (m, 3H, Ar–H), 7.21–7.251 (m, 1H, Ar–H), 7.481 (s, 1H, Ar–H); ¹³C NMR (CDCl₃, δ ppm): 13.92 (1C, CH₃), 43.14 (1C, C-4), 55.93 (1C, OCH₃), 55.99 (1C, OCH₃), 59.10 (1C, C-5), 108.29 (1C), 116.62 (1C), 111.33 (1C), 113.85 (1C), 119.26 (1C), 119.39 (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): 560.3 (M+)

Figure 5. A perspective view of a supramolecular network by hydrogen bond interactions of the molecules 5d along the a-axis.

Figure 6. A perspective view of a supramolecular network by hydrogen bond interactions of the molecules 5d along the b-axis.
414.2 (M+, 33), 412.22 (M+, 100); Anal. Calcd. (found) for C_{22}H_{21}ClN_{2}O_{2}S (%): C, 63.99 (63.92); H, 5.13 (5.10); N, 6.78 (6.72).

2.3. X-ray diffraction studies

The brown colored prismatic defect free single crystal of approximate dimension 0.27 × 0.24 × 0.21 mm³ was chosen for X-ray diffraction studies. The X-ray intensity data of the molecule 5d were collected using Rigaku XtaLAB Mini diffractometer with X-ray generator operating at 50 kV, 12 mA and MoKα radiation. Data were collected with θ fixed at 54°, for different settings of ϕ (0° and 360°), the scan width of 0.5° with exposure time of 3 s and the sample to detector distance of 50 mm. The complete data sets were processed by CRYSTAL CLEAR [24]. The crystal structures were solved by SHELXS and SHELXL programs [25, 26]. The geometrical calculations were performed with PLATON [27]. The molecular and packing diagrams were generated using MERCURY [28].

2.4. Biological activity

2.4.1. DPPH radical scavenging activity

The activity of compounds 5(a–i) was performed by a Blois method [29], with different concentrations (2.5, 5.0, 7.5 and 10.0 μM) in methanol. The absorbance was read against blank at 517 nm.

2.4.2. Hydroxyl radical scavenging activity

The experiments were performed according to reported procedure [30]. A solution of phosphate buffer (0.1 mL); 2-deoxyribose (0.2 mL), compounds, 5(a–i) (2.5, 5.0, 7.5 and 10.0 μM), Hydrogen peroxide (0.1 mL, 10 mM), ascorbic acid (0.1 mL, 1 mM), EDTA (0.1 mL), and Ferric chloride (0.01 mL, 100 mM) was incubated at 37 °C for 60 min. After this, a cold 2.8% trichloroacetic acid (1mL) followed by 1% thiobarbituric acid (1mL, 1g/100mL of 0.05 N NaOH) were added, and kept for 15 min in boiling water. The absorbance measured at 535 nm.

2.5. Molecular docking studies

The co-ordinates of Catalase (CAT) (PDB id: 2CAG) and CuZn superoxide dismutase (CuZnSOD) (PDB id: 1CB4) were collected from the Brookhaven Protein Data Bank [31]. The minimal energy of ligands was computed by OPLS 2005. Proteins prepared by retrieving into workspace, and their structure were corrected by prime software module. Water molecules from CAT and CuZnSOD were removed beyond 5 Å from the hetero atoms. The interaction between the receptor, and water molecules were optimized during protein pepwizard [32]. OPLS 2005 force field applied to the protein to restrain minimization and RMSD of 0.30 Å was set to converge heavy atoms before start of docking. Each ligand was docked into the receptor grid of radii 20 Å, and the docking calculation was performed. ADME/Tox properties were calculated with ADME/Tox prediction program [33].

3. Results and discussion

The current investigation presents the synthesis of new pyrazole derivatives, structural characterisation by spectral and crystallographic studies; which follows the assessment of new compounds for their antioxidant activities. Further, to substantiate with the experimental results, the molecular docking and ADMET analysis was performed to substantiate the experimental results. The flowchart showing the methodology is presented on Figure 1. A library of trisubstituted pyrazoles prepared were presented in Figure 2.

3.1. Chemistry

A two-step synthesis of different tri-substituted pyrazole products was performed. In the first step, the required chalcones, 3(a–f) were prepared from thiophene-2-aldehyde 1, with various substituted acetophenone, 2(a–f) in line with our earlier report [34]. In the second step, the
Chalcones 3(a-f) were subjected to Amberlyst-15 (10%, w/w) catalyzed (3 + 2) annulation reaction with phenylhydrazine hydrochloride 4(a-b) in acetonitrile as solvent. The reaction was performed under swirling conditions. The reaction produced thienyl-pyrazoles, 5(a-i) in good yields. In an alternative method, the compounds 5(a-i) were also obtained by reflux conditions in 30% acetic acid medium without an added catalyst. In this research, cyclocondensation of the chalcones, and phenylhydrazine hydrochlorides produced desired trisubstituted pyrazole derivatives (Figure 3).

Amberlyst-15, (divinylbenzene-styrenesulfonic acid) is a versatile catalyst for the reactions under non-aqueous conditions, it promotes the reaction through its –SO₃H proton, in esterification, Prins cyclization, and crossed-aldol condensation etc., [35]. We found that Amberlyst-15 catalyzed synthesis of pyrazole derivatives 5(a-i) occurs in less time with minimum heat energy compared, and an improved product yields (>4–9%) comparable to the conventional reflux conditions method in acetic acid (Table 1). The recovered catalyst can be re-used efficiently.

3.2. Spectroscopic studies

In the ¹H NMR spectra, compounds 5(a-i) show that –CH₂- group of the pyrazole ring diastereotopic; and failed to show the signals of C=C
protons of 3(a-f), confirming the (3 + 2) annulation. In $^1$H NMR spectrum, compound 5i show a doublet of doublets for C-4-Ha at δ 3.146–3.207 (J = 7.6, 17.2 Hz) ppm; C4-Hb at δ 3.767–3.839 (J = 12.0, 16.8 Hz) ppm, and C5-Ha at δ 5.379–5.428 (J = 7.6, 12.0 Hz) ppm. It shows singlets for CH3 at δ 2.304 ppm, for two OCH3 at δ 3.903, δ 3.977 ppm, and multiplet at δ 6.750–6.851 ppm, δ 7.039–7.089 ppm, δ 7.215–7.481 ppm for aromatic protons. In $^{13}$C NMR spectrum, it shows signals for C-4, C-5 and C-3 carbons of pyrazoline ring at δ 43.14, 59.10 and 150.26 ppm, respectively. It also shows signals for CH3 at δ 13.92 ppm, for two OCH3 carbons at δ 55.93 and δ 55.99 ppm. In mass spectrum, it shows peaks at m/z 415.2 (M$^+$+2) (34%), 413.2 (M$^+$). It shows comparable CHN analysis data with the theoretical values. Analytical and spectral data ($^1$H & $^{13}$C NMR, MS) for all compounds 5(a-i) were in full agreement with the proposed structures, and also structurally related pyrazoles [35]. Furthermore, the structure of one compound 5d, of the series was provided by crystallographic studies.

### 3.3. Single crystal X-ray diffraction studies

The crystallographic data and structure refinement data of 5d are depicted in Table 2. The ORTEP of the molecule with the thermal ellipsoids drawn with 50% probability is shown in Figure 4. The bond length, bond angles and torsion angles are summarized in Tables 3, 4 and 5. The compound has four aromatic rings, viz; a pyrazole, thiophene, and two chlorophenyl rings. Thiophene ring is planar (r.m.s. deviation is 0.007(5) Å).

![Figure 10. The optimized structure of molecule 5d.](image)

![Figure 11. FM orbitals (HOMO-LUMO) with energy gap of a molecule 5d.](image)

![Figure 12. Molecular electrostatic potential map of a molecule 5d.](image)

### Table 6. FMO energies and chemical reactive descriptors of molecule 5d.

| Parameters       | Value [B3LYP/6-311++G(d,p)] (eV) |
|------------------|----------------------------------|
| $E_{\text{HOMO}}$ | -5.3005                          |
| $E_{\text{LUMO}}$ | -1.4749                          |
| $E_{\text{gap}}$  | 3.8256                           |
| Ionization potential (I) | 5.3005                        |
| Electron affinity (A) | 1.4749                        |
| Electronegativity (χ) | 3.3877                        |
| Chemical hardness (η) | 1.9128                         |
| Global softness (σ) | 0.5228                          |
| Electrophilicity (ω) | 2.9999                         |
| Chemical potential (μ) | -3.8777                       |
| Dipole moment (D) | 2.9618                           |

Where, $\chi = (I + A)/2$, $\eta = (I-A)/2$, $\sigma = 1/\eta$ and $\omega = \mu^2/2\eta$.

### Table 7. DPPH and hydroxyl radical scavenging activity of the compounds, 5(a-i).

| Compounds | DPPH radical assay | Hydroxyl radical assay |
|-----------|--------------------|------------------------|
|           | $IC_{50}$ (µM)$^a$ | $IC_{50}$ (µM)$^a$     |
| 5a        | 0.807 ± 0.01       | 3.534 ± 0.01           |
| 5b        | 0.488 ± 0.02       | 1.439 ± 0.01           |
| 5c        | 0.529 ± 0.02       | 1.813 ± 0.01           |
| 5d        | 0.673 ± 0.01       | 2.641 ± 0.02           |
| 5e        | 0.616 ± 0.03       | 1.965 ± 0.04           |
| 5f        | 0.694 ± 0.04       | 2.649 ± 0.02           |
| 5g        | 0.245 ± 0.01       | 0.905 ± 0.01           |
| 5h        | 0.284 ± 0.02       | 0.892 ± 0.01           |
| 5i        | 0.491 ± 0.01       | 1.913 ± 0.04           |
| AA$^*$    | 0.483 ± 0.01       | –                      |
| BHA$^{**}$| –                  | 1.739 ± 0.01           |

$^a$Ascorbic acid; $^{**}$Butylated hydroxyanisole were used as controls; $^a^b$Values represent mean ± SEM (n = 3).
Å) with maximum deviation (0.006(6) Å) observed for atoms C2 and C3. Pyrazole ring is little non-planar (r.m.s. deviation is 0.096(5) Å) with maximum deviation of 0.082(6) Å for atom C7. Among two chlorophenyl rings, 3-dichlorophenyl ring is planar (r.m.s. deviation is 0.010(6) Å) with maximum deviation (0.012(6) Å) observed for atom C17. Similarly, 4-chlorophenyl ring is also planar (r.m.s. deviation is 0.007(6) Å) with a deviation of 0.008(7) Å for atom C21. The methylthiophene ring is fixed at C7, 3-dichlorophenyl ring fixed at N11, 4-chlorophenyl ring is fixed at C9 and benzodioxole ring is fixed at C11 atom of the pyrazole ring. The chlorine atoms substituted to the corresponding phenyl rings are in the same plane of the rings.

A dihedral angle of 76.3(3)° between the pyrazole and thiophene ring indicates that they lie in different planes. Similarly, the angle between thiophene to 3-chlorophenyl and 4-chlorophenyl rings are 86.5(3)° and 74.1(3)° respectively. The dihedral angle of 13.5(3)° and 4.0(3)° between pyrazole ring with 3-chlorophenyl and 4-chlorophenyl rings indicates that these coplanar. The analyzed Cremer and Pople puckering parameters ($Q = 0.136(6)$ Å and $\phi = 78(2)$°) were in agreement with the structurally identical molecules \cite{36, 37}. The ring has envelope conformation on C7 atom with rotation parameters value of $P = 238.9(14)$° and $\tau = 14.1(3)$°. The C–H–N hydrogen bond and C–H–π and π–π interactions stabilized the structure: The C17–H17–N10 intramolecular hydrogen bond interactions forms five membered pseudo ring (N10/N11/C12/C17/H17). Also the other molecular interactions such as, C–H–π interaction: C2–H2–Cg1 with a C–Cg distance of 3.512(6) Å, H–Cg distance of 2.77 Å and C–H–Cg angle of 137°; C7–H7–Cg3 with a C–Cg distance of 3.735(6) Å, H–Cg distance of 2.93 Å and C–H–Cg angle of 140°; C15–H15–Cg4 with a C–Cg distance of 3.567(7) Å, H–Cg distance of 2.74 Å and C–H–Cg angle of 148°. π–π interaction: Cg2–Cg3 with a Cg–Cg distance of 3.956(4) Å, α =

Figure 13. The putative binding pose of compound 5g with Catalase (PDB ID: 2CAG); the ligand 5g is represented as green wire mesh deeply embedded into active site.
13.5(3)°, \( \beta = 22.7°, \gamma = 28.5° \) and a perpendicular distance of \( \text{Cg}_2 \) on ring \( \text{Cg}_3 = 3.649(2) \) Å. The packing of the molecules viewed across \( a, b \) and \( c \)-axis showing the layered stacking are depicted in Figures 5, 6, and 7, respectively.

### 3.4. Hirshfeld surface analysis

With CrystalExplorer 17.5, the 3D d norm was mapped on Hirshfeld surface (HS) with colour scale in between -0.583 au (blue) to 1.359 au (red). The calculated volume of the Hirshfeld surface is 451.41 Å³ with an area of 402.38 Å². The 2D fingerprint plots were generated at 0.6–2.8 Å, with \( d_i \) and \( d_e \) distance scales.

In the \( d_{\text{norm}} \) (Figure 8a), colour codes indicate the different molecular interactions; red with negative \( d_{\text{norm}} \), white with zero \( d_{\text{norm}} \), and blue with positive \( d_{\text{norm}} \) indicates the short contacts, intermolecular distances equal to van der Waals radii, and longer contacts [38, 39], respectively. Figure 9 shows the expanded 2D fingerprint plots [40] with the \( d_i \) and \( d_e \) distance scales. The fingerprint plot reveals the contribution of overall

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**Figure 14.** The putative binding pose of standard ascorbic acid with Catalase (PDB ID: 2CAG); Ascorbic acid is represented as green wire mesh deeply embedded into active site.
contacts (Figure 9a) including each individual intermolecular contact to the total molecular HS. The contributions of various interactions; H–H (30.3%), C–H (28.2%) [Figure 9b], Cl–H (24.0%) [Figure 9d], S–H (7.4%) [Figure 9e], N–H (4.6%) [Figure 9f], C–Cl (1.9%), S–Cl (1.0%), Cl–Cl (0.9%), C–S (0.6%) and C–N (0.1%) were quantified through 2D fingerprint plots. The contribution of H–H contacts will be of 30.3% to the total Hirshfeld surface. The shape index, curvedness of HS were generated (Figure 8b and c) to analyze the π-stacking interactions [41, 42, 43].

3.5. Density functional theory (DFT) calculations

The electronic and chemical active regions of a compounds were identified with the quantum chemical calculations, FMO energies, and MEP surface analysis. The structural coordinates are optimized (Figure 10) in gas phase using DFT method with B3LYP hybrid functional and 6–311+ +G(d,p) basis set. The calculated structural parameters were compared with experimental results. The optimized structure substantiates the experimental findings with the correlation coefficient values; bond lengths (0.9929), bond angles (0.9954) and torsion angles (0.9995) (Tables 3, 4 and 5). Further, the FMO energies (E_{HOMO}, E_{LUMO} and E_p) and chemical reactive descriptors [44, 45, 46, 47] were calculated (Table 6).

The polarizability and chemical reactivity of a compound was assessed on the band gap energy. The FMO HOMO-LUMO energies with the calculated energy gap of $E_{gap} = 3.8256 \text{ eV}$ (Figure 11). The red and green colors on the molecular surface indicates the positive and negative phases of the wave functions. The MEP map drawn in the range of $-3.100 \text{ e}^{-2} \text{ au}$ (red) to $+3.100 \text{ e}^{-2} \text{ au}$ (blue) (Figure 12). The positive (blue), and negative (green) regions of MEP represent an electrophilic and nucleophilic reactivity. The red regions are related to the electrophilic attack. The red and light blue colors are spread over chlorine and nitrogen, hydrogens [48], respectively.

3.6. Radical scavenging activity

The radicals are highly reactive and least stable species, formed during various metabolic processes that cause cellular damages in living cells, such as capable to break DNA and cause strand breakage [49]. In recent times, antioxidant display radical scavenging activity acts as anticancer, anti-inflammatory and antiaging agents [50]. Therefore, the antioxidant ability of new compounds 5(a-i) was assessed by DPPH and Hydroxyl radical assay using ascorbic acid (AA) and Butylated hydroxyanisole (BHA), as control treatment. The experiments were performed in triplicates with varied concentrations. The IC50 values were computed by using graphpad Prism using equation $Y_{Bottom} + \frac{(Top-Bottom)}{(1 + 10((LogIC_{50}-X)^{\text{HillSlope}}))}$ from the observed percentage inhibition of the compounds 5(a-i) (Table 7).

The preliminary assessment results indicated that each compound of series 5(a-i) exhibit modest to good radical scavenging activities as compared with respective standards AA and BHA. Of the nine tested pyrazoles, five shown moderate to potent abilities in both the assays, indicating their reducing abilities. The plausible mechanism involves the transfer of acidic H-atom by a compound to DPPH to form DPPH-H. The most active compounds were 5g and 5b (IC50 = 0.245 ± 0.01, and 0.284 ± 0.02 μM, respectively). These have potent RSA than ascorbic acid (IC50 = 0.483 ± 0.01μM), indicating that, the electron donating methyl and methoxy substituents in 3-substituted aromatic ring increases the antioxidant activity of pyrazoles. The two molecules 5b (IC50 = 0.488 ± 0.02μM) and 5i (IC50 = 0.491 ± 0.01μM) shows good RSA, but lower than ascorbic acid activity. The rest of the compounds 5c (IC50 = 0.488 ± 0.02μM), 5e (IC50 = 0.488 ± 0.02μM), 5d (IC50 = 0.488 ± 0.02μM), 5f (IC50 = 0.488 ± 0.02μM), and 5a (IC50 = 0.488 ± 0.02μM), displayed moderate activities. Interestingly, all of these compounds showed the better antioxidant properties than the structurally related compounds, 5-(3,4-dichlorophenyl)-3’-naphthalen-2-yl-1’-phenyl-3,4-dihydro-1’H-[3,4]bi pyrazoles reported, which showed DPPH radical scavenging activities in the range of IC50 = 9.66 ± 0.34 to 12.02 ± 0.63μM [51].

In the Hydroxyl radical scavenging assay; the most active molecules were 5g (IC50 = 0.905 ± 0.01μM), and 5h (IC50 = 0.892 ± 0.01μM). They exhibited potent RSA than BHA (IC50 = 1.739 ± 0.01μM). The compounds 5b (IC50 = 1.439 ± 0.01μM), and 5i (IC50 = 1.913 ± 0.04μM) shows good RSA, but lower than BHA. The rest of the compounds 5e (IC50 = 1.813 ± 0.01μM), 5f (IC50 = 1.965 ± 0.04μM), 5d (IC50 = 2.641 ± 0.02μM), 5f (IC50 = 2.649 ± 0.02μM), and 5a (IC50 = 3.534 ± 0.01μM), displayed moderate activities. However, all new compounds show better activities compared with the reported similar pyrazoles; like 1,3-diaryl-4-(aryl-propenonyl)-pyrazoles (IC50 = 13.5–13.5μM) [52], 3-(2-bromophenyl)-4-(4,5-diphenyl-1H-imidazo-2-yl)-1-(p-tolyl)-1H-pyrazole (IC50 = 10.2μM) [53], N,N-bis(3, 5-dimethyl-1H-pyrazol-1-yl)ethylthiazol-2-amine (IC50 = 14.76μM) [54], suggesting that the new compounds of the present work could act as potent antioxidants. The presence of substituents on the two phenyl rings at 1,3 positions of pyrazole nucleus influences the RSA. The molecule 5a of the series, with two unsubstituted benzene rings at 1 and 3 positions of pyrazole exhibited least activity in both assays. It was observed that the presence of the electron donating 4-methyl, 4-methoxy, and 3,4-dimethoxy groups on C-3 substituted aromatic ring enhanced the DPPH and OH radical scavenging activities.

3.7. Molecular docking and ADME/Tox predictions

Molecular docking studies for newly synthesized compounds suggest that the ligand 5h and 5g comparatively possess better antioxidant activity among 5(a-i), as compared with the standard antioxidant molecule

| Protein | Catalase (PDB id: 2CAG) | CuZn superoxide dismutase (PDB id: 1CB4) |
|---------|------------------------|------------------------------------------|
| Ligand  | Docking Score           | $E_{HOMO}$                                | $E_{LUMO}$ | XP Hbond | Docking Score | $E_{HOMO}$ | $E_{LUMO}$ | XP Hbond |
|         | (kcal/mol)              | (kcal/mol)                                | (kcal/mol) | (kcal/mol) | (kcal/mol)    | (kcal/mol) | (kcal/mol) | (kcal/mol) |
| 5a      | -3.26                   | -31.73                                   | -42.14     | -0.27     | 1.93          | -30.29     | -39.26     | 0.00      |
| 5b      | -3.74                   | -33.95                                   | -42.02     | -0.70     | -1.20         | -33.28     | -42.85     | -0.05     |
| 5c      | 0.07                    | -39.67                                   | -44.00     | 0.00      | -1.08         | -32.85     | -41.16     | -0.21     |
| 5d      | -3.90                   | -36.88                                   | -48.85     | -0.70     | 1.85          | -32.80     | -41.69     | 0.00      |
| 5e      | -2.00                   | -30.77                                   | -45.65     | -0.13     | 0.16          | -32.89     | -39.33     | 0.00      |
| 5f      | -2.31                   | -32.28                                   | -46.86     | -0.48     | 1.88          | -34.93     | -45.06     | -0.08     |
| 5g      | -5.41                   | -39.09                                   | -60.37     | -0.57     | -1.23         | -33.78     | -43.78     | -0.20     |
| 5h      | -4.40                   | -41.81                                   | -49.69     | -0.01     | 1.56          | -33.04     | -44.76     | -0.35     |
| 5i      | -4.06                   | -47.53                                   | -61.02     | -0.02     | 1.85          | -34.92     | -48.88     | -0.38     |
| Ascorbic acid | -9.25                | -23.53                                   | -37.25     | -2.53     | -4.24         | -25.72     | -26.66     | -2.11     |
Antioxidants play an important role in various patho-physiological disease conditions such as, cancer, chronic inflammation, diabetics, arthritis, neuro-degenerative disorder. Hence decrease in free radicals in these diseases’ conditions, enhance the patho-physiological condition towards the normal [31]. The ligand 5g forms π-π stacking with Catalase via Trp337 and Phe140 (Figure 13) which are closely resided at active site, whereas ascorbic acid forms salt bridge with Arg333 and with Protoporphyrin IX containing Fe (Figure 14). Three-dimensional view of 5g and ascorbic acid suggest that it is deeply embedded into active site surrounded with active site amino acids which is very important in aiding catalysis with dock score of -5.41 and -9.25 (kcal/mol) (Table 8).

Superoxide dismutase (SOD) is another very important enzyme which acts as antioxidant defense against oxidative stress in the body. The ligand 5g form π-cation with Arg113 (Figure 15); while ascorbic acid form hydrogen bond with Ile111 and Gly106. The compound binds at Cu–Zn domain of SOD led to increase in an antioxidant activity and decrease in oxidative stress [52]. ADME/Tox properties depict that the ligands possess druggable properties, with 95% of available drugs which are in market. The results molecular docking (Table 8) and ADME

![Figure 15. Putative binding pose of compound 5g with CuZn superoxide dismutase (PDB ID: 1CB4); the ligand is represented as green wire mesh deeply embedded into active site.](image)
4. Conclusion

In summary, a novel thiopeone-pyrazole pharmacophores 5(a-i) were synthesized using a re-useable Amberlyst-15 catalyst, and assessed for their in vitro DPPH and Hydroxyl radical scavenging abilities. The fine structure of the compound 5d is confirmed by crystallographic studies. The analysis revealed the presence of C-H...N type H-bond and stabilized by C-H..π and π-π interactions. The chemically reactive regions were identified by HSA, and DFT studies. All the new compounds showed promising DPPH and Hydroxyl radical scavenging activities. However, two compounds 5g and 5h showed excellent activities comparable with standards ascorbic acid and BHA, respectively; which was substantiated by docking and ADMET analysis. Therefore, the compounds 5g and 5h can be used as lead antioxidants for further investigations.

Additional information
No additional information is available for this paper.

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References

[1] D. Farbstein, A. Kozak-Blickstein, A.P. Levy, Antioxidant vitamins and their use in preventing cardiovascular disease, Molecules 15 (11) (2010) 8098–8110.
[2] S. Naveen, Karthik Kumara, A. Dileep Kumar, K. Ajay Kumar, A. Zarrouk, I. Warad, N.K. Lokanath, Synthesis, characterization, crystal structure, Hirshfield surface analysis, antioxidant properties and DFT calculations of a novel pyrazole derivative: ethyl 1-(2,4-dimethylphenyl)-3-methyl-5-phenyl-1H-pyrazole-4-carboxylate, J. Mol. Struct. 1226 (A) (2021) 129550.
[3] J.C. Jayaveeran, C. Praveen, Y. Arun, A.A.M. Prince, P.T. Perumal, Cycloisomerization of acetylenic oximes and hydrazones under gold catalysis: synthesis and cytotoxic evaluation of isoxazoles and pyrazoles, J. Chem. Sci. 128 (2016) 73–83.
[4] H. Zhang, Q. Wei, G. Zhu, J. Qu, B. Wang, A facile and expeditious approach to substituted 1H-pyrazoles catalyzed by iodine, Tetrahedron Lett. 57 (2016) 2633–2637.
[5] A. Shaheeni, H. Sepahvand, M.K. Nejad, A re-engineering approach: synthesis of pyrazole(1,2-a)pyrazoles and pyrazole(2,3-c)pyrazoles via an isocyanide-based four-component reaction under solvent-free conditions, Tetrahedron Lett. 57 (2016) 1432–1437.
[6] A. Dileep Kumar, C.B. Vagish, D.M. Lokeshwari, R. Sowmya, K. Ajay Kumar, Design, synthesis, characterization, evaluation for anticancer and cytotoxic properties of new pyrazole carbothioamides, Asian J. Org. Chem. 6 (1) (2021) 53–58.
[7] P. Fizero, L. Bialy, A.W. Brown, W. Czechylsky, M. Mendez, J.P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harrity, J. P.A. Harri
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