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Chalcone-Derived Dihydropyrazoles as Dual Antiproliferative and Antioxidant Agents: Insights from Synthesis, Assays, and EGFR Docking

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Abstract

Chalcones are conjugated 1,3-unsaturated ketones that have a variety of pharmacological properties, such as antidiabetic, antioxidant and anticancer actions. Current study showed that, a chalcone, (*E*)-1,3-diphenylprop-2-en-1-one (**1)** was synthesized by reacting benzaldehyde with acetophenone. This compound was subsequently added with hydrazine hydrate and hydrazine hydrate in the attendance of acetic acid to yield two pyrazole derivatives, namely 3,5-diphenyl-4,5-dihydro-1*H*-pyrazole **(2)** and 1-(3,5-diphenyl-4,5-dihydropyrazol-1-yl) ethanone (**3)**. The synthesized compounds were characterized using FTIR and ^{1}H NMR spectroscopy. Compounds **1** to **3** demonstrated significant cytotoxic activity against the HeLa cell line. Additionally, they exhibited notable antioxidant properties. *In silico* studies were performed to evaluate the drug-likeness, pharmacokinetic profiles and molecular docking interconnections with the epidermal growth factor receptor (EGFR). These computational analyses provided insights into the potentiality of the compounds for drug-receptor binding and their pharmacokinetic behaviors. This work reports, for the first time, the comparative biological and computational studies conducted here. However, further detailed investigations are required to fully understand their mechanisms of action.

Key Words: (*E*)-1,3-diphenyl-prop-2-en-1-one, pyrazole, HeLa cell, antioxidant, anticancer.

Introduction

Chalcones are an important class of organic compounds characterized by the presence of two aromatic rings joined together by a three-carbon α, β-unsaturated carbonyl system. This structural framework plays a critical role in defining their physicochemical properties and biological activities. Chalcones have been extensively studied in medicinal chemistry due to their remarkable spectrum of biological activities, which include antioxidant, anticancer, antidiabetic, antifungal, antiviral, antimalarial, anti-inflammatory effects etc. (Dao *et al*., 2011). Furthermore, chalcones serve as versatile precursors in the synthesis of a wide range of heterocyclic compounds, such as oxazines, pyrazoles, isoxazoles, pyrazolines and so on (Kumar *et al.,* 2013). These heterocyclic systems are often associated with enhanced pharmacological properties.

Among the derivatives of chalcones, pyrazoles hold a distinct position due to their nitrogen-containing heterocyclic structure, which is a key motif in pharmaceutical and agricultural chemistry. Pyrazole derivatives are recognized for their extensive range of pharmacological activities, including their ability to act as anticancer, antioxidant, analgesic, antipyretic, antitubercular,

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anti-inflammatory, antidepressant, anticonvulsant agents, etc. These properties make pyrazole an attractive scaffold for the development of therapeutic agents targeting a variety of diseases (Kumar and Jayaroopa, 2013).

Despite the availability of numerous chemotherapeutic agents for cancer treatment, this disease remains a global health challenge, affecting millions of individuals each year. A significant limitation of existing therapies is the occurrence of adverse effects, the development of drug resistance, and high cost. This has necessitated the exploration of novel therapeutic agents with improved efficacy and reduced side effects (Albratty and Alhazmi, 2022; Mahapatra *et al*, 2021; Shaik *et al.,* 2020). Pyrazole-based compounds have emerged as promising candidates of new anticancer drugs, owing to their ability to interact with critical molecular targets involved in cancer progression. In addition to their anticancer potential, pyrazole derivatives have been studied for their antioxidant properties, which enable them to mitigate oxidative shock - a condition characterized by a disparity between free radicals and the antioxidant defenses of the body. Oxidative stress is known to damage cellular structures, impair immune function and contribute to the progression of various diseases (Albratty and Alhazmi, 2022; Rani *et al.*, 2017).

The synthesis of chalcones and pyrazole derivatives has garnered significant attention due to their versatile biological activities and potential therapeutic applications. Chalcones are typically synthesized through the Claisen-Schmidt condensation, a reaction between an aromatic ketone and an aromatic aldehyde (Ali *et al.,* 2020). This reaction is widely used because it allows the introduction of various substituents, enabling fine-tuning of the biological activities of the resulting compounds. On the other hand, pyrazoles are commonly synthesized through cyclization reactions involving hydrazines and α, β-unsaturated carbonyl compounds. The introduction of functional groups at specific positions on the pyrazole ring has been shown to significantly influence their biological activity (Sharma and Kumar, 2019).

Recent advances in medicinal chemistry have highlighted the potential of hybrid molecules that combine the structural features of chalcones and pyrazoles (Patel *et al.,* 2021). These hybrid compounds often exhibit synergistic pharmacological activities, surpassing the bioactivity of their individual components (Singh *et al.,* 2022). For instance, chalcone-pyrazole hybrids have demonstrated potent antimicrobial, anticancer, and anti-inflammatory activities *in vitro* (Verma *et al.,* 2023). Animal studies have further supported their therapeutic potential, revealing their ability to reduce tumor growth and inflammation. The enhanced activity of these hybrids is believed to stem from their ability to interact with multiple molecular targets, such as enzymes and receptors involved in disease progression (Chaudhary *et al.,* 2020). Furthermore, ongoing research aims to optimize the pharmacokinetic properties of these hybrids, such as their bioavailability, metabolic stability and safety profile, to maximize their therapeutic utility.

In the present study, the chalcone compound *(E)*-1,3-diphenyl-prop-2-en-1-one **(1)** was synthesized via a condensation reaction between acetophenone and benzaldehyde under controlled conditions. Following this, two derivatives of pyrazole were prepared. The first derivative named after 3,5-diphenyl-4,5-dihydro-1*H*-pyrazole **(2)**, was synthesized by reacting the chalcone with hydrazine hydrate, resulting in the cyclization of the α, β-unsaturated carbonyl system. The second derivative named after 1-(3,5-diphenyl-4,5-dihydropyrazol-1-yl) ethanone **(3)**, was synthesized by further modifying the pyrazole structure through a reaction involving hydrazine and acetic acid. Their antioxidant potential was assessed using free radical scavenging assays and total antioxidant capacity assays, which provide insights into their ability to neutralize reactive oxygen species and prevent oxidative damage. The anticancer properties of the compounds were evaluated using an antiproliferative assay on the HeLa cell line, a widely used model for studying cervical cancer.

Additionally, *in silico* drug likeness analyses were conducted to explore the pharmacokinetic prospects of the synthesized compounds. Molecular docking studies were also performed to inspect the interaction of the compounds with the epidermal growth factor receptor (EGFR), a critical target in cancer therapy. For the first time, this study presents comparative biological and computational investigations carried out here.

Materials and Methods

General experimental design and equipment: Melting points were measured using capillary tube. IR spectra were observed by an FTIR spectrophotometer. On a Bruker DPX-600 spectrometer (600-MHz), ^{1}H NMR spectra were acquired using TMS as an internal reference. Thin-layer chromatography (TLC) employed on precoated silica gel 60 F_{254} (E. Merck), while column chromatography employed on silica gel (60-120 mesh). The reagents were supplied by Merck (Germany) and Loba Chemie (India).

*Synthesis of (E)-1,3-diphenyl-prop-2-en-1-one **(1)**:* In a beaker, a mixture of 0.005 mole (0.6 g) of acetophenone and 0.005 mole (0.530 g) of benzaldehyde was dissolved in 10 ml of ethanol. A fifty percent aqueous solution of NaOH was then added dropwise to the mixture, while stirring continuously using a magnetic stirrer for 15-20 minutes at room temperature (Figure 1). Thin layer chromatography (TLC) was conducted at hourly intervals to monitor the progress of the reaction. The reaction mixture was then stored in a refrigerator overnight and a light-yellow solid compound was obtained (Díaz-Carrillo *et al*., 2018).

Yield 92%; Light-yellow solid; mp. 53-55 $^{\circ}$C; IR (cm^{-1}): 3061 (Csp^2-H stretching), 1660 (C=O stretching), 1575 (C=C stretching), 1602 and 1496 (C=C stretching, aromatic ring), 1447-1337 (C-C stretching), and 1216 (C-O stretching); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41 (3H, br. s, H-6, H-7, H-8), 7.50 (2H, t, J=7.2 Hz, H-11, H-13), 7.55 (1H, d, J=16.2 Hz, H-2), 7.59 (1H, m, H-12), 7.63 (2H, m, H-5, H-9), 7.81 (1H, d, J=16.2 Hz, H-3), 8.03 (2H, d, J=7.8 Hz, H-10, H-14).

*Synthesis of 3,5-diphenyl-4,5-dihydro-1H-pyrazole **(2)**:* In a beaker, a mixture of 0.005 mole (1.040 g) of (*E*)-1,3-diphenylprop-2-en-1-one **(1)** and 0.005 mole (0.25 g) of hydrazine hydrate was dissolved in 20 ml of ethanol. This solution was then transferred to a round-bottom flask and refluxed at 70-75°C for 10 hours (Figure 1). Thin layer chromatography (TLC) was carried out at hourly intervals to monitor the reaction. After cooling down, a light-brown solid was observed. Later, ethanol was used to recrystallize it to purify further (Osman *et al*., 2012).

Yield 70%; Light-brown solid; mp. 88-90 $^{\circ}$C; IR (cm^{-1}): 3061 (C-H stretching, aromatic ring), 1583 (C=N stretching), 1104 (C-N stretching), and 838 (C-N scissoring); ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.23 (1H, m, H-4a), 3.84 (1H, m, H-4b), 5.65 (1H, m, H-5), 6.85 (1H, br.s, pyrazole -NH), 7.2-7.74 (10H, m, Ar-H).

*Synthesis of 1-(3,5-diphenyl-4,5-dihydropyrazol-1-yl) ethanone **(3)**:* In a beaker, a solution containing 0.005 mole (1.040 g) of (*E*)-1,3-diphenylprop-2-en-1-one (**1**), 0.005 mole (0.25 g) of hydrazine hydrate and 7 ml of acetic acid was prepared in 20 ml of ethanol. This mixture was then transferred to a round-bottom flask and refluxed at a temperature of 70-75°C. After 14 hours of reflux, a deep-yellow solid was formed (Figure 1). Later, ethanol-based recrystallization was done (Zhou *et al*., 2013).

Yield 81%; Deep-yellow solid; mp. 110-112 ºC; IR (cm^{-1}): 3062 (C-H stretching, aromatic ring), 1659 (C=O stretching), 1596 (C=N stretching), 1143 (C-N stretching), and 864 (C-N scissoring); ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.49 (3H, s, 7-$COCH_3$), 3.16 (1H, br.d, J=17.4 Hz, H-4a), 3.77 (1H, dd, J=16.8, 12 Hz, H-4b), 5.64 (1H, br. d, J=12 Hz, H-5), 7.26-7.8 (10H, m, Ar-H).

Evaluation of antiproliferative properties: The qualitative cell viability assay is a critical method for evaluating the cytotoxicity of test compounds on HeLa cells, a human cervical cancer model that is widely used in research. This assay allows

researchers to observe morphological changes, cell adherence, and proliferation directly under a microscope, providing insights into the health and survival of the cells. The principle is based on the ability of viable cells to maintain normal morphology and adhesion, while non-viable cells may detach, shrink, or undergo lysis due to compound-induced stress. This method is important for preliminary

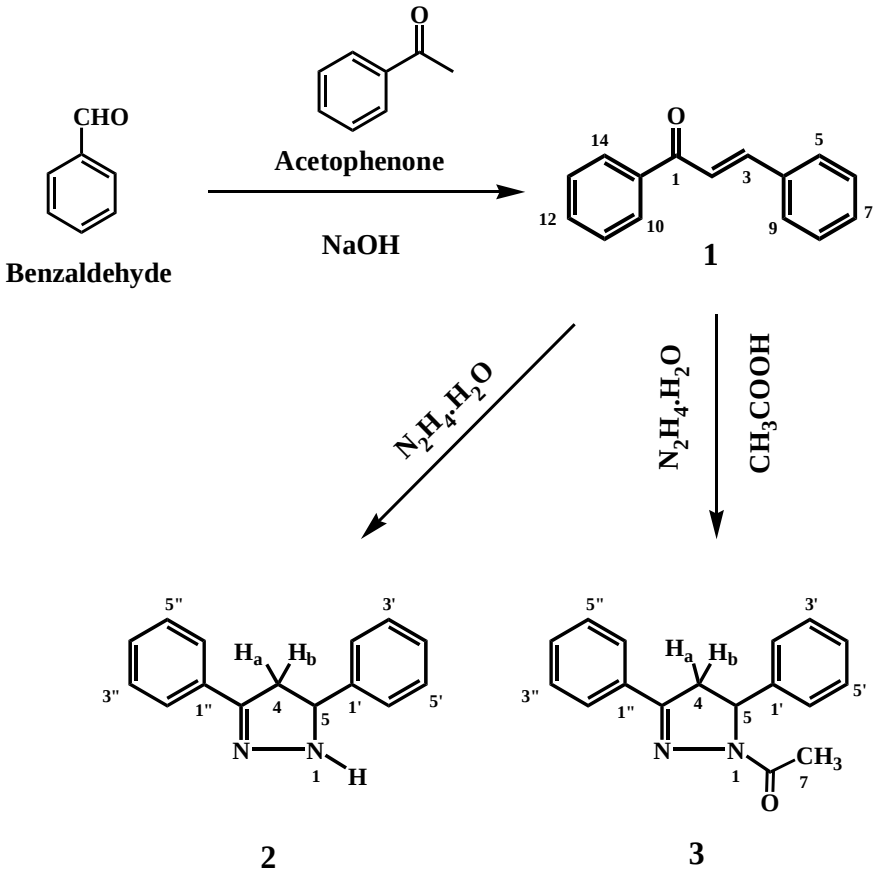

Figure 1. Illustration of the synthetic pathway of compounds **1-3**.

$$\% \text{ Inhibition of free radical} = \frac{\text{Absorbance in nm of DPPH} - \text{Absorbance in nm of Sample}}{\text{Absorbance in nm of DPPH}} \times 100$$

screening of potential therapeutic agents and assessing their anticancer effects. Additionally, it serves as a cost-effective and straightforward approach to identifying promising compounds for further quantitative analysis and drug development (El Habbash, 2017). The HeLa cell line was grown in Dulbecco's Modified Eagle Medium (DMEM) for this assay. To maintain sterile conditions, the medium was supplemented with one percent of penicillin together with streptomycin (one to one ratio) and 0.2 percent of gentamycin, while 10 percent of fetal bovine serum (FBS) was added as a nutrient source. A stock solution of the test compound was prepared by dissolving three mg of the substance in 3 ml of DMSO, maintaining a final concentration of 1 mg/ml and ensuring DMSO constituted less than 5% of the aqueous phase. Cells were dispersed at a density of 4.0×10^4 cells per 200 µl in 48-well plates and incubated at 37 °C with 5 percent of CO_2 to sustain optimal pH conditions. After 24 hours of incubation, 50 µl of the filtered solution of the test compounds was included to each well. The plates were then returned to the incubator under identical conditions for an additional 24 hours. Following the incubation

period, cell viability was examined using an inverted light microscope to observe changes in morphology and adherence (Ganji *et al*., 2023).

Assessment of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity: The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay is commonly utilized to assess the antioxidant activity of various compounds. In this assay, antioxidants neutralize the DPPH radical by donating an electron or hydrogen atom, leading to a color change from purple to yellow as the radical is reduced. The degree of this color shift is directly related to the ability of the compounds to scavenge free radicals. This assay is highly valued due to its simplicity, low cost and reliability, making it an effective tool for evaluating both natural and synthetic antioxidant agents. It plays a crucial role in identifying potential candidates for treating conditions associated with oxidative stress. This common technique is responsible for assessing antioxidant activity of a substance, which gauges how well it scavenges free radicals (Brand-Williams *et al*., 1995a). The free radical scavenging of the compounds was estimated using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. To perform the assay, One and a half milliliters of a DPPH solution, prepared in MeOH, was mixed with one milliliter of the compound solution, also dissolved in methanol. The mixture was then incubated in the dark for 30 to 60 minutes to prevent any light-induced changes that could interfere with the results. After incubation, the absorbance of the resulting solution was measured at 517 nm using methanol as a blank to account for the solvent. To quantify the free radical scavenging potential of the test compound, the absorbance of the DPPH solution without the compound was also recorded. The percentage inhibition was then calculated based on the difference in absorbance between the test solution and the DPPH solution alone, and this data was used to construct a % inhibition curve. The IC_{50} value, which denoted the required concentration of the test compounds to inhibit fifty percent of the DPPH radicals, was derived from this curve. This method provided valuable insights into the antioxidant potential of the tested compounds (Sökmen and Akram, 2016).

Estimation of total antioxidant capacity: The total antioxidant capacity (TAC) assay is a widely used method for assessing the overall antioxidant potential of a sample by evaluating its ability to counteract free radicals. The principle of this assay involves measuring the reduction of a colored reagent when antioxidants present in the sample donate electrons or hydrogen atoms to stabilize the free radicals, resulting in a detectable change in absorbance. Unlike methods that target individual antioxidants, the TAC assay offers a holistic view of the combined antioxidant activity within a sample. This assay is significant as it provides an insight into the ability of the sample to mitigate oxidative stress, a contributing factor in various chronic health conditions such as cancer, cardiovascular diseases and neurodegenerative disorders. The total antioxidant capacity assay was conducted following the method outlined by Prieto *et al*., 1999a, which involved the use of a reagent composed of 0.6M sulfuric acid, 28M sodium phosphate and 4M ammonium molybdate. To perform the assay, 0.1 ml of sample, dissolved in methanol at varying serial dilutions ranging from 200 to 25 µg/ml, was added to Eppendorf tubes containing the reagent mixture. The samples were then subjected to heating in a water bath at 95°C for 90 minutes to facilitate the reaction. After this incubation period, the absorbance of the resulting solution was measured at 695 nm using a spectrophotometer. The principle behind this assay was that antioxidants present in the sample interact with the reagent, leading to a change in color, which was directly proportional to the antioxidant capacity of the sample. In this study, a higher absorbance indicated a greater total antioxidant capacity, reflecting the ability of the sample to neutralize free radicals as well as reactive oxygen species (Prieto *et al*., 1999b).

Calculation of drug likeness properties: The drug-likeness, physicochemical properties, and pharmacokinetic features of compounds **1-3** were estimated using SwissADME, a comprehensive and

user-friendly online tool. SwissADME provides predictions on key drug-like characteristics, such as solubility, permeability and lipophilicity, which are essential for assessing the potential of the compounds as a therapeutic agent. The tool estimates a range of pharmacokinetic properties such as absorption, distribution, metabolism, and excretion (ADME) by analyzing the chemical structure of the compounds.

These predictions are valuable for understanding how the compounds might behave in biological systems and their likelihood of achieving therapeutic efficacy. By utilizing SwissADME, the study provided an early-stage assessment of the suitability of the compounds for drug development and discovery (http://www.swissadme.ch/) (Daina *et al*., 2017a).

In silico molecular docking studies: Epidermal growth factor receptor (EGFR) is a crucial target in the treatment of cancer, as inhibiting its activity can significantly aid in cancer therapy. Compounds that act as EGFR inhibitors have shown promising results in combating cancer, and a growing library of pyrazole-containing compounds has been explored as potential EGFR inhibitors (Kurban *et al*., 2023). In this study, compounds **1-3**, alongside a well-established EGFR inhibitor named after Erlotinib, were subjected to molecular docking analysis to evaluate their binding affinity with EGFR. The three-dimensional structures of these compounds were obtained from the PubChem database as SDF format (https://pubchem.ncbi.nlm.nih.gov/). Additionally, the crystal structure of EGFR, having the PDB ID of 1M17, was retrieved in PDB format from the RCSB Protein Data Bank (https://www.rcsb.org/) (Stamos *et al*., 2002), with a resolution of 2.60 Å. Using PyMOL version 2.5.2 software (Schrodinger, LLC), unnecessary atoms, molecules and ligands were removed from the EGFR structure to prepare it for docking analysis (Lill and Danielson, 2011). The enzymatic receptor-binding domain was also eliminated and the protein chains were stored in pdbqt format, which was suitable for molecular docking analysis (Morris and Lim-Wilby, 2008). The docking study was performed using the PyRx software (Dallakyan and Olson, 2015). Binding affinities (in kcal/mol), non-covalent interactions and the 2D and 3D representations of the protein-ligand docking complexes were generated using BIOVIA Discovery Studio. These studies provided profound insights into the binding interactions and potential efficacy of the synthesized compounds as EGFR inhibitors.

Results and Discussion

Compound **1** was a light-yellow solid and after TLC over silica gel PF_{254}, spraying with vanillin-sulfuric acid, and heating at 90 °C for 5 minutes, it showed a yellow spot. The chemical was soluble in methanol, chloroform, ethyl acetate, and others. The IR data of it displayed characteristic peaks at frequency (cm^{-1}): 3062 (Csp^2-H stretching), 1661 (C=O stretching), 1576 (C=C stretching), 1601 and 1497 (C=C stretching of aromatic ring), 1445-1335 (C-C stretching) and 1215 (C-O stretching) (Chovatia *et al*., 2006a). The ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound **1** exhibited resonances of five aromatic protons as multiplets at δ 7.41 (H-6, H-7 and H-8) and 7.63 (H-5 and H-9). The triplet at δ 7.50 (J=7.8 Hz) could be integrated for ArH-11 and ArH-13. In addition, a deshielded broad doublet for ArH-10 and ArH-14 was seen at δ 8.03 (J= 7.8 Hz), which might be ascribed to a neighboring electronegative keto group (C-1) in a side chain. Another multiplet at δ 7.59 indicated the presence of ArH-12. Additionally, two doublets detected at δ 7.55 and δ 7.81 with greater J value (16.2 Hz) could be allotted to H-2 and H-3 in an (*E*) alkene. Based on the inspection of the above-mentioned IR and NMR data with earlier published data (Mameda *et al*., 2016; Hu *et al*., 2004; Ibieta *et al*., 2016), the structure of compound **1** was established as a chalcone *(E)*-1,3-diphenylprop-2-en-1-one.

Compound **2** appeared as light-brown solid. When it was subjected to TLC (thin-layer chromatography) over silica gel PF_{254}, it appeared as dark spot-on UV and as yellow spot while spraying with vanillin-sulfuric acid and heating at a temperature range of 80-85 °C for a duration of 5-10

minutes. The compound showed solubility in various solvents such ethanol, chloroform and ethyl acetate. The IR data of the compound **2** showed characteristic peaks at frequency (cm^{-1}): 3061 (C-H stretching, aromatic ring), 1583 (C=N stretching), 1104 (C-N stretching and 838 (C-N scissoring) (Chovatia *et al*., 2006b). When compound **2** was subjected to ^1H NMR analysis in $CDCl_3$ solvent at 600 Hz, and it exhibited multiplets at δ 3.23, 3.84 and 5.65 for H-4a, H-4b and H-5, respectively for a pyrazole ring. The –NH (H-1) was appeared as broad singlet at δ 6.85. Hydrogens of aromatic ring showed chemical shift at δ 7.20-7.74 for 10 protons. By comparing the NMR data with previously published references of related pyrazoles (Safaei-Ghomi *et al*. 2006a), the compound **2** was confirmed as 3,5diphenyl-4,5dihydro-1*H*-pyrazole.

Compound **3** appeared as deep-yellow solid. When it was subjected to TLC (thin layer chromatography) over silica gel PF_{254}, it appeared as dark spot-on UV and as yellow spot while spraying with vanillin-sulfuric acid and heating at a temperature range of 80-85 °C for a duration of 5-10 minutes. The compound showed solubility in various solvents such ethanol, chloroform and ethyl acetate. The IR data of the compound **3** showed characteristic peaks at frequency (cm^{-1}): 3062 (C-H stretching, aromatic ring), 1659 (C=O stretching), 1596 (C=N stretching), 1143 (C-N stretching) and 864 (C-N scissoring) (Chovatia *et al*., 2006c). When compound **3** was subjected to ^1H NMR analysis in $CDCl_3$ solvent at 600 Hz, and it exhibited a broad doublet at δ 3.16 (J = 17.4 Hz), a double doublet at 3.77 (J = 16.8, 12 Hz), and another broad doublet at δ 5.64 (J = 12 Hz) for H-4a, H-4b and H-5, respectively for a pyrazole nucleus. The 7-$COCH_3$ appeared as a broad singlet at δ 2.49. Hydrogens of aromatic ring showed chemical shift at δ 7.26-7.8 for 10 protons. By comparing the NMR data with previously published references of related pyrazoles (Safaei-Ghomi *et al*., 2006b), the compound **3** was confirmed as 1-(3,5-diphenyl-4,5-dihydropyrazol-1-yl) ethanone.

The HeLa cell line, originating from Henrietta Lacks cervical cancer cells in 1951, had become one of the most widely used human cancer cell lines in the field of biomedical research. These immortalized cells had played a crucial role in advancing scientific knowledge across numerous disciplines, including toxicity testing, drug discovery, and cancer research. HeLa cells had been indispensable for investigating the potential anticancer effects of new compounds and therapeutic interventions. A key component of these investigations was the evaluation of cytotoxicity, which involved in assessing the survival of cells when exposed to various substances. Cytotoxicity was typically quantified by comparing the number of live cells to dead or damaged cells, as this ratio provides excellent insight into the toxicity of the sample being tested. In the present cytotoxicity assay, HeLa cells were exposed to several experimental compounds, and their survival was monitored using a hemocytometer-based cell counting technique. The findings from this assay demonstrated that the control group, consisting only of media, showed a 100% survival rate, indicating no toxic effects. Similarly, when the media contained a vehicle (the solvent needed for the dissolution purpose of the subject compounds), the survival rate remained above 95%, suggesting that the vehicle itself did not adversely affect the HeLa cells. In contrast, treatment with compounds **1**, **2** and **3**, when dissolved in the media, led to a dramatic decrease in cell survival, with all three compounds resulting in a survival rate of less than 5%. This suggested that these compounds exerted strong cytotoxic effects, significantly reducing cell viability. These observations were made using a hemocytometer, a device for accurately counting cells, under an inverted phase-contrast microscope. This method allowed for clear differentiation between viable and non-viable cells, ensuring that the cytotoxicity results were both accurate and consistent. The results are mentioned in table 1.

A widely utilized method in drug discovery for evaluating the antioxidant potential of various compounds concerned as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. This method offers a simple, cost-effective approach for assessing the ability of a substance to neutralize free radicals by

donating an electron or hydrogen atom. The DPPH assay is depicted as particularly valuable during the early stages of drug development when researchers aimed to identify compounds that may help mitigate diseases or conditions related to oxidative stress. Oxidative stress plays a substantial part in the pathogenetic pathway of many chronic ailments, including cancer, neuronal degeneration-based disorders and cardiovascular diseases, making antioxidant compounds a focal point in the search for therapeutic agents. In this particular study, the DPPH assay revealed a clear, dose-dependent decrease in the absorbance of DPPH radicals, which is a direct indication of the scavenging activity of the tested substances. As the concentration of the compounds increased, there was a corresponding reduction in the radical absorbance, demonstrating the effectiveness of the compounds in neutralizing free radicals. This observation suggests that the substances investigated possess robust antioxidant properties, capable of counteracting oxidative damage by scavenging harmful free radicals. These results are consistent with findings from previous studies, further confirming the strong antioxidant activity of the compounds. The ability to quench free radicals is a critical characteristic for potential therapeutic agents that could alleviate oxidative stress-related diseases. The dose-dependent nature of the response observed in this assay delivers profound views into the potency of the compounds, indicating that their antioxidant capabilities may be influenced by their concentration. This could strengthen the potential of the investigated compounds as candidates for further development in the context of oxidative stress-related therapies (Brand-Williams *et al*., 1995b). Higher quantities exhibited stronger antioxidant activity, suggesting that they could be helpful in the battle against oxidative stress. In case of DPPH free radical scavenging assay, the maximum scavenging activity was shown by **1**, (IC_{50} =34.6 µg/ml). The results are mentioned in table 2.

Table 1. HeLa cell mediated cell viability assay of synthesized compounds 1-3.

Sample	Survival of cells in HeLa medium	Cytotoxicity
Media without vehicle	Hundred percent	Negative
Media with Vehicle	More than ninety five percent	Negative
1	Less than five percent	Positive
2	Less than five percent	Positive
3	Less than five percent	Positive

The total antioxidant capacity (TAC) assay is another valuable method for evaluating the antioxidant ability of compounds, focusing on their ability to reduce oxidative stress. In this experiment, a significant reduction in the phosphomolybdenum complex was observed by the synthesized compounds **1-3**, which is widely recognized as an indicator of potent antioxidant activity. This reduction reflected the capability of the tested compounds to diminish reactive species and counters oxidative damage, which is a key factor in preventing cellular damage caused by free radicals. The findings from the TAC assay aligned with those obtained from the DPPH assay, reinforcing the evidence that the samples possess strong antioxidant properties. These complementary results underscored the potential of the compounds to mitigate oxidative damage, which is a common factor in a range of diseases, including cancer, cardiovascular conditions and neurodegenerative disorders. The consistency between the two assays, DPPH and TAC, further supported the reliability of the antioxidant capabilities of the substances being tested. Both assays demonstrated that the samples exhibit effective scavenging actions against free radicals, suggesting that they could be explored for their therapeutic potential in treating diseases linked to

oxidative stress. Additionally, in the context of the TAC assay, compound **1** stood out as the one demonstrating the highest antioxidant activity. This indicated that compound **1** was particularly effective at neutralizing oxidative species, making it a strong candidate for further discovery and potential therapeutic application in the management of oxidative stress-related diseases. Taken together, the results from both the DPPH and TAC assays provided compelling evidence for antioxidant capabilities of the samples, holding up their prospects as candidates for the development of drugs aimed at reducing oxidative damage and improving overall health. The results are described in figure 2.

Table 2. Assessment of IC_{50} using DPPH free radical sequestering method.

Sample	IC_{50} ± SD (μg/ml)
BHT	10.7 ± 0.03
1	34.6 ± 0.25
2	35.8 ± 0.15
3	53.9 ± 0.30

Expression of results were done as mean ± standard deviation (SD), where sample size was 3.

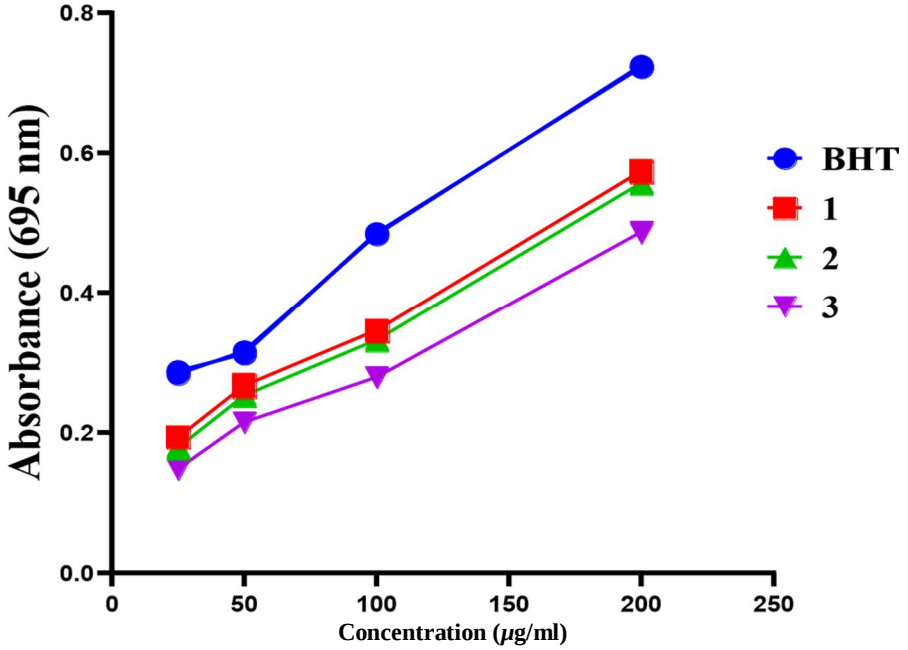

Figure 2. Comparative estimation of the total antioxidant activity of compounds 1-3 and the standard.

The drug-likeness, physicochemical properties, and pharmacokinetic profiles of compounds **1**, **2** and **3** were assessed using the SwissADME web tool (http://www.swissadme.ch/) (Daina *et al.*, 2017b). Compounds **1-3** showed the superior intestinal absorption indicating their good bioavailability. The blood-brain barrier (BBB) permeability test findings for all three substances were also positive, indicating that they could be able to pass across the BBB. For these compounds, the topological polar surface area (TPSA) values varied from 17.07 Å² to 32.67 Å², suggesting moderate polarity. After more investigation, it was shown that compounds **1**, **2** and **3** effectively inhibited cytochrome P-450 enzymes, including 2C19 and 2D6, as well as combinations of 2C9 and 2C19. However, cytochrome P450 enzymes 1A2 and 3A4 were not inhibited by any of the substances. According to Chen *et al.*, 2020, the characteristics for compounds **1-3** were consistent with Lipinski's rule of five, a widely recognized guideline for determining a compound's drug-likeness. The web tool also used other drug-likeness filters,

such as those suggested by Veber, Ghose, Egan and Muegge. Compound **1** demonstrated a breach of the Muegge filter, while all other compounds satisfied the requirements. Notwithstanding this slight variation, every molecule showed promising drug-likeness potential. According to this, compounds **1**, **2** and **3** had favorable qualities that could lead to their further development as possible therapeutic agents. The findings are listed in table 3.

Table 3. Drug likeliness, pharmacokinetics and physicochemical properties of compounds 1-3 measured using *in silico* method.

Frameworks	1	2	3
A) Physicochemical features			
Molecular formula	$C_{15}H_{12}O$	$C_{15}H_{14}N2$	$C_{17}H_{16}N_2O$
Volume	201.85	213.90	249.83
Molecular weight (MW)	208.26	222.29	264.32
Rotatable bond (RB) number	3	2	3
MLOGP	3.44	3.01	2.95
H-bond acceptor (HBA) number	1	1	2
Topological polar surface area (TPSA)	17.07 $Å^2$	24.39 $Å^2$	32.67 $Å^2$
Molar refractivity (MR)	66.25	77.12	87.03
H-bond donor (HBD)	Null	One	Null
XLOGP	3.08	3.33	2.70
B) Pharmacokinetics			
Gastro-Intestinal Absorption	High	High	High
Inhibitor of cyt. P450 2C9 enzyme	Negative	Negative	Positive
Substrate of P-glycoprotein	Negative	Negative	Negative
Inhibitor of cyt. P450 1A2 enzyme	Negative	Negative	Negative
Inhibitor of cyt. P450 2D6 enzyme	Negative	Positive	Negative
Inhibitor of cyt. P450 2C19 enzyme	Positive	Negative	Positive
Permeation of blood brain barrier	Positive	Positive	Positive
Inhibitor of cyt. P450 3A4 enzyme	Negative	Negative	Negative
C) Filters of drug likeness			
Rule of lipinski	Positive	Positive	Positive
Rule of veber	Positive	Positive	Positive
Rule of ghose	Positive	Positive	Positive
Score of bioavailability	0.55	0.55	0.55
Rule of egan	Positive	Positive	Positive
Rule of muegge	Negative, 1 objection	Positive	Positive
Compound lead likeliness	Positive	Positive	Positive

Here, Cyt. means cytochrome, MLOGP: Moriguchi octanol-water partition coefficient; XLOGP: atom-additive method-based octanol/water partition coefficient. The SwissADME filter criteria are as follows: Lipinski: MLOGP ≤ 4.15; HBD ≤ 5; HBA ≤ 10; MW ≤ 500, Veber: TPSA ≤ 140 $Å^2$; Rotatable bonds ≤ 10. Egan: WLOGP ≤ 5.88; TPSA ≤ 131.6, Ghose: 40 ≤ MR ≤ 130; 20 ≤ atoms ≤ 70; − 0.4 ≤ WLOGP ≤ 5.6; 160 ≤ MW ≤ 480, Muegge: Number of carbons > 4; − 2 ≤ XLOGP ≤ 5; RB ≤ 15; TPSA ≤ 157; Number of heteroatoms > 1; HBA ≤ 10; 200 ≤ MW ≤ 600; HBD ≤ 5; Number of rings ≤ 7.

Table 4. The binding potentialities of the compounds 1-3, and Erlotinib.

Sample	Binding capacity (Kcal/mol)	Interactions (Residual three letter code of amino acids)
Erlotinib (Standard inhibitor)	-7.0	Lys 721, Asp 831, Val 702, Met 769, Gln 767, Leu 694, Leu 768, Ala 719, Leu 764
1	-7.3	Lys 721, Leu 764, Val 702, Met 742, Leu 694, Ala 719, Leu 820
2	-7.9	Lys 721, Val 702, Met 769, Ala 719, Leu 694, Leu 820
3	-8.3	Phe 699, Val 702, Ala 719, Lys 721

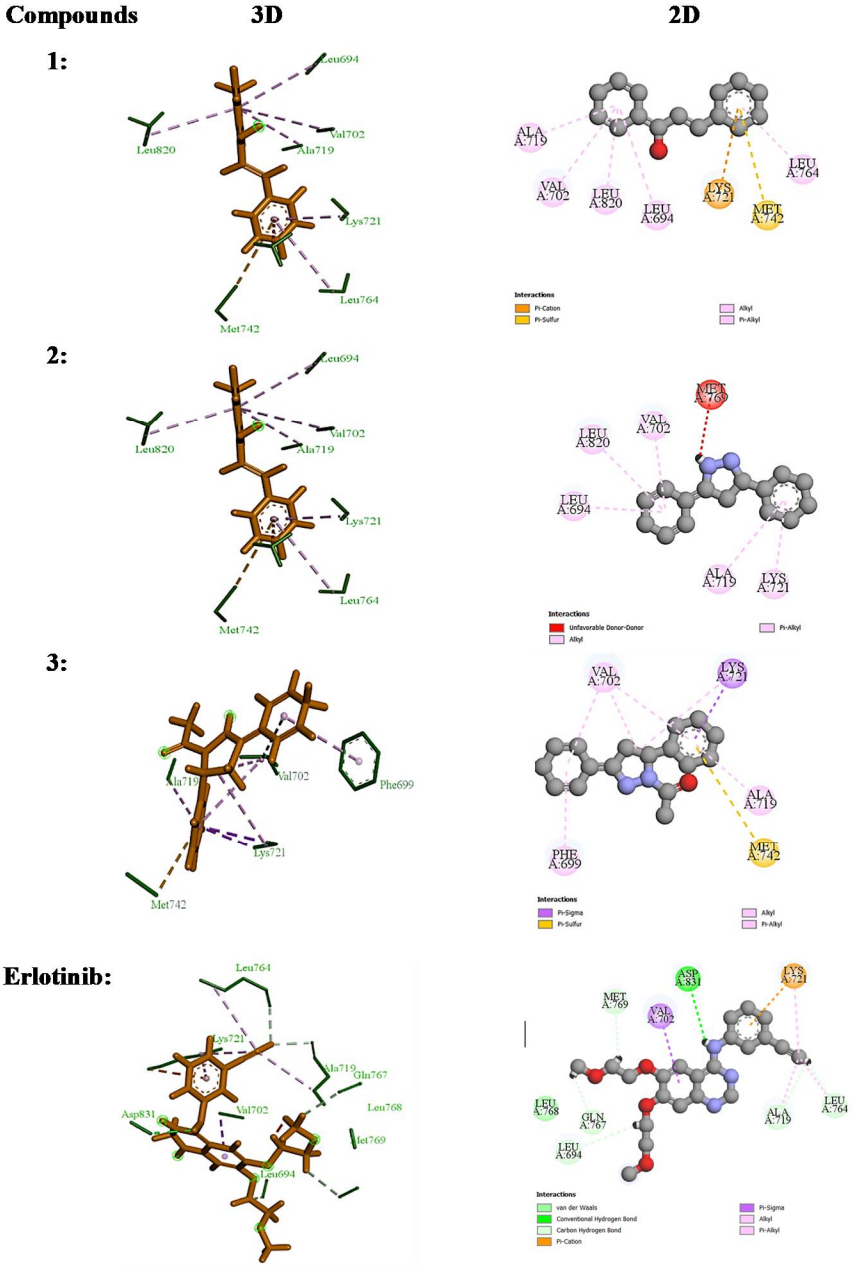

Figure 3. Molecular docking assessment of synthesized compounds **1-3** with erlotinib.

Molecular docking studies predicted that all compounds **1, 2 and 3** exhibit strong inhibitory potential against epidermal growth factor receptor (EGFR). The detailed results of these studies are presented in table 4 and figure 3. The binding affinities of the compounds were ranked in the following order: compound **3** > compound **2** > compound **1** > Erlotinib. As compound 3 had the most potent binding interaction with EGFR, it might be a promising candidate for further investigation as an EGFR inhibitor.

Conclusions

In summary, the synthesis of a chalcone compound, named *(E)*-1,3-diphenyl-prop-2-en-1-one **(1)**, was achieved, followed by its conversion into two pyrazole derivatives: 3,5-diphenyl-4,5-dihydro-1*H*-pyrazole **(2)** and 1-(3,5-diphenyl-4,5-dihydropyrazol-1-yl) ethanone **(3)**. The cytotoxic potential of compounds **1-3** was assessed using a qualitative microscopic HeLa cell assay, where all three compounds demonstrated significant toxicity, with less than 5% cell survival. Additionally, compounds **1-3** exhibited notable antioxidant activity, highlighting their potential in combating oxidative stress. *In silico* predictions provided insights into the drug-likeness, pharmacokinetic profiles and physicochemical properties of these compounds. Molecular docking studies against EGFR further supported their potential as inhibitors of this target. Given their promising characteristics, compounds **1-3** could serve as valuable lead candidates for further research. For the first time, this study reported the comparative biological and computational evaluations conducted in this work. However, further extensive investigations are necessary to gain a complete understanding of their underlying mechanisms of action.

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