Synthesis and Assessment of Antiproliferative, Antioxidant, and *In Silico* Features of *(E)*-3-(4-nitrophenyl)-1-phenyl-prop-2-en-1-one and Its Two Pyrazole Derivatives

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ABSTRACT: Chalcones are unsaturated 1,3-diaryl ketones, which can act as precursors of many bioactive heterocyclic compounds like pyrimidines, pyrazoles, and so on. Pyrazoles are also well-known to offer hundreds of bioactive moieties. Cancer and oxidative stress are two vital concerns in the present world. In the present investigation, a chalcone named as (*E*)-3-(4-nitrophenyl)-1-phenyl-prop-2-en-1-one (**1)** was produced by the reaction of 4-nitrobenzaldehyde with acetophenone. It was then treated with hydrazine hydrate, and hydrazine hydrate with acetic acid to obtain two pyrazoles, 5-(4-nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (**2)**, and 1-(5-(4-nitrophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl) ethenone (**3)**, respectively. The structures of the compounds were deduced by using FT-IR, ^{1}H NMR, and ^{13}C NMR analyses. Compounds **1-3** showed noticeable cytotoxicity on HeLa cell line. Antioxidant potentials of compounds **1-3** were also prominent. Additionally, *in silico* drug likeness properties and drug-receptor docking with epidermal growth factor receptor (EGFR) were also performed. It provided ideas on their pharmacokinetic and drug-receptor binding features. Based on the overall study, the compounds **1-3** might be considered as lead molecule. Further comprehensive studies are required to know their detail mechanism of the observed biological activities.

Key words: 4-nitrochalcone, pyrazole, hela cell, antioxidant, anticancer.

INTRODUCTION

The structure of chalcones is made up of two aromatic rings bridged by a three-carbon α,β-unsaturated carbonyl moiety. They have been widely used in medicinal chemistry as antioxidant, anticancer, antidiabetic, antifungal, antiviral, antimalarial, anti-inflammatory elements and more.[1] Heterocycle integration onto chalcone scaffolds is an intriguing approach to medication development. By introducing heterocycles containing nitrogen, oxygen, or sulphur into the chalcone core, scientists can adjust the pharmacological characteristics, effectiveness and selectivity of drugs.[2] The well-known nitrogen heterocycle class, pyrazole, and its derivatives, because of their diverse biological function, have a prominent place in pharmaceutical and pesticide chemistry. Numerous properties of them, including antibacterial, analgesic, anti-tubercular, anticancer, anti-inflammatory, antidepressant, anticonvulsant, antipyretic, antioxidant, and so on have been reported.[3]

Currently, thousands of people throughout the world are fighting against cancer, and there are many different types of chemotherapeutic medicines available. Nevertheless, they have disadvantages, including adverse effects, drug resistance, high cost, etc. The production of novel anticancer medications requires the production and evaluation of many types of compounds. Pyrazole derivatives might represent a promising class of anticancer agents due to their ability to target multiple mechanisms involved in cancer growth, survival, and metastasis. Because of their structural adaptability, powerful and selective inhibitors against important targets might be developed.[4-6]

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The oxidation process in the human body produces free radicals. These free radicals have the potential to cause oxidative stress and damage cellular components.[7-9] It weakens the defense system of body against infections.[10,11] Pyrazole derivatives might be promising candidates as antioxidants due to their ability to inhibit free radicals, chelate metal ions, and modify antioxidant enzyme systems. Their structural variation and tendency to interact with oxidative stress pathways make them potential therapeutic elements for diseases where oxidative stress is a contributing factor, including neurodegenerative diseases, cardiovascular diseases, diabetes, and cancer.[6,12]

Based on the aforementioned facts, in this study, a nitrochalcone, (*E*)-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one (**1)**, was produced by condensing acetophenone with 4- nitrobenzaldehyde. Moreover, two dihydropyrazoles, 5-(4-nitrophenyl)-1,3-diphenyl-4,5-dihydro-1*H*-pyrazole (**2)**, and 1-(5-(4-nitrophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl) ethenone (**3)** were synthesized by treating the chalcone **1** with hydrazine hydrate, and hydrazine hydrate with acetic acid, separately. All these synthesized compounds **1-3** have been passed through *in vitro* radical scavenging and total antioxidant capacity assays, as well as antiproliferative assay using HeLa cell line to determine their antioxidant, and anticancer potentials, respectively. In addition, *in silico* drug likeliness properties and docking studies with EGFR (epidermal growth factor receptor) were also performed.

MATERIALS AND METHODS

General experimental design and equipment. Using a Gallenkamp (England) melting point equipment, melting points were measured. A Shimadzu UV-visible spectrometer was used to obtain UV data, while an FTIR spectrophotometer was used to observe IR spectra. Using TMS as an internal reference, ^{1}H as well as ^{13}C NMR spectra were obtained on a Bruker DPX-600 spectrometer (600-MHz). The ESI-MS spectra were recorded on a 6400 series triple quadrupole-time-of-flight mass spectrometer (Agilent, Palo Alto, CA). Precoated silica gel 60 F_{254} (E. Merck) was used for thin-layer chromatography (TLC), and silica gel (60-120 mesh) was used in column chromatography. The suppliers of the reagents were Loba Chemie (India) and Merck (Germany).

Synthesis of (*E)*-3-(4-nitrophenyl)-1-phenyl-prop-2-en-1-one (1). In a beaker, 0.005 mole (0.6g) of acetophenone, and 0.005 mole (0.755g) 4-nitrobenzaldehyde were mixed with 15 ml of ethanol. Fifty percent aqueous solution of NaOH was slowly added to the mixture with continuous stirring with rotating magnet for 60 minutes at room temperature. TLC was performed for monitoring and the mixture was kept in a refrigerator for 12 hours. Later, an orange-yellow solid compound was obtained.[13] It was then recrystallized using ethanol.

Yield 78%; Orange-yellow solid; mp. 162-165 $^{\circ}C$; R_f value 0.8 (ethyl acetate : *n*-hexane = 2 : 8); IR (cm^{-1}): 1656 (C=O stretching, 1596 (C=C stretching), 1577 (C=C stretching of aromatic ring), 1412-1446 (C-C stretching), 1334 (N-O stretching), 1217 (C-O stretching), 844 (C-N scissoring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (2H, d, J= 7.2 Hz, H-11, H-13), 7.63 (1H, t, H-12), 7.67 (1H, d, J= 16 Hz, H-2), 7.80 (2H, d, J= 8.8 Hz, H-5, H-9), 7.82 (1H, d, J= 16 Hz, H-3), 8.05 (2H, d, J= 7.2 Hz, H-10, H-14), 8.30 (2H, d, J= 8.8 Hz, H-6, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ 189.79 (C=O), 148.68, 141.64, 141.17, 137.65, 133.50, 129.07, 128.95, 128.72, 125.85,124.34. ESI-MS *m/z* 254 $[M+H]^+$.

Synthesis of 5-(4-nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (2). In a beaker, 0.005 mole (1.265g) of 4-nitrochalcone (**1)** and 0.005 mole (0.25g) hydrazine hydrate (equivalent to 0.25 ml) was mixed in 40 ml of ethanol. The combining mixture was refluxed at 70-80 $^{\circ}C$ for 18 hours and TLC was performed at every hour interval. After completion of reaction, the reaction mixture was cooled in an ice water bath. A reddish-brown solid was appeared as a precipitate.[14] It was then recrystallized using ethanol for further purification.

Yield 56%; Reddish-brown solid; mp. 190-195 °C; R_f value 0.6 (ethyl acetate : *n*-hexane = 2 : 8); IR (cm^{-1}): 3070 (C-H stretching, aromatic ring), 1596 (C=N stretching), 1340 (N-O stretching), 1105 (C-N stretching), 844 (C-N scissoring); ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.32 (1H, dd, J= 18, 9 Hz, H-4a), 3.43 (1H, d, J= 18 Hz, H-4b), 5.45 (1H, d, J= 9 Hz, H-5), 7.63 (1H, s, pyrazole NH-1), 7.48-8.23 (9H, m, Ar-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 150.34 (C=N), 147.58, 136.37, 134.18, 129.19, 129.08, 129.00, 128.75, 128.37, 128.31, 126.72, 124.39, 123.98, 58.62 (Ar-CH-N), 47.13 (CH_2); ESI-MS *m/z* 268 $[M+H]^+$.

Synthesis of 1-(5-(4-nitrophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl) ethenone (3). In a beaker, 0.005 mole (1.265g) of *(E)*-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one (**1)** and 0.005 mole (0.25g) hydrazine hydrate (equivalent to 0.25 ml), and 10 ml acetic acid were mixed in 40 ml of ethanol. The mixture was refluxed at 70-80 °C for 24 hours and TLC was performed at every hour interval. A deep reddish-brown solid was formed when the mixture was cooled in an ice water bath.[15] It was then recrystallized using ethanol for further purification.

Yield 68%; Deep reddish-brown solid; mp. 125-128 °C; R_f value 0.5 (ethyl acetate : *n*-hexane = 2 : 8); IR (cm^{-1}): 2945 (C-H stretching, aromatic ring), 1732 (C=O stretching), 1598 (C=N stretching), 1336 (N-O stretching), 1105 (C-N stretching), 834 (C-N scissoring); ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.43 (3H, s, 7-$COCH_3$), 3.13 (1H, d, J=18 Hz, H-4a), 3.81 (1H, dd, J= 18, 12 Hz, H-4b), 5.65 (1H, d, J= 12 Hz, H-5), 7.40-8.18 (9H, m, Ar-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.20 (C=O), 153.80 (C=N), 148.97, 147.54, 130.99, 130.84, 129.01, 126.82, 126.76, 124.46, 59.59 (Ar-CH-N), 42.20 (CH_2); ESI-MS *m/z* 310 $[M+H]^+$.

Antiproliferative assay. Antiproliferative assay using HeLa cells, derived from human cervical cancer, is a common method used to assess the ability of a compound to inhibit the proliferation (growth and division) of cancer cells. HeLa cells are widely used in cancer research due to their robust growth and established use in testing anticancer compounds.[16] In the present study, DMEM media was used to maintain the HeLa. Here, 1% penicillin-streptomycin (1:1) and 0.2% gentamycin were used to keep the media in sterile condition. 10% fetal bovine serum (FBS), which acts as a source of nutrients of the growth media, was also utilised in the media. Five milligrams of desired compound were dissolved in 4 ml of DMSO solvent (5% in water), and the final concentration of solution was 1.25 mg/ml. Cells (2.0×10^4/100 µl) were seeded onto 48-well plate and incubated at 37 °C with 5% CO_2 to regulate the pH of the medium. Next day after cell seeding, 50 µl sample was added to each well. The cell culture plate was then placed in carbon dioxide incubator at 37 °C with 5% CO_2. Cytotoxicity was assessed using an inverted light microscope following a 24-hour incubation period.[16]

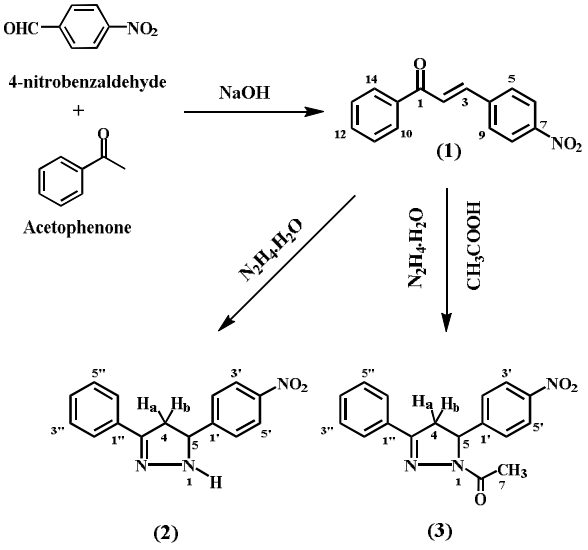


Figure 1. Scheme of the synthesis of compounds **1-3**.

Estimation of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging. A prevalent technique for assessing antioxidant activity any compound is the DPPH test, which gauges how well it scavenges free radicals. This test evaluates the antioxidant capacity of a range of biological and chemical components in an easy, fast, and accurate manner.[30] It is assessed by using the stable DPPH free radical. Here, 1.5 ml of DPPH solution in methanol was added with 1 ml of compound solution

(100 – 3.125 µg/ml) in methanol. A methanol blank was used to measure the absorbance at 517 nm after the combination was incubated for 30 to 60 minutes without light. The absorbance of the DPPH solution was also taken for calculation purpose and the plot of percentage inhibition versus concentration was used to calculate the half-maximal inhibitory concentration (IC50) value.[17] The equation for the calculation of % free radical scavenging is as follows:

$$\% \text{ Scavenging} = \frac{\text{Absorbance of DPPH} - \text{Absorbance of Sample}}{\text{Absorbance of DPPH}} \times 100$$

Total antioxidant capacity assay. A common technique for determining a sample's overall antioxidant potential is the total antioxidant capacity (TAC) assay, which measures the sample's capacity to neutralize free radicals or reactive oxygen species (ROS). The technique typically uses a reagent that contains a molybdenum-based complex made by ammonium molybdate (4 M), sodium phosphate (28 M), and sulphuric acid (0.6 M). This reagent reacts with the antioxidants in the sample to produce a color shift that can be measured spectrophotometrically, which is how the TAC assay works. Here, this reagent was mixed with 0.1 ml of samples, dissolved in methanol of different concentrations (200-25 µg/ml) in eppendorf tubes. The samples were then submerged for ninety minutes at 95 °C in a water bath. A measurement of their absorbance was made at 695 nm. The greater the absorbance, the upper the total antioxidant capacity is claimed.[18]

Drug likeness properties. The physicochemical properties, pharmacokinetics and drug likeliness status of compounds **1- 3** were evaluated using SwissADME web-based tool (http://www.swissadme.ch/). By uploading molecular structure or inputting its Simplified Molecular Input Line Entry System, the tool can provide a comprehensive analysis across several categories.[19] The parameters used in this *in silico* analysis can be seen in Table 3.

Molecular docking studies. EGFR is a cell surface receptor that plays a key role in cell proliferation, survival, and differentiation. It is a critical target in cancer therapy, particularly in cancers where EGFR is overexpressed or mutated, such as non-small cell lung cancer, colorectal cancer, and head and neck cancers. When in disorder, it can contribute to uncontrolled cell growth and tumorigenesis. EGFR inhibitors may be very promising in the fight against cancer. Consequently, a number of novel EGFR inhibitor candidates with pyrazole rings have been reported.[20] On this basis, compounds **1-3** along with a known EGFR inhibitor, erlotinib, were subjected to docking study. The 3D conformers (SDF format) of the compounds were obtained from the online databases PubChem (https://pubchem.ncbi.nlm.nih.gov/).[21] The RCSB Protein Data Bank website (https://www.rcsb.org/) provided the PDB format for the crystal structure of the EGFR (PDB ID: 1M17).[22] The downloaded enzyme had a resolution of 2.60 Å. PyMOL version 2.5.2 software (Schrodinger, LLC) was utilized to eliminate undesired atoms and molecules, including ligands, from the protein structures.[23] The excess protein chains were removed from the crystal structure by using PyMOL after the receptor-binding domain (RBD) of the enzyme was removed. PDB formats were used to store the protein chains in order to facilitate molecular docking.[24] Molecular dockings on the synthesized ligands against the target were carried out using the PyRx software.[25] The auto-dock grid dimensions were x=26.1663, y=9.6467, z=59.2302 for grid center; x=170, y=178, z=139 for number of points, and 0.3750 Å for space. The binding affinity (kcal/mol) values for every protein-ligand combination, noncovalent interactions, and 2D and 3D schematic representations of the protein-ligand docking complexes were acquired from BIOVIA Discovery Studio.

RESULTS AND DISCUSSION

Compound **1** appeared as orange-yellow solid and displayed a yellow spot in TLC over silica gel PF_{254}, followed by spraying with vanillin-sulfuric acid and heating at a temperature range of 90 °C for 5 minutes. The compound was soluble in various solvents such as methanol, ethanol, chloroform, ethyl acetate, etc. The IR data of the compound **1** showed

characteristic peaks at frequency (cm^{-1}): 1656 (C=O stretching, 1596 (C=C stretching), 1577 (C=C stretching of aromatic ring), 1412-1446 (C-C stretching), 1217 (C-O stretching), 1334 (N-O stretching) and 844 (C-N scissoring).[26] The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1** exhibited a doublet at δ 7.55 (J=7.2 Hz) integrated for two aromatic protons (H-11 and H-13). A triplet was observed at δ 7.63 of one proton intensity (H-12). Two doublets at δ 7.67 (J= 16 Hz) and δ 7.82 (J= 16 Hz) integrated for one proton each could be assigned for H-2 and H-3, respectively. The larger coupling constants indicated the presence of an *E* alkene moiety in compound **1**.[13] Three doublets of two aromatic protons each at δ 7.80 (J= 8.8 Hz), δ 8.05 (J= 7.2 Hz), δ 8.80 (J= 8.8 Hz) demonstrated the presence of H-5, H-9, H-10, H-14, H-6 and H-8, respectively. Based on the enquiry of the above-mentioned NMR data with previously published data,[13,27] the structure of **1** was confirmed as a nitrochalcone, (*E*)-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one. In addition, ^{13}C NMR records of compound **1** were found similar to that of previously published data.[13,27]

Compound **2** appeared as reddish-brown solid. When it was subjected to TLC (thin-layer chromatography) over silica gel PF_{254}, it appeared as a dark spot-on UV and as yellow spot while spraying with vanillin-sulfuric acid and heating at 90 °C for a duration of 5-10 minutes. The compound shows solubility in various solvents such ethanol, chloroform, ethyl acetate, etc. The IR data of the compound **2** showed characteristic peaks at frequency (cm^{-1}): 3070 (C-H stretching of aromatic ring), 1596 (C=N stretching), 1340 (N-O stretching), 1105 (C-N stretching) and 844 (C-N scissoring).[26] When compound **2** was subjected to NMR analysis in $CDCl_3$ solvent at 600 Hz, it exhibited a double doublet δ 3.32 (J= 18, 9 Hz), a doublet δ 3.43 (J= 18 Hz) and another doublet δ 5.45 (J= 9 Hz) of one proton each indicated the presence of H-4a, H-4b and H-5, respectively of a pyrazole ring. The NH-1 appeared as broad singlet at δ 7.63. Resonances of nine aromatic protons were seen between δ 7.48 - 8.23. By comparing the NMR data with previously published references of related pyrazoles,[28] the compound **2** was confirmed as 5-(4-nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole. In addition, ^{13}C NMR data of compound **2** were found similar to that of previously published data.[28]

Compound **3** appeared as deep 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 90°C for a duration of 5-10 minutes. The compound showed solubility in various solvents such ethanol, chloroform, ethyl acetate, etc. The IR data of the compound **3** showed characteristic peaks at frequency (cm^{-1}): 2945 (C-H stretching of aromatic ring, weak), 1732 (C=O stretching), 1598 (C=N stretching), 1336 (N-O stretching), 1105 (C-N stretching) and 834 (C-N scissoring).[26] When compound **3** was subjected to NMR analysis in $CDCl_3$ at 600 Hz, it exhibited doublet at δ 3.13 (J= 18 Hz), double doublets at δ 3.81 (J= 18, 12 Hz) and another doublet at δ 5.65 (J= 12 Hz), which could be consigned for H-4a, H-4b and H-5, respectively of a pyrazoline ring. The 7-$COCH_3$ was appeared as a singlet at δ 2.43. Resonances of nine aromatic protons were seen between δ 7.40 - 8.18. By comparing the NMR data with previously published references of related pyrazoles,[28] the compound **3** was confirmed as 1-(5-(4-nitrophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl) ethenone. In addition, ^{13}C NMR data of compound **3** were found similar to that of previously published data.[28]

In biomedical research, one of the most popular human cancer cell lines is the HeLa cell line. Henrietta Lacks' cervical carcinoma in 1951 served as the source of these cells, which have made a substantial contribution to our knowledge of toxicity testing, drug screening, and cancer biology. Assessing the possible anticancer effects of new chemicals or treatment agents requires the use of cytotoxicity tests with the HeLa cell line. The number of viable cells and non-viable cells is a good indicator to identify whether a compound is cytotoxic or not. In the present cytotoxicity assay against HeLa cells,

only media showed hundred percent survival rate, and the media with vehicle showed more than ninety five percent of survival rate of HeLa cells. The survival rate of HeLa cells was below 5% for all of the compounds **1-3** dissolved in the media. All the results were obtained from cell counting method using hemocytometer under inverted phase microscope. The effects of **1-3** are depicted in table 1.

Table 1. Cell viability assay of compounds 1-3 using HeLa cells.

Sample	% Survival of HeLa cells	Cytotoxicity
Media	100%	Not Cytotoxic
Media + Vehicle	>95%	Not Cytotoxic
1	<5%	Cytotoxic
2	<5%	Cytotoxic
3	<5%	Cytotoxic

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a popular technique for assessing a compound's antioxidant capacity in drug discovery. A straightforward and economical method of assessing a compound's capacity to neutralise free radicals by donating an electron or hydrogen atom. The DPPH assay is very useful in the early phases of drug discovery when looking for possible substances that can lessen illnesses or symptoms linked to oxidative stress. In the current assessment, the assay demonstrated a distinct dose-dependent reduction in DPPH radical absorbance, indicating a strong scavenging action of the investigated substances. These findings are in line with earlier research, indicating that, the samples have strong antioxidant qualities, can counteract free radicals.[30] In the case of DPPH oriented free radical scavenging assay, the highest scavenging activity was shown by **1**, (IC_{50} =35.9 µg/ml). The results are mentioned in table 2. In case of total antioxidant capacity assay, the highest antioxidant activity was also shown by **1**. The results are depicted in figure 2. In both cases, the order of antioxidant activity was as, compound **1** > compound **2** > compound **3**.

Table 2. IC_{50} data of DPPH free radical scavenging assay of compounds 1-3.

Compound	IC_{50} ± SD (µg/ml)
BHT	10.7 ± 0.03
1	35.9 ± 0.40
2	47.1 ± 0.61
3	60.7 ± 0.40

Results were expressed as mean ± standard deviation (SD), where n=3.

In total antioxidant capacity assay, an antioxidant sample is added to a solution containing molybdate ions under acidic conditions. The antioxidants in the sample reduce the molybdate ions, forming a green-colored complex. The intensity of the color is directly related to the antioxidant substances. In the present study (Figure 2), the dose-dependent antioxidant potentials were seen for compounds **1-3** and these results were consistent with the aforesaid DPPH assay, which further supports the capacity of a sample to reduce oxidative damage.[18] When considered collectively, the samples **1-3** might have therapeutic value in conditions associated with oxidative stress.

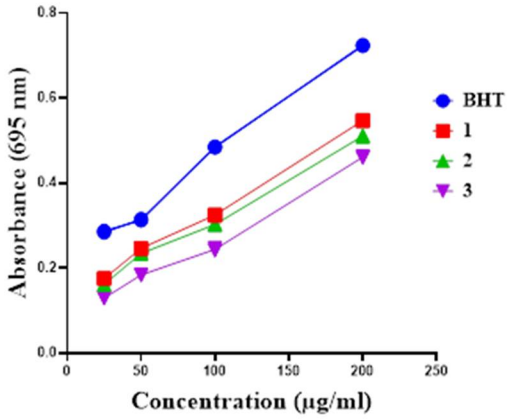


Figure 2. Total antioxidant capacity assay of compounds **1-3**, and BHT.

The physicochemical, pharmacokinetics, and drug likeliness status of compounds **1 - 3** were evaluated using SwissADME internet-based tool (http://www.swissadme.ch/)[19] (Table 3). Compounds **1-3** demonstrated good intestinal absorption. In case of blood–brain barrier penetration, compound **1-2** showed positive results. Topological polar surface

area (TPSA) ranged from 62.89 Å^2 to 78.49 Å^2**.** Compound **1-3** were also found as good inhibitor of cytochrome P450- 1A2, and 2C9, but failed to inhibit cytochrome P450- 2D6 and 3A4. The parameters for compounds **1-3** were consistent with the Lipinski's rule.[29] Other methods of drug likeliness calculation (Veber, Ghose, Egan, and Mueggein filters) were also explored and found no violation. All the compounds demonstrated positive drug likeliness. The results are mentioned in table 3.

Table 3. *In silico* physicochemical, pharmacokinetics, and drug likeliness properties of compounds 1-3.

Parameters	1	2	3
A) Physicochemical Properties			
Formula	$C_{15}H_{11}NO_3$	$C_{15}H_{13}N_3O_2$	$C_{17}H_{15}N_3O_3$
Molecular weight (MW)	253.25	267.28	309.32
Volume	225.19	237.24	273.16
Number of heavy atoms	19	20	23
Number of aromatic heavy atom	12	12	12
Number of rotatable bond (RB)	4	3	4
H-bond acceptor (HBA) number	3	3	4
H-bond donor (HBD) number	0	1	0
Molar Refractivity (MR)	75.07	85.94	95.85
MLOGP	2.26	1.93	1.94
XLOGP	2.91	3.16	2.53
TPSA (Topological Polar Surface Area)	62.89 Å^2	70.21 Å^2	78.49 Å^2
B) Pharmacokinetics			
GI Absorption	High	High	High
BBB (Blood Brain Barrier) permeation	Yes	Yes	No
P-glycoprotein substrate	No	No	No
Inhibitor of Cyt. P450 1A2	Yes	Yes	No
Inhibitor of Cyt. P450 2C9	Yes	Yes	Yes
Inhibitor of Cyt. P450 2C19	Yes	Yes	Yes
Inhibitor of Cyt. P450 2D6	No	No	No
Inhibitor of Cyt. P450 3A4	No	No	No
C) Drug likeliness filters			
Lipinski rule	Agreed, 0 Violation	Yes, 0 Violation	Yes, 0 Violation
Ghose rule	Agreed	Agreed	Agreed
Veber rule	Agreed	Agreed	Agreed
Egan rule	Agreed	Agreed	Agreed
Muegge rule	Agreed	Agreed	Agreed
Bioavailability value	0.55	0.55	0.55
Lead likeliness	Agreed	Agreed	Agreed

Here, Cyt., Cytochrome; MLOGP: Moriguchi concept of octanol-water partition coefficient; XLOGP: Octanol/water partition coefficient (atom-additive method); WLOGP: atomistic method to calculate lo partition coefficient;

Filter criteria: Lipinski: MLOGP ≤ 4.15; MW ≤ 500; HBD ≤ 5; HBA ≤ 10; Veber: TPSA ≤ 140 Å^2; Rotatable bonds ≤ 10; Egan: WLOGP ≤ 5.88; TPSA ≤ 131.6; Ghose: 40 ≤ MR ≤ 130; 160 ≤ MW ≤ 480; − 0.4 ≤ WLOGP ≤ 5.6; 20 ≤ atoms ≤ 70; Muegge: HBA ≤ 10; HBD ≤ 5; RB ≤ 15; − 2 ≤ XLOGP ≤ 5; 200 ≤ MW ≤ 600; TPSA ≤ 157; Heteroatom number > 1; Ring number ≤ 7; Number of carbons > 4.

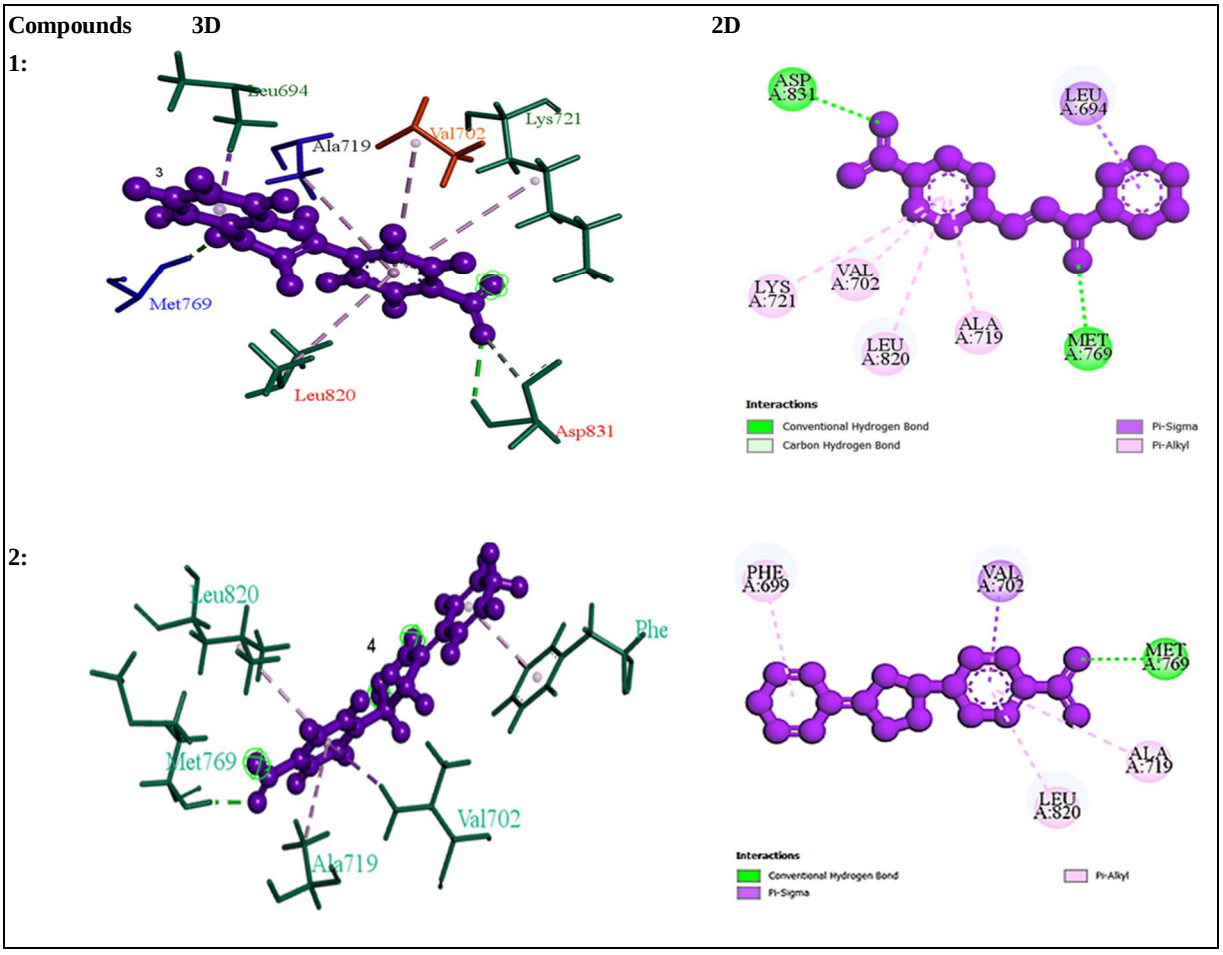
A number of malignancies have been associated to aberrant EGFR signalling, which makes it a desirable target for therapeutic approaches. Small compounds that can alter EGFR activity are quite important in this situation. Heterocyclic compounds like pyrazole have shown promise as EGFR inhibitor scaffolds. To forecast the interactions between molecules **1-3** and EGFR, docking simulations were

run. Understanding the intermolecular interactions that stabilize the complex, the binding pose (conformation), and the binding affinity are the objectives. It was anticipated that all of the compounds **1-3** would be strong EGFR inhibitors. The outcomes are stated in Table **4**, and Figure **3**. The order of binding affinity was as follows:

compound **3** > compound **2** > compound **1** > erlotinib

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

Compounds	Binding affinity (Kcal/mol)	Interactions (three letter code of amino acid residue)
Erlotinib (Standard inhibitor)	-7.0	Ala 719, Lys 721, Leu 764, Gln 767, Leu 768, Met 769, Asp 831, Leu 694, Val 702
1	-7.5	Ala 719, Lys 721, Leu 694, Leu 820, Asp 831, Val 702, Met 769
2	-8.2	Met 769, Leu 820, Ala 719, Phe 699, Val 702
3	-8.4	Phe 699, Leu 820, Ala 719, Val 702



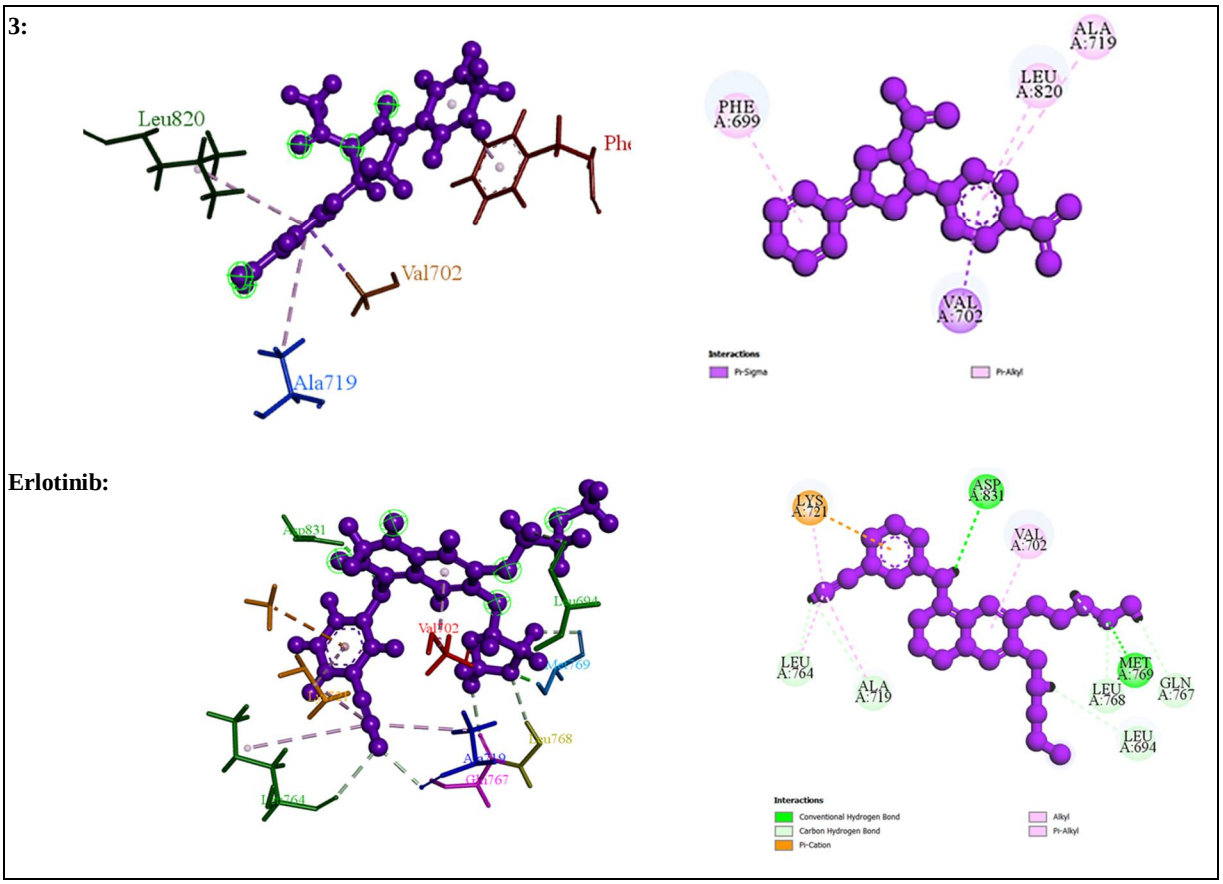


Figure 3. 3D (left) and 2D (right) docking images of compounds **1-3**, and an EGRF inhibitor, erlotinib.

CONCLUSION

In conclusion, a nitrochalcone, *(E)*-3-(4-nitrophenyl)-1-phenyl-prop-2-en-1-one (**1**) and its two dihydropyrazoles, 5-(4-nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (**2**), and 1-(5-(4-nitrophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl) ethenone (**3**) were synthesized. Compounds **1-3** might be considered cytotoxic, as they showed less than 5% cell survival in HeLa cell cytotoxicity assay. Compounds **1-3** also showed significant antioxidant potential. The *in silico* prediction provided their images on physio-chemical, pharmacokinetic and drug likeness properties as well as molecular docking features against EGFR. Compounds **1-3** might be considered as lead molecules primarily. Further bioactivity studies are required to know their detail chemical properties and biochemical functions.

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