SYNTHESIS, ANTIOXIDANT ACTIVITY AND MOLECULAR DOCKING
OF SOME NEW THIAZOLYL-PYRAZOLINYL-PYRAN-2-ONE
DERIVATIVES


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Abstract – 3-[2-(5-Aryl-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4-yl]-4-hydroxy-6-methyl-2H-pyran-2-ones 6a-h were synthesized via the ring closure reactions of 3-phenyl-5-aryl-4,5-dihydropyrazole-1-carbothioamides 3a-h with 3-(bromoacetyl)-4-hydroxy-6-methyl-2H-pyran-2-one 5. The new synthesized thiazolyl-pyrazolinylin-pyran-2-one compounds were evaluated for their potential antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method. The molecular docking of the synthesized compounds on DPPH oxidase protein showed a high binding affinity, confirming the experimental results recorded. The synthesized compounds deserve a consideration for further analyses regarding the control of oxidative stress and their use as possible antioxidant agents in the pharmaceutical industry.
INTRODUCTION

Heterocyclic compounds, especially five- or six-membered cyclic compounds, rank first among various classes of organic compounds for their diverse biological activities and applications. These compounds exhibit numerous chemotherapeutic and pharmacological activities. Among them, chalcones represent an essential family of natural as well as synthetic products. Some of them possess a wide range of pharmacological activities such as antimicrobial, antitumor, anticancer, antituberculous, anti-inflammatory, antioxidant, antituberculou, and antileishmanial properties. The presence of the reactive α,β-unsaturated carbonyl group in chalcones turns out to be the main reason for their biological activity.

In the present work, the chalcones were prepared according to the Claisen-Schmidt method by condensation of acetophenone with various aromatic aldehydes (Scheme 1). Owing to numerous pharmacological and biological applications, thiazole derivatives have received considerable attention from the scientific community. Thiazole has been reported in the literature with various effects such as antifungal, anti-HIV, antituberculous, antiviral and antioxidant agent activity. Furthermore, the thiazolyl-pyrazoline ring is also a biologically valuable structural unit. Currently, various compounds based on the pyrazole nucleus are known to have analgesic, anti-inflammatory, antipyretic, antiarrhythmic, muscle relaxant, psychoanaleptic, antidiabetic, anticonvulsant, hypotensive and antibacterial activities. Led by these considerations, it seemed interesting to us to synthesize novel hybrid heterocycles, associating the thiazolyl-pyrazoline motif with pyran-2-one and to investigate their antioxidant activity.

RESULTS AND DISCUSSION

Chemistry

3-Phenyl-5-aryl-4,5-dihydropyrazole-carbothioamides 3a-h were prepared by the reaction of 1-phenyl-3-arylprop-2-en-1-ones 2a-h with thiosemicarbazide and sodium hydroxide in ethanol according to a reported procedure. The reaction mechanism involves the formation of hydrazone followed by subsequent addition of N–H on the olefinic bond of the propenone moiety.

The required 3-(bromoacetyl)-4-hydroxy-6-methyl-2H-pyran-2-one 5 is not commercially available. It was easily obtained via the selective α-monobromination of dehydroacetic acid (DHAA) 4 in 70% yield according to a known procedure. Reaction of the thioamide derivatives 3a-h with 5 yielded 4-hydroxy-6-methyl-3-[2-(5-aryl-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl]-2H-pyran-2-ones 6a-h as shown in Scheme 1.

The structures and purities of the synthesized products were deduced from NMR data and mass spectrometry. As a representative example, the 1H NMR spectrum of 6c shows two singlets at 2.21 and 5.84 ppm which are attributed to the proton resonances of the methyl group and the H-4 proton of the
pyrone ring, respectively. The upfield doublet of the doublet signals at 3.35 ppm (Ha) and 3.98 ppm (Hb) was identified as characteristic of the pyrazoline (Hb) geminal proton. Moreover, the vicinal proton (Hx) appeared at 5.52 ppm. The aromatic proton of thiazole appeared as a singlet at 7.59 ppm.

\[ \text{Scheme 1. Synthesis of 3-[2-(5-aryl-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4-yl]-4-hydroxy-6-methyl-2H-pyran-2-ones 6a-h} \]

**Antioxidant activity**

All the synthesized compounds 6a-h were evaluated for their in vitro antioxidant activity by DPPH radical scavenging assay at different concentrations. Figure 1 shows the variation of absorbance versus concentration of the different compounds 6a-h and of the standard ascorbic acid. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH• (or inhibition %) was calculated as follows:

\[ \text{RSA} \% = \left[ \left( \frac{\text{Ac} - \text{As}}{\text{Ac}} \right) \times 100 \right] \]

Where Ac is the absorbance of the control (absorbance of DPPH• EtOH solution without sample), and As is the absorbance of the tested compound after 60 min incubation. According to the results obtained, all the synthesized compounds presented a very good activity almost
similar for some compounds to that of ascorbic acid taken as reference. This can be explained by the presence of a hydroxyl group on the 6-methyl-2H-pyran-2-one ring.

Compound 6g presented the best result (IC$_{50}$ = 0.73 mg/mL) followed by compound 6a (IC$_{50}$ = 0.96 mg/mL) then compound 6e (IC$_{50}$ = 1.06 mg/mL) and compound 6h (IC$_{50}$ = 1.08 mg/mL). Compounds 6f and 6d were the least active. The presence of a sulfur heterocycle in compound 6g in addition to the hydroxyl group at position 4 on 6-methyl-2H-pyran-2-one gave this molecule better inhibitory power compared to the other compounds.

For compounds 6a-f, which have a simple or substituted benzene ring, the antioxidant activity varied according to the nature of the substituent. For compound 6a, the absence of a substituent on the benzene ring gave the molecule a better power of inhibition resulting from the presence of the hydroxyl group present on the 6-methyl-2H-pyran-2-one.

Antioxidant activity remained stable in compounds 6e and 6b; the presence of electron-donating substituents did not influence negatively the inhibition power of the compounds, unlike the presence of halogen (Cl). The two compounds 6f and 6d presenting a halogen group (Cl) in positions 2,6- and 2,4 led to a remarkable reduction in the antioxidant activity.

According to the obtained results, it can be noted that the presence of halogen (Cl) in positions 2,6 on the benzene ring generated a remarkable drop in activity (IC$_{50}$ =18.07 mg/mL) compared to the presence of halogen in positions 2,4 (IC$_{50}$ = 6.28 mg/mL).

Compound 6h also exhibited very good antioxidant activity (IC$_{50}$ =1.08 mg/mL). The presence of the -NH group preventing indole in addition to the hydroxyl group on 6-methyl-2H-pyran-2-one conferred on the molecule a good power of inhibition.

![Variation of absorbance versus concentration of the different compounds 6a-h and ascorbic acid](image)

**Figure 1.** Variation of absorbance versus concentration of the different compounds 6a-h and ascorbic acid.
Molecular docking

Binding modes of the synthesized compounds with NADPH oxidase protein were predicted by docking studies with Autodock 4. As the studied compounds are chiral, both enantiomers (S) and (R) were analysed for their binding energies and binding modes.\textsuperscript{32} The docking poses obtained from the Discovery Studio visualizer are shown in Figure 1.

The docking simulations of the main synthesized compounds with NADPH oxidase protein showed a higher binding affinity for (S)-enantiomer of 6g followed by (S)-enantiomer of 6a. These results, presented in Table 1, prove the significant affinities of the synthesized compounds for NADPH oxidase protein. It is worth noting that the negative value of binding energy change (ΔG) reveals that the binding process is spontaneous and that the compound can be accepted as a drug.\textsuperscript{33}

Table 1. Binding energies (ΔG) Predicted interacting residues for synthesized compounds 6a and 6g with their (R) enantiomer and (S) enantiomer with NADPH oxidase protein.

<table>
<thead>
<tr>
<th>Compound Interaction</th>
<th>6a (R) enantiomer</th>
<th>6a (S) enantiomer</th>
<th>6g (R) enantiomer</th>
<th>6g (S) enantiomer</th>
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<td>Gly180 (1.79 Å)</td>
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</table>

Visualisation of docked poses of the studied compounds showed that they occupied the binding site composed of aromatic residues as determined experimentally.\textsuperscript{33} The thiophene ring of the (S)-enantiomer of compound 6g, having the best experimental antioxidant activity (0.73 mg/mL), was located in the vicinity of the nitrogen atom of the amino group of residue Lys187 (3.16 Å), leading to π-cation interaction. This interaction was conserved in the docked conformations of both enantiomers of compound 6a. It occurs between the phenyl ring on position 5 of the dihydropyrazole and the nitrogen atom of the amino group of side chains of residues Lys187.
The phenyl ring of the thiazole ring of the (S)-enantiomer of compound 6a, was arranged almost perpendicular to the imidazole ring of His181, leading to attractive, noncovalent interactions, and $\pi-\pi$ stacking (Table 1 and Figure 2). This interaction was conserved in the docked conformation (R)-enantiomer of the same compound whereas this interaction was not present in the case of the enantiomers of compound 6g.

Besides, the (S)-enantiomer of compound 6g was engaged in four hydrogen bonding interactions with the amino acid residues of the NADPH oxidase protein. The hydrogen atom of the amino group side chain of residue Lys187 formed two hydrogen bonds with the hydroxide group and nitrogen atom of the thiazole ring (1.97 Å and 2.08 Å, respectively). These results are in good agreement with previous studies which state that the residues Lys213, His181 and Lys187 play a determining role in ligand binding by forming hydrogen bonds or by stabilizing the charge of bound NADPH molecules. In addition, the carbonyl oxygen backbone of residue Ile243 is involved in hydrogen bond interaction with the hydroxyl group of the (S)-6g compound (1.80 Å). The fourth hydrogen bond interaction occurs between the backbone nitrogen of residue Ser157 and the carbonyl oxygen of the pyranone ring (1.99 Å). Two of these hydrogen-bonding interactions were retained in the docked conformation of (R)-6g, namely those with Lys187 and Ser157.

On the other hand, both (R) and (S) enantiomers of compound 6a maintain the hydrogen bond interaction with residue Lys187. Both enantiomers of compound 6a are also involved in a hydrogen bonding interaction with the NH backbone of residue Gly180 via its hydroxyl group (2.33 Å and 2.09 Å), which might contribute to the inhibitor binding.

Moreover, the phenyl ring at position 3 of the dihydropyrazole ring was pointing directly toward the residues side chain of two residues in the channel terminating at the Re-face, namely Pro298 and Leu299, leading to favorable hydrophobic interactions. The thiophene ring was located in the vicinity of residues Phe245, Gly244, Ile243, Cys242, and Tyr188, also leading to favorable hydrophobic interactions. Furthermore, other interactions were observed between the (S)-enantiomer of compound 6g and active site residues of the NADPH oxidase protein such as polar and charged (positive and negative) with Ser328, His181, Ser157, Lys187, Glu182, Asp179, Lys187, Arg183, Lys213, which may stabilize the ligand in the active site and increase its affinity. Most of these interactions were conserved in the docked conformation of the (R)-enantiomer of 6g. Comparison between the four selected docked conformations shows that two novel hydrophobic interactions were observed in the docked conformations of the two enantiomers of compound 6a with residues Val214 and Ile160. Finally, comparison between docked conformations of the (R) and (S)-enantiomers of compound 6g, the most potent compound, showed that the (S) enantiomer of compound 6g bound with NADPH oxidase protein is more stable than the (R) enantiomer of 6g bound to the selected protein. In contrast, docking results suggest that, in the case of
compound 6a, the complex (R)-6a bound to the NADPH oxidase protein complex is more stable due to a higher number of favorable interactions and hydrogen bonds, both polar and positively/negatively charged, between this enantiomer and the studied receptor.

In view of the above analysis, the docking results obtained in the present study showed that compounds 6a and 6g form hydrogen bonds and favorable hydrophobic interactions with conserved residues of the binding site of NADPH oxidase protein such as Ile160, His181 and Lys187, thus stabilizing the ligand in the active site of the protein. These results prove significant antioxidant activities, which deserve consideration for further analyses concerning the control of oxidative stress and their use as possible antioxidant agents in the pharmaceutical industry.

**Figure 2.** 2D Views of binding modes of compounds A-(S) and (R)-6a and B-(S) and (R)-6g NADPH oxidase protein
EXPERIMENTAL

The chemical reagents and solvents (Fluka products) used were of analytical grade and were used without further purification. Melting points of samples were measured using open capillary tubes determined on a Stuart SMP3 melting point apparatus and are uncorrected. The infrared spectra were recorded in the region 4000–400 cm$^{-1}$ on a BRUKER TENSOR 27 IR spectrophotometer without KBr. Electronic spectra were measured on a JENWAY 6800 ultraviolet-visible spectrophotometer; measurements were made from 200 to 800 nm.

$^1$H and $^{13}$C NMR spectra were recorded in CDCl$_3$ or DMSO-$d_6$ solutions on a Bruker Avance 300 (300 MHz for $^1$H and 75 MHz for $^{13}$C) spectrometer. Chemical shifts are reported in parts per million (δ, ppm) using TMS as internal reference and coupling constants (J) are given in hertz (Hz). The following abbreviations were used for the $^1$H NMR spectra multiplicities: br. s: broad singlet, s: singlet, d: doublet, t: triplet, q: quartet, qt: quintuplet, m: multiplet. Electrospray ionization high-resolution mass spectrometry experiments were performed with a hybrid tandem quadrupole/time-of flight (QTOF) instrument, equipped with a pneumatically assisted electrospray (Zspray) ion source (Micromass, Manchester, U.K.) operated in positive mode. The progress of the reactions was monitored throughout by TLC plates (silica gel G) using mobile phase, chloroform: methanol (5:1), and the spots were identified by iodine vapors or UV light.

**Synthesis**

The synthetic route to compounds 3a-h and 6a-h is shown in Scheme 1 using a procedure slightly modified from that in the literature. 35,36

**General procedure of synthesis of 3,5-diphenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide derivatives.** A mixture of substituted chalcone 2 (1 mmol) and thiosemicarbazide (1 mmol) was dissolved in 10 mL of anhydrous EtOH and stirred vigorously. Pellets of NaOH (1.5 mol) were added to the mixture and the reaction was refluxed for 6 h. After completion of the reaction (controlled by TLC), the products obtained were collected by filtration, washed with hot EtOH to give the pure 3,5-diphenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (3a-h).

3,5-Diphenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (3a), pale yellow solid was obtained. Yield: 72%. Mp: 201–203 °C (203–204 °C).$^{37-41}$ $^1$H NMR (300 MHz, CDCl$_3$) δppm: 3.24 (dd, $J_{gem}$ = 17.7 Hz, $J_{cis}$ = 3.6 Hz, CH-pyrazoline, 1H), 3.87 (dd, $J_{gem}$ = 17.7 Hz, $J_{trans}$ = 11.4 Hz, CH-pyrazoline, 1H), 6.07 (dd, $J_{trans}$ = 11.4 Hz, $J_{cis}$ = 3.9 Hz, Ar-H, 1H), 6.11 (br s, NH, 1H), 7.14 (br s, NH, 1H), 7.26–7.24 (m, Ar-H, 2H), 7.39–7.30 (m, Ar-H, 3H), 7.48–7.43 (m, Ar-H, 3H), 7.77–7.74 (m, Ar-H, 2H).

5-(4-Dimethylaminophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (3b), white solid was obtained. Yield: 91%. Mp: 199–201 °C (198–200 °C).$^{42}$ $^1$H NMR (300 MHz, CDCl$_3$) δppm: 2.78 (s, N(CH$_3$)$_2$, 6H), 3.27 (dd, $J_{gem}$ = 17.7 Hz, $J_{cis}$ = 8.1 Hz, CH-pyrazoline, 1H), 3.82 (dd, $J_{gem}$ = 17.7 Hz, $J_{trans}$ = 11.4 Hz, CH-pyrazoline, 1H), 7.01–7.03 (m, Ar-H, 2H), 7.09–7.08 (m, Ar-H, 3H), 7.38–7.30 (m, Ar-H, 3H), 7.47–7.42 (m, Ar-H, 3H), 7.76–7.73 (m, Ar-H, 2H).
= 12.6 Hz, CH-pyrazoline, 1H), 6.02 (dd, \(J_{\text{trans}} = 12.6\) Hz, \(J_{\text{cis}} = 8.1\) Hz, CH-pyrazoline, 1H), 6.09 (br s, NH, 1H), 6.74 (d, \(J = 8.4\) Hz, Ar-H, 2H), 7.09 (br s, NH, 1H), 7.19 (d, \(J = 8.4\) Hz, Ar-H, 2H), 7.48–7.41 (m, Ar-H, 3H), 7.78–7.74 (2H, m, Ar-H).

5-(4-Chlorophenyl)-3-phenyl-4,5-dihydro-1\(H\)-pyrazole-1-carbothioamide (3c), white solid was obtained. Yield: 80\%. Mp: 175–177 °C (174–178 °C).\(^{13}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) ppm: 3.19 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{cis}} = 3.9\) Hz, CH-pyrazoline, 1H), 3.87 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{trans}} = 11.4\) Hz, CH-pyrazoline, 1H), 6.03 (dd, \(J_{\text{trans}} = 11.4\) Hz, \(J_{\text{cis}} = 3.9\) Hz, CH-pyrazoline, 1H), 6.18 (br s, NH, 1H), 7.14 (br s, NH, 1H), 7.19 (d, \(J = 8.7\) Hz, Ar-H, 2H), 7.31 (d, \(J = 8.7\) Hz, Ar-H, 2H), 7.90–7.43 (m, Ar-H, 3H), 7.76–7.73 (m, Ar-H, 2H).

5-(2,6-Dichlorophenyl)-3-phenyl-4,5-dihydro-1\(H\)-pyrazole-1-carbothioamide (3d), beige solid was obtained. Yield: 35\%. Mp: 210–212 °C (209-210 °C).\(^{42}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) ppm: 3.34 (dd, \(J_{\text{gem}} = 18.0\) Hz, \(J_{\text{cis}} = 7.8\) Hz, CH-pyrazoline, 1H), 3.88 (dd, \(J_{\text{gem}} = 18.0\) Hz, \(J_{\text{trans}} = 12.6\) Hz, CH-pyrazoline, 1H), 6.02 (br s, NH, 1H), 6.51 (dd, \(J_{\text{trans}} = 12.6\) Hz, \(J_{\text{cis}} = 7.8\) Hz, CH-pyrazoline, 1H), 7.19 (d, \(J = 8.1\) Hz, Ar-H, 1H), 7.27 (br s, NH, 1H), 7.37 (dd, \(J = 7.8, 1.2\) Hz, Ar-H, 1H), 7.51–7.45 (m, Ar-H, 3H), 7.63 (dd, \(J = 8.1, 1.2\) Hz, Ar-H, 1H), 7.78–7.75 (m, Ar-H, 2H).

5-(3-Methoxyphenyl)-3-phenyl-4,5-dihydro-1\(H\)-pyrazole-1-carbothioamide (3e), white solid was obtained. Yield: 93\%. Mp: 169–171 °C (172 °C).\(^{40,44}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) ppm: 3.23 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{cis}} = 3.6\) Hz CH-pyrazoline, 1H), 3.79 (s, OCH\(_3\), 3H), 3.86 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{trans}} = 11.4\) Hz CH-pyrazoline, 1H), 6.02 (dd, \(J_{\text{trans}} = 11.4\) Hz, \(J_{\text{cis}} = 3.6\) Hz, CH-pyrazoline, 1H), 6.06 (br s, NH, 1H), 6.87 (d, \(J = 8.7\) Hz, Ar-H, 2H), 7.12 (br s, NH, 1H), 7.18 (d, \(J = 8.7\) Hz, Ar-H, 2H), 7.49–7.42 (m, Ar-H, 3H), 7.78–7.74 (m, Ar-H, 2H).

5-(2,4-Dichlorophenyl)-3-phenyl-4,5-dihydro-1\(H\)-pyrazole-1-carbothioamide (3f), white solid was obtained. Yield: 87\%. Mp: 219–221 °C (217–220 °C).\(^{40,41}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) ppm: 3.12 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{cis}} = 3.9\) Hz, CH-pyrazoline, 1H), 3.86 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{trans}} = 11.7\) Hz, CH-pyrazoline, 1H), 6.21 (br s, NH, 1H), 6.31 (dd, \(J_{\text{trans}} = 11.7\) Hz, \(J_{\text{cis}} = 3.9\) Hz, CH-pyrazoline, 1H), 7.03 (d, \(J = 8.4\) Hz, Ar-H, 1H), 7.15 (br s, NH, 1H), 7.22 (dd, \(J = 8.4, 2.1\) Hz, Ar-H, 1H), 7.52–7.41 (m, Ar-H, 4H), 7.75–7.72 (m, Ar-H, 2H).

3-Phenyl-5-thiopen-3-yl-4,5-dihydro-1\(H\)-pyrazole-1-carbothioamide (3g), brown solid was obtained. Yield: 59\%. Mp: 181–183 °C (184 °C).\(^{45}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) ppm: 3.29 (dd, \(J_{\text{gem}} = 17.4\) Hz, \(J_{\text{cis}} = 3.3\) Hz, CH-pyrazoline, 1H), 3.79 (dd, \(J_{\text{gem}} = 17.4\) Hz, \(J_{\text{trans}} = 11.1\) Hz, CH-pyrazoline, 1H), 6.09 (br s, NH, 1H), 6.20 (dd, \(J_{\text{trans}} = 11.1\) Hz, \(J_{\text{cis}} = 3.3\) Hz, CH-pyrazoline, 1H), 7.01 (dd, \(J = 5.1, 1.2\) Hz, Ar-H, 1H), 7.09 (1H, NH, br s), 7.24 (dd, \(J = 3.0, 1.2\) Hz, Ar-H, 1H), 7.29 (dd, \(J = 5.1, 3.0\) Hz, Ar-H, 1H), 7.90–7.43 (m, Ar-H, 3H), 7.78–7.75 (m, Ar-H, 2H).

5-(1\(H\)-Indol-2-yl)-3-phenyl-4,5-dihydro-1\(H\)-pyrazole-1-carbothioamide (3h), Pale yellow crystal was
obtained. Yield: 48%. Mp: 183–185 °C (185 °C).\(^1\)H NMR (300 MHz, CDCl\(_3\)) δ ppm: 3.49 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{cis}} = 3.6\) Hz, CH-pyrazoline, 1H), 3.86 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{trans}} = 11.4\) Hz, CH-pyrazoline, 1H), 6.02 (br s, NH, 1H), 6.38 (dd, \(J_{\text{trans}} = 11.4\) Hz, \(J_{\text{cis}} = 3.6\) Hz, CH-pyrazoline, 1H), 7.05 (td, \(J = 7.8, 0.9\) Hz, CH-indole, 1H), 7.10 (br s, NH, 1H), 7.18 (td, \(J = 7.8, 0.9\) Hz, CH-indole, 1H), 7.27 (br s, CH-indole, 1H), 7.40–7.36 (m, CH-indole, 2H), 7.51–7.53 (m, Ar-H, 3H), 7.83–7.79 (m, Ar-H, 2H), 8.18 (br s, NH, 1H).

General procedure for the synthesis of 4-hydroxy-6-methyl-3-[2-(5-aryl-3-phenyl-4,5-dihydro-pyrazol-1-yl)thiazol-4-yl]-2H-pyran-2-ones. To a mixture of substituted 3,5-diphenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide 3 (2 mmol) in EtOH (10 mL) was added 3-bromoacetyl-4-hydroxy-6-methyl-2H-pyran-2-one 5 (2.1 mmol). The resulting mixture was stirred under reflux for 8 h. The progress of the reaction was monitored by TLC. After cooling to room temperature, the precipitate obtained were filtered, and then recrystallized from MeOH to get the pure desired compounds 6a-h.

3-[2-(3,5-Diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-thiazol-4-yl]-4-hydroxy-6-methyl-2H-pyran-2-one (6a), pale yellow powder was obtained. Yield: 90%. Mp: 242–244 °C. IR ν max (cm\(^{-1}\)): 1742 (CO=O), 1691 (C=N), 1589 (CH ar), 1200, (C-O), 687 (CH ar). \(^1\)H NMR (300 MHz, CDCl\(_3\)) δ ppm: 2.22 (s, CH\(_3\), 3H), 3.31 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{cis}} = 7.5\) Hz, CH-pyrazoline, 1H), 3.98 (dd, \(J_{\text{gem}} = 17.7\) Hz, \(J_{\text{trans}} = 12.0\) Hz, CH-pyrazoline, 1H), 5.50 (dd, \(J_{\text{trans}} = 12.0\) Hz, \(J_{\text{cis}} = 7.5\) Hz, CH-pyrazoline, 1H), 5.86 (s, H-pyrole, 1H), 7.40–7.31 (m, Ar-H, 5H), 7.48–7.45 (m, Ar-H, 3H), 7.61 (1H, s, CH-thiazole), 7.79–7.76 (m, Ar-H, 2H), 7.35. (br s, OH-pyrole, 1H). \(^13\)C NMR (75 MHz, CDCl\(_3\)) δ ppm: 19.9, 44.3, 64.5, 101.4, 106.6, 126.6 (2C), 127.4 (2C), 128.8 (2C), 129.5 (2C), 130.4, 130.7, 134.2, 139.1, 152.9, 161.0, 168.3. HRMS (ESI) m/z [M+H]+ calcd for C\(_{26}\)H\(_{25}\)N\(_{2}\)O\(_{3}\): 430.1219, found: 430.1244.

3-[2-(5-(4-Dimethylaminophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4-yl]-4-hydroxy-6-methyl-2H-pyran-2-one (6b), light brown powder was obtained. Yield: 78.1%. Mp: 254–256 °C. IR ν max (cm\(^{-1}\)): 1739 (CO-O), 1690 (C=N), 1589 (C=Car), 1203 (C-O), 687 (CHar). UV: \(\lambda_{\text{abs}} = 349–263\) nm.\(^1\)H NMR (300 MHz, DMSO-d\(_6\)) δ ppm: 2.20 (s, CH\(_3\), 3H), 2.97 (s, N-CH\(_3\), 6H), 3.32 (dd, \(J_{\text{gem}} = 18.0\) Hz, \(J_{\text{cis}} = 6.7\) Hz, CH-pyrazoline, 1H), 4.07 (dd, \(J_{\text{gem}} = 18.0\) Hz, \(J_{\text{trans}} = 11.7\) Hz, CH-pyrazoline, 1H), 5.63 (dd, \(J_{\text{trans}} = 11.7\) Hz, \(J_{\text{cis}} = 6.7\) Hz, CH-pyrazoline, 1H), 6.12 (s, H-pyrole, 1H), 7.02 (br s, Ar-H, 1H), 7.30 (d, \(J = 7.8\) Hz, Ar-H, 2H), 7.43 (s, CH-thiazole, 1H), 7.51–7.49 (m, Ar-H, 4H), 7.82–7.79 (m, Ar-H, 2H), 13.73 (s, OH-pyrole, 1H). \(^13\)C-NMR (75 MHz, DMSO) δ ppm: 19.8, 64.5 (2C), 67.3, 88.2, 91.1, 94.8, 101.3 (2C), 105.1, 127.1 (2C), 127.3, 127.6 (2C), 129.4 (2C), 130.9, 131.1, 143.5, 149.8, 155.1, 161.3, 162.4, 164.1, 168.5. HRMS (ESI) m/z [M+H]+ calcd for C\(_{26}\)H\(_{25}\)N\(_{2}\)O\(_{3}\): 473.1641; found: 473.1666.

3-[2-(5-(4-Chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4-yl]-4-hydroxy-6-methyl-2H-pyran-2-one (6c), green powder was obtained. Yield: 58%. Mp: 269–271 °C. IR ν max (cm\(^{-1}\)): 3159 (=C=H), 1693 (C=N), 1579 (C=C), 1209 (C-O), 758 (CHar), 684 (CHar). UV: \(\lambda_{\text{abs}} = 344–272\) nm;\(^1\)H NMR (300 °C).
3-Hydrate-3\{-5-(2,6-Dichlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrAzol-1-yl\}thiazol-4-yl\}-4-hydroxy-6-methyl-2
H-pyran-2-one (6d), brown powder was obtained. Yield: 74%. Mp: 228–229 °C. IR νmax (cm−1): 1743 (C=O), 1673
(C=N, Pyr), 1578 (C=C, Ar), 748 (C-H, Ar). 1H NMR (300 MHz, CDCl3) δ: 2.21 (s, CH3, 3H), 3.32 (dd, Jgem = 17.7 Hz, Jcis = 7.2 Hz, CH-pyrazolone, 1H), 5.48 (dd, Jtrans = 12 Hz, Jcis = 7.2 Hz, CH-pyrazolone, 1H), 5.86 (s, H-pyron, 1H), 6.92 (d, J = 8.7 Hz, Ar-H, 2H), 7.30 (d, J = 8.7 Hz, Ar-H, 2H), 7.44–7.46 (m, Ar-H, 3H), 7.58 (s, CH-thiazole, 1H), 7.75–7.78 (m, Ar-H, 2H), 7.81–7.88 (m, Ar-H, 2H), 13.27 (s, CH-pyrazolone, 1H). 13C-NMR (75 MHz, CDCl3) δ: 19.9, 24.7, 44.4, 60.3, 64.6, 95.6, 101.4, 105.5, 126.3, 126.5 (2C), 127.2, 127.4, 127.7, 128.3, 128.9, 129.0, 129.8, 130.2, 130.4, 130.8, 133.4, 135.5, 152.7, 160.8, 168.2. HRMS (ESI) m/z [M+H]+ calcd for
C26H19ClN3O3S: 498.0440; found: 498.0466.

4-Hydroxy-3\{-2\{-5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrAzol-1-yl\}thiazol-4-yl\}-6-methyl-2H-
pyran-2-one (6e), yellow powder was obtained. Yield: 74%; Mp: 226–228 °C; IR νmax (cm−1): 1743 (C=O), 1673
(C=N, Pyr), 1578 (C=C, Ar), 748 (C-H, Ar). 1H NMR (300 MHz, CDCl3) δ: 2.22 (s, CH3, 3H), 3.32 (dd, Jgem = 17.7 Hz, Jcis = 7.2 Hz, CH-pyrazolone, 1H), 5.86 (s, H-pyron, 1H), 6.92 (d, J = 8.7 Hz, Ar-H, 2H), 7.30 (d, J = 8.7 Hz, Ar-H, 2H), 7.44–7.46 (m, Ar-H, 3H), 7.58 (s, CH-thiazole, 1H), 7.75–7.78 (m, Ar-H, 2H), 13.70 (s, CH-pyrazolone, 1H). 13C-NMR (75 MHz, CDCl3) δ: 19.9, 24.7, 44.4, 55.3, 64.6, 95.6, 101.4, 106.3, 114.6 (2C), 126.5 (2C), 127.2 (2C), 128.8 (2C), 130.3, 131.0, 132.7, 143.8, 152.9, 159.5, 160.9, 162.7, 163.9, 168.4. HRMS (ESI) m/z [M+H]+ calcd for
C28H22N2O4S: 460.1325; found: 460.1351.

3\{-2\{-5-(2,4-Dichlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrAzol-1-yl\}thiazol-4-yl\}-4-hydroxy-6-methyl-2
H-pyran-2-one (6f), yellow powder was obtained. Yield: 74%. Mp: 226–228 °C; IR νmax (cm−1): 1743 (C=O), 1673
(C=N, Pyr), 1578 (C=C, Ar), 748 (C-H, Ar). 1H NMR (300 MHz, CDCl3) δ: 2.22 (s, CH3, 3H), 3.32 (dd, Jgem = 17.7 Hz, Jcis = 7.5 Hz, CH-pyrazolone, 1H), 5.86 (s, H-pyron, 1H), 6.89 (dd, Jtrans = 12.0 Hz, Jcis = 7.5 Hz, CH-pyrazolone, 1H), 7.25 (d, J = 0.9 Hz, Ar-H, 2H), 7.47–7.45 (3H, m, Ar-H), 7.53 (s, CH-thiazole, 1H), 7.65 (s, Ar-H, 1H), 7.79–7.86 (m, Ar-H, 2H), 13.54 (s, CH-pyrazolone, 1H). 13C-NMR (75 MHz, CDCl3) δ:
19.8, 42.6, 61.9, 95.5, 101.4, 105.8, 126.6 (2C), 128.0, 128.1, 128.8 (2C), 130.0, 130.5, 130.6, 132.6, 134.5, 136.1, 143.9, 153.2, 161.1, 163.8, 168.4. HRMS (ESI) m/z [M+H]^+ calcd for C_{24}H_{18}Cl_{2}N_{5}O_{5}S: 498.0440; found: 498.0466.

4-Hydroxy-6-methyl-3-(2-(3-phenyl-5-thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4-yl)-2H-pyran-2-one (6g), pale yellow powder was obtained. Yield: 90%. Mp: 266–268 °C. IR ν max (cm⁻¹): 1741 (CO-O), 1681(C=N, Pyr), 1572 (C=C, Ar), 1213 (C-O), 762 (C-H, Ar). ¹H NMR (300 MHz, CDCl₃) δ: 2.23 (s, CH₃, 3H), 3.37 (dd, J_{gem} = 17.7 Hz, J_{cis} = 7.5 Hz, CH-pyrazoline, 1H), 3.92 (dd, J_{gem} = 17.7 Hz, J_{trans} = 11.7 Hz, CH-pyrazoline, 1H), 5.66 (dd, J_{trans} = 11.7 Hz, J_{cis} = 7.5 Hz, CH-pyrazoline, 1H), 5.89 (s, H-pyrene, 1H), 7.07–7.05 (m, CH-thiazole, 1H), 7.36 (d, J = 1.2 Hz, CH-thiazole, 2H), 7.47–7.45 (3H, m, ArH), 7.60 (s, CH-thiazole, 1H), 7.79–7.77 (m, Ar-H, 2H), 13.74 (s, OH-pyrene, 1H). ¹³C-NMR (75 MHz, CDCl₃): δ 19.8, 43.3, 60.8, 101.4, 105.5, 122.4, 122.7, 124.9, 126.5 (2C), 127.7, 128.8 (2C), 130.4, 130.9, 136.8, 140.9, 153.1, 157.3, 161.0, 164.0, 168.4. HRMS (ESI) m/z [M+H]^+ calcd for C_{22}H_{18}N_{5}O_{5}S_{2}: 436.0784; found: 436.0808.

4-Hydroxy-3-[2-[5-(1H-indol-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]thiazol-4-yl]-6-methyl-2H-pyran-2-one (6h), yellow powder was obtained. Yield: 60%. Mp: 279–281 °C. IR ν max (cm⁻¹): 1746 (CO-O), 1698 (C=N, Pyr), 1569 (C=C, Ar), 1209 (C-O), 755 (C-H, Ar), 1058 (C-N). ¹H NMR (300 MHz, DMSO-d₆) δ: 2.19 (s, CH₃, 3H), 3.50 (dd, J_{gem} = 18.0 Hz, J_{cis} = 6.9 Hz, CH-pyrazoline, 1H), 4.08 (dd, J_{gem} = 18.0 Hz, J_{trans} = 12.0 Hz, CH-pyrazoline, 1H), 5.98 (dd, J_{trans} = 12.0 Hz, J_{cis} = 6.9 Hz, CH-pyrazoline, 1H), 6.11 (s, H-pyrene, 1H), 6.91 (t, J = 7.2 Hz , CH-indole, 1H), 7.06 (t, J = 7.2 Hz, CH-indole, 1H), 7.19 (d, J = 8.1 Hz, CH-indole, 1H), 7.34 (s, CH-thiazole, 1H), 7.38 (d, J = 8.1 Hz, CH-indole, 1H), 7.53–7.51 (m, Ar-H, 4H), 7.89–7.86 (m, Ar-H, 2H), 11.22 (s, N-H indole, 1H), 14.13 (s, OH-pyrene, 1H). ¹³C-NMR (75 MHz, DMSO-d₆): δ 19.8, 42.5, 59.0, 94.8, 101.3, 104.5, 112.6, 113.6, 118.4, 119.6, 122.0, 124.4, 125.0, 127.0 (2C), 129.4 (2C), 130.8, 131.2, 137.5, 143.5, 155.2, 161.7, 162.3, 163.9, 168.6. HRMS (ESI) m/z [M+H]^+ calcd for C_{26}H_{21}N_{4}O_{5}S: 469.1328; found: 469.1355.

**In vitro antioxidant evaluation**

The DPPH test is a simple technique and requires only a vis spectrophotometer or an electronic paramagnetic resonance (EPR) spectrometer. However, DPPH is not a natural radical but the mechanism of reaction with antioxidants is similar to that with peroxyl radicals ROO⁻.⁴⁶

The DPPH neutralization test is based on donating electrons from the antioxidants in order to neutralize the DPPH radical. The reaction is accompanied by changing the DPPH color measured at 517 nm, and discoloration acts as an indicator of antioxidant activity. Antioxidant activity by the DPPH neutralization method is often reported as EC50, which is defined as the efficient concentration of the antioxidant necessary to reduce the initial DPPH concentration by 50%. In addition, TEC50 may be used, which is the necessary time to reach the equilibrium state with EC50.³¹
prepared by dissolving 4 mg DPPH• in 100 mL EtOH. Compounds 6a-g, were dissolved in DMSO to obtain a solution of 10-1 M. Test compounds were further diluted with DMSO to obtain final concentrations of 0.05, 0.025 and 0.0125 mol/L for all the compounds, whereas the standard (ascorbic acid, AA) was diluted to 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.0015625, 0.00078125 mol/L solutions respectively. Wells were loaded with 40 µL of tested sample and then with 2 mL of DPPH• solution. All assays were carried out in triplicate. Negative control wells were loaded with 40 µL of DMSO and 2 mL of DPPH• solution. After vortexing, the mixtures were incubated at room temperature for 1 h in darkness at 25 °C, and then the absorbance of the plate was recorded at 517 nm. A blank containing only EtOH with DMSO was used as the control. Each measurement was performed in triplicate.

Molecular docking studies
In order to understand the antioxidant activities of the synthesized compounds at the atomic level, molecular docking studies were performed. The crystallographic structure PDB ID 2CDU with resolution of 1.80 Å corresponding to the NADPH oxidase protein complexed with its inhibitor ADP was downloaded from the Royal Collaboratory for Structural Bioinformatics, RCSB Protein Data Bank. NADPH oxidase is a primary target for antioxidant effects. All heteroatoms, non-receptor atoms; water and other ions were removed. Polar hydrogen atoms and partial Gasteiger charges were performed using AutodockTools 1.5.7. The amino acids nearest to ADP and optimized structures of the synthesized compounds were treated as flexible during the molecular docking simulations while the rest of the protein was kept rigid. Molecular docking studies were performed on the prepared compounds to predict the binding mode, binding energies and orientation at the active site of the selected NADPH oxidase protein. Thus, a grid map of 56×42×54 points around the modeled domain was used. The spacing dimensions of the grid were 0.375 Å. The Lamarckian genetic algorithm (LGA) as implemented in Autodock 4 software with default docking parameters was used. At the end of docking, the best conformation of each compound was analyzed for its binding interactions.

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