

Design, Synthesis, Structural Characterization, and Anticancer Evaluation of Novel Pyrazole–Thiohydantoin Hybrids

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ABSTRACT:

Synthesis of Seven Novel Pyrazole-Futilized Thiohydantoins (TH1-TH7) through Hydrate Formation, Vilsmeier-Haack Cyclization, Condensation of Thiosemicarbazones and Intramolecular Cyclization Reaction with Ethyl Chloroacetate has been successfully carried out. The synthesis resulted in compounds with Satisfactory to excellent yields. Structural elucidation of the synthesized compounds has been achieved utilizing Fourier Transform Infrared Spectroscopy, ¹H and ¹³C Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry, which revealed the successful preparation of target thiohydantoins. Evaluation of the cytotoxic activity of the synthesized thiohydantoins has been conducted on the human Mammary carcinoma cell line (MCF-7) utilizing the MTT Assay. It was found that among the compounds tested, TH3 showed the highest cytotoxic activity with an IC₅₀ value of 82.98 µg/mL, followed by TH6 and TH4, thus suggesting the importance of having a para-fluoro substituent on the biological activity of the pyrazole-thiohydantoin scaffold. According to molecular docking studies, Suitable interactions between the synthesized derivatives and the target active site were Acquired, consistent with the experimental cytotoxic data and providing the likely binding modes. A structure-activity relationship study suggests that electron-withdrawing groups have a significant effect on the biological activity of the synthesized compounds. Despite the low antiproliferative activity of these derivatives, it can be concluded from the results of this study that pyrazole-thiohydantoin hybrid derivatives can serve as Satisfactory leads for the development of new potent anti-cancer drugs.

KEYWORDS: Pyrazole derivatives, Thiohydantoin, Cytotoxic activity, MCF-7 cells; Molecular docking.

1. Introduction

Mammary carcinoma continues to represent the commonest form of cancer and one of the main causes of cancer deaths in women across the world[1], [2] Despite the advancements made in the diagnosis and management of Mammary carcinoma, there is a need for the development of more effective treatment methods because of the nature of the condition, which makes it hard to manage. This calls for the discovery of new drugs with high efficiency.[3]

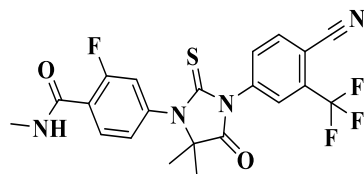
Among the experimental models available for Mammary carcinoma research, the MCF-7 human breast adenocarcinoma cell line is one of the most extensively utilized in vitro systems for evaluating the anticancer potential of newly synthesized compounds[4], [5], [6]. As an estrogen receptor-positive (ER⁺) cell line, MCF-7 closely mimics hormone-responsive breast tumors and provides a reliable platform for investigating cytotoxicity, antiproliferative activity, and the molecular mechanisms underlying anticancer drug action. Consequently, it has become a standard model for the preliminary biological assessment of potential chemotherapeutic candidates[7], [8], [9].

There is considerable importance of heterocyclic molecules including nitrogen and sulfur moieties in medicinal chemistry owing to their significant structural diversity and biological activity spectra[3], [10]. Thiohydantoin (2-thioxoimidazolidin-4-one) analogs belong to the group of compounds that have received considerable recognition on account of their diversified pharmacological effects[7], [11], such as antibacterial[12], antiparasitic, anti-inflammatory[13], [14], antioxidant[15], [16], and [17], [18] effects. The presence of both nitrogen and sulfur moieties in the heterocyclic system enables their Suitable binding to biological targets, thus making them valuable structures for drug development.

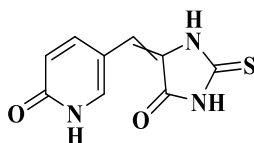
Pharmaceutical relevance of thiohydantoins can also be observed in the way that such compounds are utilized in the formation of biologically active compounds and drugs of clinical importance. Thus, analogs based on the thiohydantoin scaffold showed Satisfactory anticancer potential, whereas some 5-arylidene-2-thiohydantoin compounds were found to be highly active in the inhibition of mutant isocitrate dehydrogenase (IDH), which represents one of the important enzymes participating in the metabolism and cancer development. Moreover, due to the structural diversity of the thiohydantoin scaffold, hybridization with other pharmacophores is likely, improving target selectivity and overcoming drug resistance [19], [20].

Though a wide range of thiohydantoin analogs have been reported so far, the development of structurally different analogs having greater anticancer efficacy is the main goal of medicinal chemistry. This can be accomplished by altering the core of the thiohydantoin molecule and thus Acquiring more cytotoxic analogs for Mammary carcinoma cells while expanding current structure–activity relationship knowledge.

Although several investigations have been carried out concerning parole and thiohydantoin derivatives, research involving the combined utilization of these two biologically significant frameworks within a molecular compound is still rare. In addition, the influence of different electron-withdrawing groups attached to the aromatic ring of pyrazole-linked thiohydantoin derivatives has not been extensively studied before. This prompted the current investigation to focus on the synthesis of a novel series of parole thiohydantoin derivative compounds (TH1–TH7), study the chemical structure of these derivatives utilizing spectral techniques, and also investigate the cytotoxicity of these compounds on MCF-7 Mammary carcinoma cells, as well as molecular docking studies of their binding mode. The findings are expected to provide useful insights into the structure–activity relationship of this class of compounds and support the future design of more potent anticancer agents.



Enzalutamide



5-substituted arylidene-2-thiohydantoin

2 Practical Section

2.1 Chemistry

Melting points of all the synthetic compounds were measured without making any adjustments. A Bruker FTIR ALPHA II spectrophotometer was utilized to record FT-IR spectra in the 4000–400 cm^{-1} spectral range. It were recorded using the KBr disk technique. A Bruker Avance-NEO spectrometer operating at 400 MHz was utilized to Acquire ^1H and ^{13}C NMR spectra. Chemical shifts (δ) were measured in ppm with reference to DMSO- d_6 (2.50 and 3.30 ppm, 39.5 ppm) and coupling constants (J) were measured in Hz. Multiplicities of signals were indicated by the acronyms s (singlet), d (doublet), t (triplet) and m (multiplet). An Agilent 5975 quadrupole mass spectrometer operating in EI mode with an ionization energy of 70 eV was utilized to produce mass spectra.

2.2. Synthesis and characterization

2.2.1 Synthesis of 2-phenyl-1-(1 phenylethylidene)hydrazine [HR1–7]

100 mL of ethanol and acetophenone derivative (0.05 mol) were added to a 250 mL round bottom flask. 0.05 mol of phenylhydrazine or 4-chlorophenylhydrazine was then added to this solution, which was also Supplied with a sparse drips of glacial acetic acid as a catalyst. The reaction blend was warmed to reflux with continuous stirring for 5 hours using magnetic stirrer. Once the reaction was complete, the blend was left to cool to room temperature at which point the formation of hydrazone products was observed. The solid products were purified by filtration and washed with glacial acetic acid and distilled water and dried to Acquire the pure hydrazone derivatives. Using ethyl acetate:ethanol:hexane (8:1:1, v/v/v) as the eluent, thin-layer chromatography (TLC) was utilized to track the reactions' Advancement. Rinsing the TLC plate with a small amount of ethanol showed that acetophenone had been removed from the plate [21,22].

Vilsmeier-Haack Formylation [PA1–7]

A total of 5 mL of Dichloromethane (CH_2Cl_2) and 15 mL of N, N-dimethylformamide (DMF) was added to a 250 mL round-bottom flask and continuously stirred at 0 °C. While maintaining the reaction blend's temperature at 0 °C, phosphorus oxychloride (Polls, 3 mL) was gradually added to the blend above over the course of ten to fifteen minutes. To make sure the Vilsmeier-Haack reagent formed in situ, the liquid was churned for thirty minutes. The solution of the corresponding hydrazine derivative (E)-2-phenyl-1-(1-phenylethylidene) hydrazine in CH_2Cl_2 (20 mL) was then dropwise added to the above prepared reagent at 0 °C ensuring the reaction blend stays cold. The reaction blend was warmed under reflux for one hour after being churned for one hour at 0 °C. The temperature was then gradually raised up to the room temperature. The Advancement of the reaction was Observed using TLC. The reaction blend was cooled to the room temperature and poured onto the crushed ice on Consumption of the reaction. To allow the product to completely precipitate, the blend was Permitted to stand overnight. The settled product was collected using filtration, cleaned gently with distilled water and then dried [23,24].

2.2.2 Synthesis of (E)-2-(((1,3-diphenyl-1,2-diazole-4-yl)methylene)hydrazine-1-carbothioamide [TS1–7]

An Equilar quantity of 1,3-diphenyl-1,2-diazole-4-carbaldehyde and thiosemicarbazide was stirred in ethanol, followed by adding 2–4 drips of glacial acetic acid. The reaction blend was then warmed under reflux with constant churning for 6 h. Reaction Advancement was checked intermittently utilizing thin-layer chromatography (TLC) on silica gel G coated plates, developed with ethyl acetate/ethanol/humane (1:1:8, v/v/v) solvent system. After Consumption of the reaction, the reaction blend was chilled to room temperature, and the product was settled out. The product was separated from the reaction blend by filtration and purified by washing with cold ethanol [25].

2.2.3 Synthesis of (E)-3-(((1,3-diphenyl-1,2-diazole-4-yl)methylene)amino)-2-thioxoimidazolidin-4-one (TH1–7).

Equilar amounts of 2-((1,3-diphenyl-1,2-diazole-4-yl) methylene)hydrazine-1-carbothioamide and ethyl chloroacetate were dissolved in ethanol and warmed under reflux with continuous churning for 8 h. The Advancement of the reaction was Observed by thin-layer chromatography (TLC). After Consumption of the reaction, the blend was chilled to room temperature, and the resulting solid product was collected by filtration. The crude product washed successively with humane and distilled water to remove residual impurities, then recrystallized from an appropriate solvent to afford the corresponding thiazolidinone derivative in pure form [26].

2.3.1 3-(((1,3-diphenyl-1,2-diazole -4-yl)methylene)amino)-2-thioxoimidazolidin-4-one (TH-1)

IR data: -C-H(Ar, str.): 3046, -C-H(alky., str.) 2955, -C=O(str.)1715, -C=N(str.) 1635, -C=C(Ar, str.)1595, 1531, -C=S(str.)1205, 1244, -C-H(Ar, bend.)692, 747. **¹H-NMR** data: 11.92(s, 1H, NH), 8.96 (s, 1H, CH=N), 8.43(s, 1H, CH-pyrazole), 7.39–7.99(m, 10H, Ar-H), 3.91(s, 2H, CH₂-thiohydantoin). **¹³C-NMR** data: 152.06, 149.67(C=N azomethine and pyrazole), 139.38, 132.52(Ar-C, quaternary), 30.30, 130.12, 129.33, 129.12, 128.96, 127.56, 119.31, 117.15(Ar-C and pyrazole carbons), 33.65(CH₂, thiohydantoin). **MS**(EI) m/z = 361 [M]⁺.

2.3.2 (E)-3-(((1-(4-nitrophenyl)-3-phenyl-1,2-diazole -4-yl)methylene)amino)-2-thioxoimidazolidin-4-one (TH2)

IR data: 3038 (C-H Ar, str.), 2943 (C-H alky., str.), 1708 (C=O, str.), 1645 (C=N, str.), 1596 (C=C Ar, str.), 1498 (NO₂, asym. Str.), 1335 (NO₂, sym. Str.), 1253, 1204 (C=S, str.), 850 (C-H Ar, out-of-plane bend., p-disubstituted benzene), 736, 682 (C-H Ar, out-of-plane bend., monosubstituted benzene). **¹H-NMR** data 11.95 (s, 1H, NH), 9.06 (s, 1H, CH=N), 8.51 (s, 1H, CH-pyrazole), 8.34 (d, J = 8.8 Hz, 2H, Ar-H ortho to NO₂), 7.98 (d, J = 8.8 Hz, 2H, Ar-H meta to NO₂), 7.41–7.60 (m, 5H, Ar-H), 3.92 (s, 2H, CH₂-thiohydantoin). **¹³C-NMR** data 149.24, 147.58 (C-NO₂, C=N, and pyrazole C3), 139.22, 139.14 (Ar-C, quaternary), 132.47, 130.24, 130.22, 127.97, 123.99, 119.43, 117.94 (Ar-C and pyrazole carbons), 33.80 (CH₂, thiohydantoin). **MS** (EI) m/z = 406 [M]⁺.

2.3.3 (E)-3-(((1-(4-fluorophenyl)-3-phenyl-1,2-diazole -4-yl)methylene)amino)-2-thioxoimidazolidin-4-one (TH-3)

IR data: 3053 (C-H Ar, str.), 2945 (C-H alky., str.), 1716 (C=O, str.), 1631 (C=N, str.), 1594, 1519 (C=C Ar, str.), 1319 (C=S, str.), 1211 (C-F, str.), 838 (C-H Ar, out-of-plane bend., p-disubstituted benzene), 742, 688 (C-H Ar, out-of-plane bend., monosubstituted benzene). **¹H-NMR** data : 11.91 (s, 1H, NH), 8.97 (s, 1H, CH=N), 8.43 (s, 1H, CH-pyrazole), 7.31–7.98 (m, 9H, Ar-H), 3.90 (s, 2H, CH₂-thiohydantoin). **¹³C-NMR** data: 163.96, 161.52 (d, C-F, 4-fluorophenyl), 150.87 (C=N azomethine), 139.33, 131.40, 131.32, 131.11, 130.13, 129.12, 129.09, 127.59, 119.27, 117.11, 115.87, 115.65 (Ar-C and pyrazole carbons), 33.69 (CH₂, thiohydantoin). **MS** (EI) m/z = 379 [M]⁺.

2.3.4 (E)-3-(((1-(4-bromophenyl)-3-phenyl-1,2-diazole -4-yl)methylene)amino)-2-thioxoimidazolidin-4-one (TH-4)

IR data 3047 (C-H Ar, str.), 2960 (C-H alky., str.), 1709 (C=O, str.), 1640 (C=N, str.), 1596, 1532 (C=C Ar, str.), 1249, 1207 (C=S, str.), 838 (C-H Ar, out-of-plane bend., p-disubstituted benzene), 739, 685 (C-H Ar, out-of-plane bend., monosubstituted benzene), 588, 545 (C-Br, str.). **¹H-NMR** data: 11.91 (s, 1H, NH), 9.00 (s, 1H, CH=N), 8.45 (s, 1H, CH-pyrazole), 7.55–7.98 (m, 8H, Ar-H), 3.92 (s, 2H, CH₂-thiohydantoin). **¹³C-NMR** data δ (ppm): 150.51 (C=N azomethine), 139.28, 131.86, 131.81, 131.49, 131.16, 130.15, 127.68, 119.32, 117.27 (Ar-C and pyrazole carbons), 122.41 (C-Br, 4-bromophenyl), 33.63(CH₂, thiohydantoin), **MS** (EI) m/z = 440 [M]⁺.

2.3.5 (E)-3-(((1-(4-chlorophenyl)-3-phenyl-1,2-diazole -4-yl)methylene)amino)-2-thioxoimidazolidin-4-one (TH-5)

IR data 3046 (C-H Ar, str.), 2958 (C-H alky., str.), 1715 (C=O, str.), 1633 (C=N, str.), 1531, 1496 (C=C Ar, str.), 1242, 1204 (C=S, str.), 1092 (C-Cl, str.), 815 (C-H Ar, out-of-plane bend., p-disubstituted benzene), 736, 694 (C-H Ar, out-of-plane bend., monosubstituted benzene). **¹H-NMR** data: 11.92 (s, 1H, NH), 8.98 (s, 1H, CH=N), 8.41 (s, 1H, CH-pyrazole), 7.83–8.03 (m, 4H, Ar-H, p-chlorophenyl), 7.44–7.61 (m, 5H, Ar-H, phenyl), 3.91 (s, 2H, CH₂-thiohydantoin). **¹³C-NMR** data: 174.64 (C=O/C=S, thiohydantoin), 153.52, 152.31, 149.48 (C=N azomethine and pyrazole carbons), 138.20, 132.33, 131.65, 130.37, 130.00, 129.32, 129.25, 129.14, 129.11, 129.03, 128.98, 120.93, 117.43 (Ar-C and remaining pyrazole carbons), 33.66 (CH₂, thiohydantoin). **MS** (EI) m/z = 395 [M]⁺.

2.3.6 (E)-3-(((1-(4-chlorophenyl)-3-(4-fluorophenyl)- 1,2-diazole -4-yl) methylene)amino)-2-thioxoimidazolidin-4-one (TH-6)

IR data : 3125, 3052 (C-H Ar, str.), 2953 (C-H alky., str.), 1713 (C=O, str.), 1638 (C=N, str.), 1531, 1496 (C=C Ar, str.), 1324 (C=S, str.), 1211 (C-F, str.), 1092 (C-Cl, str.), 820 (C-H Ar, out-of-plane bend., p-disubstituted benzene). **¹H-NMR** (400 MHz, DMSO-d₆) δ (ppm): 11.92 (s, 1H, NH), 8.98 (s, 1H, CH=N), 8.40 (s, 1H, CH-pyrazole), 7.30–8.00 (m, 8H, Ar-H), 3.91 (s, 2H, CH₂-thiohydantoin). **¹³C-NMR** data: 164.00, 161.56 (d, J = 244 Hz, C-F ipso), 174.68, 174.46 (C=O and C=S, thiohydantoin), 151.12, 149.44 (C=N azomethine and pyrazole carbons), 138.12 (C-Cl ipso), 131.68, 131.41, 131.32, 131.15, 130.02, 128.91, 128.88, 120.87, 117.37, 116.18, 115.90, 115.68 (Ar-C and pyrazole carbons), 33.72 (CH₂, thiohydantoin). **MS** (EI) m/z = 414 [M]⁺.

2.3.7 (E)-3-(((3-(4-bromophenyl)-1-(4-chlorophenyl)- 1,2-diazole -4-yl) methylene) amino)-2-thioxoimidazolidin-4-one (TH7)

IR data : 3135, 3056 (C-H Ar, str.), 2947 (C-H alky., str.), 1709 (C=O, str.), 1630 (C=N, str.), 1532, 1493 (C=C Ar, str.), 1209 (C=S, str.), 1083 (C-Cl, str.), 817 (C-H Ar, out-of-plane bend., p-disubstituted benzene), 498 (C-Br, str.). **¹H-NMR** data : 11.94 (s, 1H, NH), 9.00 (s, 1H, CH=N), 8.42 (s, 1H, CH-pyrazole), 7.60–8.04 (m, 8H, Ar-H), 3.92 (s, 2H, CH₂-thiohydantoin). **¹³C-NMR** data: 150.76, 149.41 (C=N azomethine and pyrazole carbons), 138.13, 138.07 (C-Cl ipso), 133.36, 132.29, 131.83, 131.77, 131.65, 131.52, 131.27, 131.15, 130.11, 130.04, 120.92, 117.52, 114.59 (Ar-C and pyrazole carbons), 122.53 (C-Br ipso). 33.76 (CH₂, thiohydantoin). **MS** (EI) m/z = 473 [M]⁺.

2.4 Anticancer Activity Evaluation Method

The MCF7 cell line, derived from a human breast carcinoma patient, was procured from the National Cell Bank of Iran (Pasteur Institute, Iran). The MCF7 cells were grown in RPMI-1640 medium (Gibco) Supplied with 10% fetal bovine serum (FBS, Gibco), 100 U/mL of penicillin, and 100 µg/mL of streptomycin. The cells were grown in an incubator at 37 °C with a moistened

atmosphere including 5% CO₂. Subcultivation was done by trypsinization utilizing trypsin/EDTA (Gibco) and washing the cells with PBS buffer.

2.5 MTT cell viability assay.

The cells were counted and their viability assessed by using the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich). In brief, the MCF7 cells were detached by trypsin, suspended and then plated in 96 well plates at a density of 1.4×10^4 cells/well with 200 μ L/well in culture media. After another 24 h of cell attachment and cell growth, the cells were subjected to the test compounds at various concentrations (3.7 – 300 μ g/mL) for 24 h at 37 °C in a moistened incubator with 5% CO₂. The culture medium was then removed and replaced with fresh medium including 200 μ L of MTT solution (0.5mg/mL PBS) per well and incubated for further 4h at 37 °C. The MTT solution was then removed and 100 μ L of dimethyl sulfoxide (DMSO) was placed in each well to dissolve the formazan product. The formazan crystals were completely dissolved by shaking in each well at 37 °C. Cells viability was evaluated by absorbance measurement at the 570 nm with a microplate reader (BioTek Wave XS2, USA).

2.6 Molecular docking study.

Molecular docking was executed using the Molecular Operating Environment (MOE) 2022.02 software. The three dimensional crystal structure of the target protein was retrieved from Protein Data Bank (PDB ID: 3ERT). All water molecules and inappropriate heteroatoms were removed from the protein during the preparation of the protein model, while retaining the essential structure of the receptor. Hydrogen atoms were added and protonation states and partial atomic charges were assigned using the Protonate 3D module of MOE.

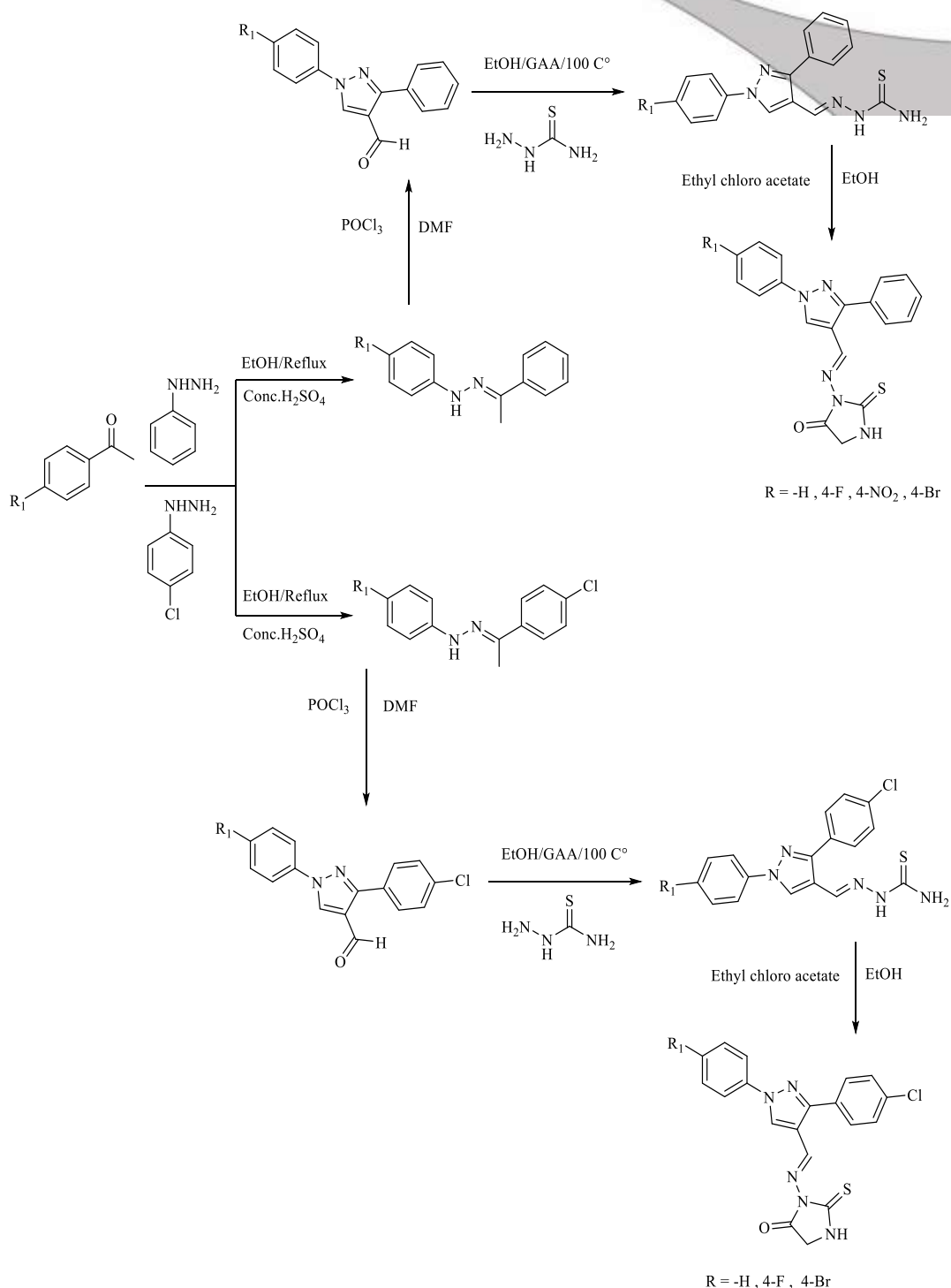
Table 1. Physical Features of synthesized compounds

Comp. No	Molecular formula	M.Wt	M.P., °C.	% Yield	Rf 70% hexane 2 ethyl acetate3	Color
TH1	C ₁₉ H ₁₅ N ₅ OS	361.42	300(dec)	70%	0.73	yellow
TH2	C ₁₉ H ₁₄ N ₆ O ₃ S	406.42	300(dec)	67%	0.70	Yellow-orange
TH3	C ₁₉ H ₁₄ FN ₅ OS	379.41	300(dec)	73%	0.68	Yellowish-white
TH4	C ₁₉ H ₁₄ BrN ₅ OS	440.32	300(dec)	64%	0.74	yellow
TH5	C ₁₉ H ₁₄ ClN ₅ OS	395.87	300(dec)	62%	0.66	Yellow
TH6	C ₁₉ H ₁₃ ClFN ₅ OS	413.86	300(dec)	71%	0.77	Yellowish-white
TH7	C ₁₉ H ₁₃ BrClN ₅ OS	474.76	300(dec)	77%	0.70	yellow

3. RESULTS AND DISCUSSION

3.1. Synthesized Compounds

A novel series of pyrazole conjugated thiohydantoin compounds (TH1–TH7) have been synthesized in an efficient four-step synthesis as represented by Scheme 1. First, substituted acetophenones were treated with phenylhydrazine or 4-chlorophenylhydrazine in ethanol with a catalytic amount of glacial acetic acid to synthesise the hydrazone precursors (HR1-HR7) in high yields (82-94%). From the relatively high yields Acquired in this step, it is evident that this condensation reaction is very efficient under mild acidic conditions. The melting points and spectroscopic data of the synthesized hydrazones were found to be in consistency with the corresponding values reported in the literature establishing the successful preparation of the synthesized hydrazones. The resulting hydrazone intermediates were then subjected to the Vilsmeier-Haack cyclization reaction using DMF and POCl₃ to afford the desired pyrazole-4-carbaldehyde products (PA1-PA7) in fair to Satisfactory yields (67-84%). The Consumption of the formylation and cyclization process proves the effectiveness of the Vilsmeier-Haack approach to form the pyrazole ring system and concurrently attach the aldehyde group needed for subsequent condensation processes. The condensation of pyrazole-4-carbaldehyde derivatives with thiosemicarbazide in the presence of refluxed ethanol as a solvent and glacial acetic acid as a catalyst led to the formation of the corresponding thiosemicarbazones with moderate to Satisfactory yields (60-80%). These intermediates were then intramolecularly reacted during reflux conditions with ethyl chloroacetate to yield the desired thiohydantoin derivatives (TH1-7) in the next step. Products Acquired on cooling were separated and purified by recrystallization from DMF to get pure products in Satisfactory yields.



Scheme 1: Synthesis of 2-thioxoimidazolidin-4-one derivatives.

The structures of the synthesized compounds were unequivocally verified by FT-IR, ¹H NMR, ¹³C NMR, and mass spectrometry. The FT-IR results give definite proof of the cyclization process and the ring formation of thiohydantoin. One important observation is the disappearance of the terminal primary amino (-NH₂) absorption peaks, which appeared at about 3300 cm⁻¹ in the thiosemicarbazone compounds. At the same time, the appearance of new broad absorption peaks in the range of 3100-3200 cm⁻¹ is noticed. These peaks belong to the N-H stretching vibration of the secondary amide in the thiohydantoin ring. In addition

to that, there are strong absorption peaks at 1708-1716 cm^{-1} belonging to the carbonyl (C=O) group of the thiohydantoin. The other distinct absorbances include those for the azomethine (C=N) group at 1630-1645 cm^{-1} and for the thiocarbonyl (C=S) group at 1204-1324 cm^{-1} .

The other method utilized to confirm the structure is ^1H NMR spectroscopy. A singlet attributable to the protons of the methylene group of the newly formed thiohydantoin ring was noted at δ 3.90-3.92 ppm for all the synthesized compounds. The observation of this singlet together with the absence of the signal of the terminal NH_2 found in the thiosemicarbazone precursors indicates a successfully formed ring. In addition, a highly deshielded singlet for the NH proton of the thiohydantoin ring was located at δ 11.91-11.94 ppm, whereas the signal for the azomethine proton (CH=N) was a singlet at δ 8.96-9.06 ppm. The aromatic protons were found in the expected range of δ 7.30-8.34 ppm, in consistency with the proposed substituted pyrazole structures.

The ^{13}C NMR data provide support for the assigned structures. The presence of a distinct signal in the range of δ 33.65 – 36.26 ppm can be ascribed to the methylene carbon in the thiohydantoin ring. The carbonyl carbon and the thiocarbonyl carbons have their expected downfield shifts, whereas the azomethine, pyrazole, and aromatic carbons show up at their characteristic positions. The observed chemical shifts were fully consistent with the proposed molecular structures.

The molecular purity of the synthesized compounds was eventually verified utilizing electron impact mass spectroscopy, which provided molecular ion peaks that agreed with to the expected molecular weights of the desired compounds. The consistency of molecular ion peaks with the expected molecular weights offers further proof of the synthesis and purity of the synthesized thiohydantoin including a pyrazole moiety.

From the analysis of all the spectroscopic techniques mentioned above, FTIR, ^1H NMR, ^{13}C NMR, and mass spectroscopy, it can be concluded that the target compounds (TH1–TH7) have been successfully prepared. This also confirms the proposed pathway for synthesizing this series of compounds.

3.2. In vitro anticancer activity against MCF-7 cells

Cytotoxic effects of the thiohydantoin derivatives (TH1-TH7) on the MCF-7 Mammary carcinoma cell line were evaluated, and their respective IC_{50} values are shown in Table 1. The observed cytotoxic activities differed in intensity and showed that the Features of the aromatic substitution influenced the biological activity of the compounds. Out of all the chemicals screened, TH3 proved to have the highest level of activity ($\text{IC}_{50} = 82.98 \mu\text{g/mL}$), followed by TH6 (126.16 $\mu\text{g/mL}$), TH4 (163.38 $\mu\text{g/mL}$), and TH5 (199.55 $\mu\text{g/mL}$), whereas TH1 displayed the lowest activity (897.05 $\mu\text{g/mL}$).

It may be safely assumed that this enhancement in the activity of TH3 may be ascribed to the effect of the fluoro group and nitro group, because both have an electron-withdrawing effect. Furthermore, TH6 (bromine including) has been found to be highly active, suggesting the potential of improving the anticancer activity of the thiohydantoin core by halogenation. Lastly, the lower activity of TH1 indicates that substitution with aromatics makes a difference in regard to cytotoxicity.

On the whole, the experiment followed the order of TH3> TH6> TH4> TH5> TH2> TH7> TH1, which shows that the presence of electron-withdrawing substituents, especially those of fluoro and nitro, has a positive effect on the antiproliferative property of the compounds under consideration.

Table.2. IC_{50} values of compounds against Mammary carcinoma cells MCF-7.

Symbol	$\text{IC}_{50}(\mu\text{g/ml})$
TH1	897.05
TH2	270.08
TH3	82.98
TH4	163.38
TH5	199.55
TH6	126.16
TH7	360.87

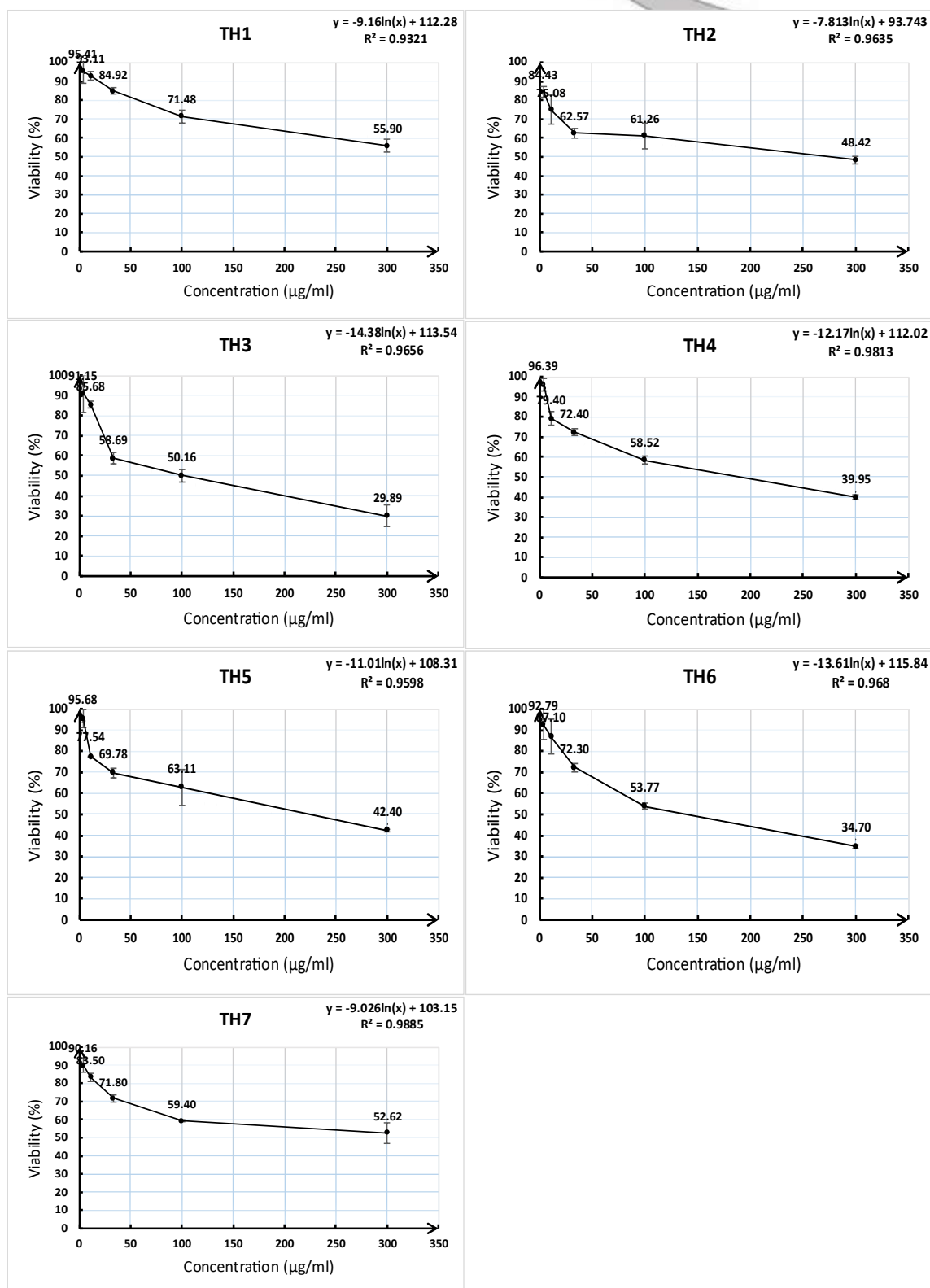


Figure 1. Illustrates the dose-dependent effects of compounds TH1–TH7 on the viability of MCF-7 Mammary carcinoma cells

3.3. Molecular Docking Study

In order to determine the likely mode of interaction of the thiohydantoin derivatives, molecular docking was Executed utilizing the target protein, and the findings are shown in Table 2. The reference ligand showed the greatest binding energy with a score of -8.33 kcal/mol, confirming the validity of the docking procedure. Among the synthesized thiohydantoins, TH2 (-7.28 kcal/mol) and TH5 (-7.25 kcal/mol) showed the best binding energies, followed by TH3 (-6.99 kcal/mol), TH1 (-6.95 kcal/mol), and TH4 (-6.93 kcal/mol). From the docking study, it can be seen that the designed compounds interact with important amino acids that are present in the active site, such as Glu353, Thr347, Met343, Met388, Gly52, and His524. Hydrogen bonding became the main type of interaction, along with π -H and hydrophobic interactions[27], [28].

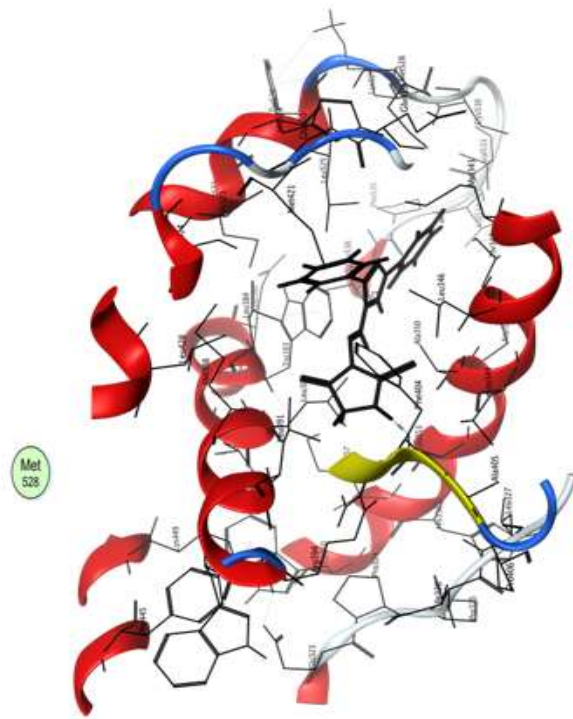
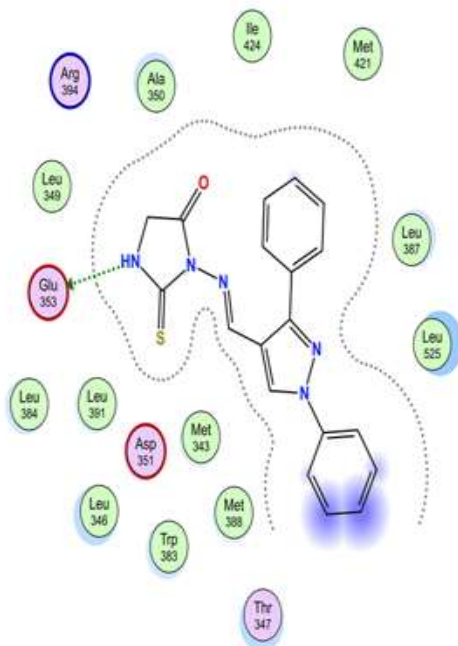
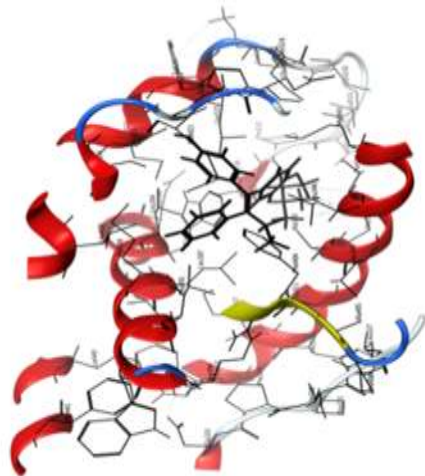
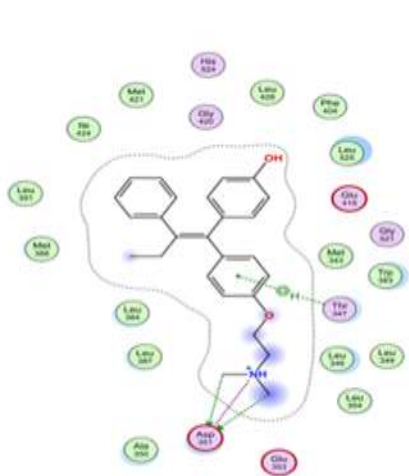
All of the ligands studied have hydrogen bond donors, hydrogen bond acceptors and π -H bonds with Thr347, but among them, TH3 has most interactions with Met343, Gly521, and His524. Although the docking score of TH3 is lower than TH2 and TH5, there are more positive interactions, suggesting a better fit of the molecule in the active site. The higher toxicity of TH3 on MCF-7 cells suggests that both the binding affinity and interaction stability may contribute to the biological effect of TH3 compounds [29], [30].

TH2 and TH5, on the other hand, had slightly better docking scores and sparser interactions with the protein, indicating that the binding energy is also majorly due to hydrophobic interaction. Overall, it may be concluded that the docking results are in Satisfactory consistency with the experimental results of cytotoxicity and that the strength and nature of the docking of both ligands and proteins have an impact on biological activity.

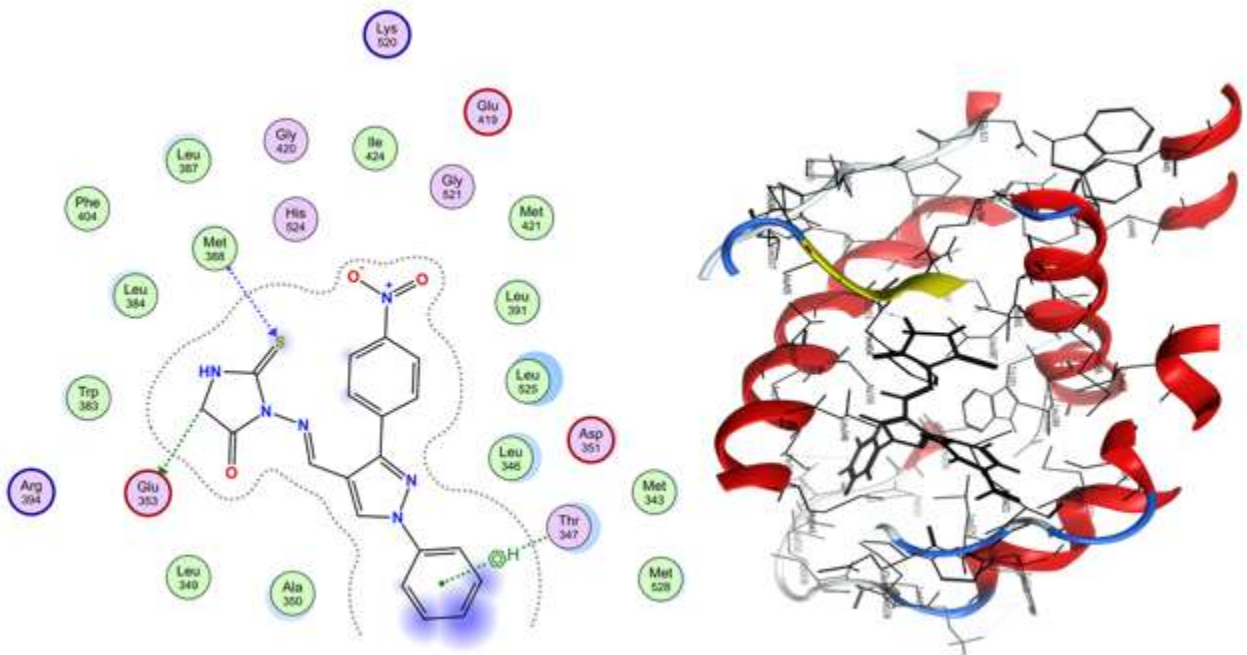
Table 2. The molecular docking results of the synthesized pyrazole–thiohydantoin derivatives against the selected target protein

Comp code	S score (kcal/mol)	RMSD (Å)	Atom of Compound	Atom of Receptor	Involved Interaction Residues	Type of Interaction bond	Distance (Å)	E (kcal/mol)
Reference	-8.33124	1.639434	C 48 C 52 N 46 6-ring	OD1 OD1 OD1 CA	ASP 351 (A) ASP 351 (A) ASP 351 (A) THR 347 (A)	H-donor H-donor Ionic π -H	3.33 3.19 3.54 4.21	-0.8 -1.0 -1.7 -0.5
TH1	-6.94815	1.754557	N 37	OE2	GLU 353 (A)	H-donor	2.99	-4.9
TH2	-7.27721	2.122857	C 36 S 1 6-ring	OE2 CA CG2	GLU 353 (A) MET 388 (A) THR 347 (A)	H-donor H-acceptor π -H	3.38 3.91 3.76	-0.6 -0.6 -0.5
TH3	-6.99142	1.561872	C 32 S 1 S 1 5-ring 6-ring	SD CA CB CA CG2	MET 343 (A) GLY 521 (A) HIS 524 (A) THR 347 (A) THR 347 (A)	H-donor H-acceptor H-acceptor π -H π -H	3.48 3.61 3.96 3.96 3.63	-0.6 -0.6 -0.5 -1.0 -0.8

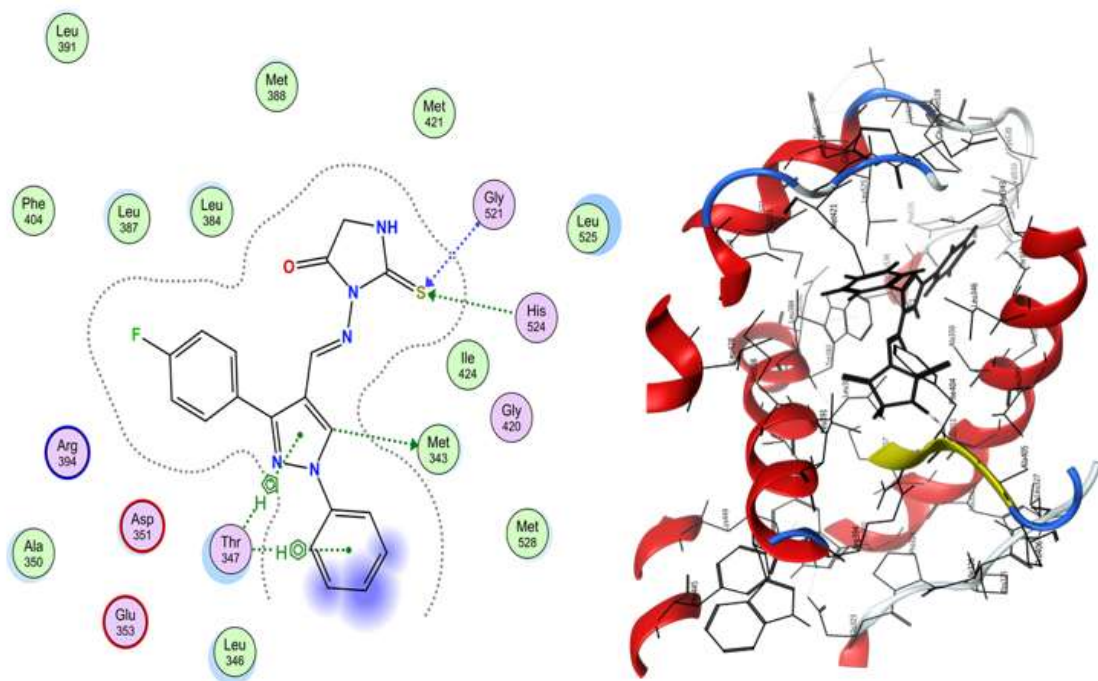
TH4	-6.93275	2.068568	C 36	OE2	GLU 353 (A)	H-donor	3.53	-0.5
TH5	-7.24673	1.647541	S 1	CA	MET 388 (A)	H-acceptor	3.75	-0.5



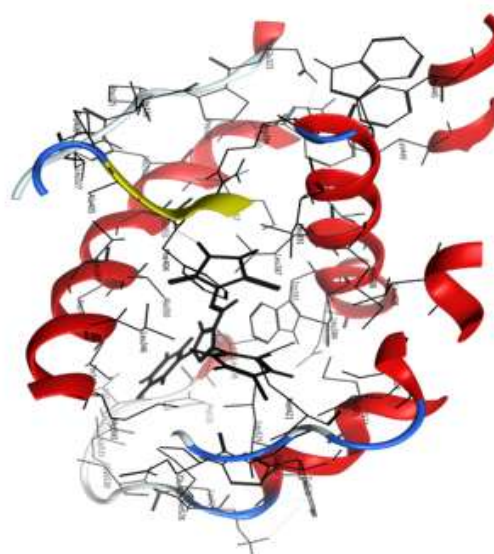
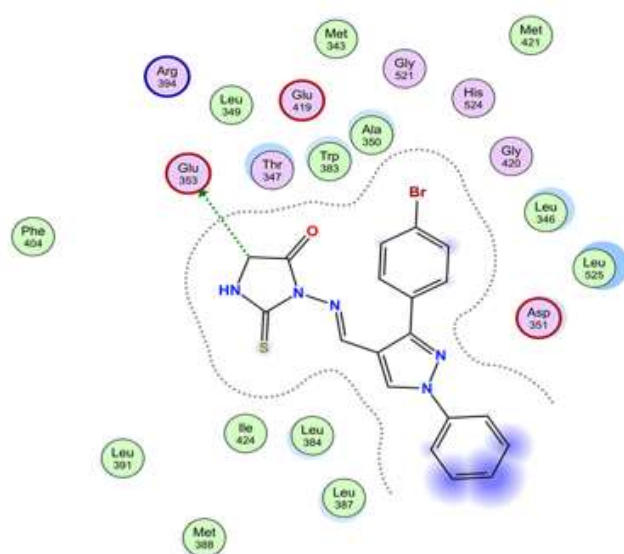
TH1



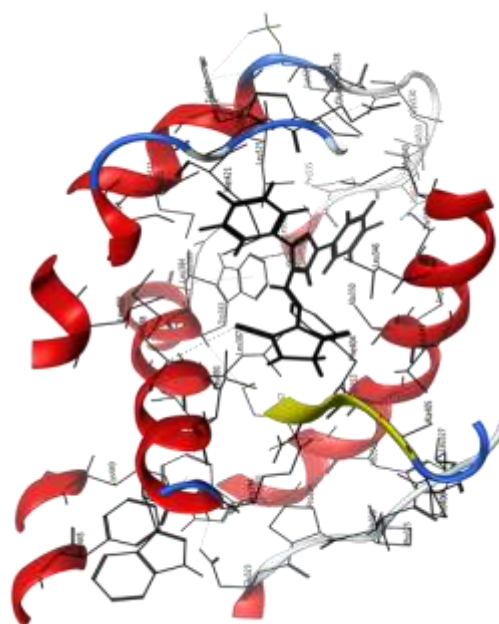
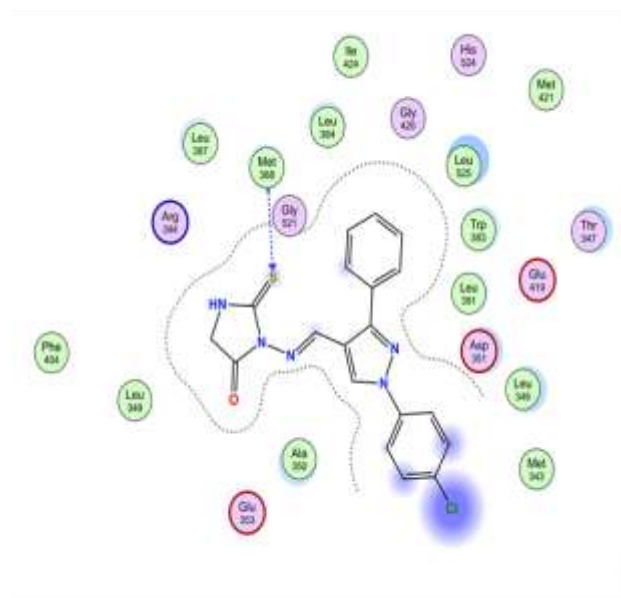
TH 2



TH 3

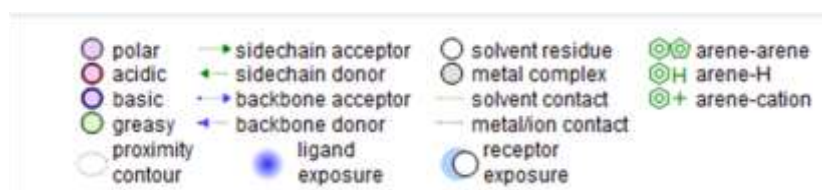


TH 4



TH 5

Figure 2. The predicted binding conformations of the reference ligand and the synthesized compounds within the active site of the target protein



4. Conclusion

Seven novel derivatives (TH1–TH7) of pyrazole-linked thiohydantoin were synthesized through a four-step synthetic protocol and were characterized on the basis of FT-IR, ¹H NMR, ¹³C NMR, and mass spectrometry analysis, which proved that the desired heterocyclic moiety had been successfully prepared. The compounds' cytotoxic activity was evaluated in the human Mammary carcinoma cell line (MCF-7) using MTT assay. All the derivatives studied exhibited cytotoxic activities, with TH3 having the maximum values (IC₅₀ = 82.98 µg/mL) and it is suggested that para-fluoro substitution is more effective for increasing the biological activity of the pyrazole-thiohydantoin moiety. Furthermore, molecular-docking analysis revealed suitable docking of synthesized derivatives with the pocket residues of the receptor binding site, which further corroborates the experimental findings, and offers the binding pose of the compounds. The SAR clearly showed that the electronic effects of the halogens were very important in controlling biological activity, although they had moderate anti-proliferative activity. Overall, it can be concluded that pyrazole linked thiohydantoin derivatives are Satisfactory anticancer lead molecules.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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