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Synthesis and pharmacokinetic profiling of a novel pyrimidine imidazole based thiosemicarbazone derivative for BRAF inhibition in lung cancer therapy

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Abstract

This study presents the design, synthesis, and comprehensive evaluation of a novel thiosemicarbazone derivative containing a pyrimidine-imidazole ($C_{11}H_{11}N_8SCl$) as a potential BRAF inhibitor for the treatment of non-small cell lung cancer. The compound was synthesized by a condensation reaction and characterized by elemental analysis, molar conductivity, SEM-EDS, FT-IR, ^1H , and ^{13}C -NMR spectral techniques. DFT calculations at the B3LYP/6-311++G(d,p) level revealed a HOMO-LUMO gap of 2.8 eV, indicating appropriate molecular stability and reactivity. Molecular docking studies with BRAF (PDB ID: 1UWH) demonstrated favorable binding affinity (-6.85 kcal/mol) with key interactions at ILE512, HIS509, HIS584, GLN529, GLU585, LYS590, VAL510, and LEU587 residues. In vitro cytotoxicity assays revealed that the compound possesses anticancer potential against H-460 cells, with a significant inhibitory effect observed at a concentration of 200 $\mu g/mL$. Combined with its favorable binding affinity toward BRAF kinase, these results suggest that the pyrimidine-imidazole thiosemicarbazone may serve as a potential lead scaffold for further development in targeted lung cancer therapy. ADME profiling confirmed the compound's compliance with Lipinski's rule of five parameters, supporting its potential as an orally active drug candidate. These findings suggest that our pyrimidine-imidazole thiosemicarbazone represents a promising scaffold for the development of targeted BRAF inhibitors for lung cancer therapy.

Keywords ADME profiling, BRAF inhibitor, DFT calculations, Molecular docking, Non-small cell lung cancer, Pyrimidine-imidazole based thiosemicarbazone

1 Introduction

Heterocyclic thiosemicarbazone compounds are ring organic compounds and are obtained by the reaction of aldehydes or ketones with thiosemicarbazides and contain nitrogen, hydrogen, carbon, and sulfur. Heterocyclic thiosemicarbazones and their metal complexes are important compounds that exhibit various antimicrobial activities, including antitumor, antifungal, antibacterial, antimycobacterial, antimalarial,



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antituberculosis, antiviral, antileishmanial, antiparasitic, anti-HIV, anti-inflammatory, and antioxidant properties [1–11]. Heterocyclic thiosemicarbazones exhibit larvicidal effects against various insects, such as *Culex pipiens pallens*, and *Plutella xylostella* [12, 13]. They also have their potential applications in the treatment of infectious and neoplastic diseases [14]. Heterocyclic thiosemicarbazones inhibit the biosynthesis of DNA and certain enzymes, such as ribonucleotide diphosphate reductase and tyrosinase [15, 16]. Heterocyclic thiosemicarbazones exhibit therapeutic properties, including cytotoxic and anticancer activity against various cell lines, such as those associated with cervical cancer, colorectal cancer, and lung cancer [17–21].

Despite advances in medical science, cancer continues to pose a profound global health burden, with lung cancer persisting as the foremost contributor to cancer-related mortality worldwide [22]. According to the most recent international epidemiological data, lung cancer is responsible for an estimated 2.3 million new cases and 1.8 million deaths annually, accounting for approximately 18% of all cancer-associated deaths [23]. Non-small cell lung cancer (NSCLC) represents roughly 85% of all lung cancer cases and is notable for its highly aggressive biological behavior and dismal clinical outlook. Despite significant advancements in therapeutic strategies over recent years, the overall five-year survival rate for individuals diagnosed with NSCLC remains less than 20%, reflecting the ongoing difficulties in achieving durable disease control and improved patient survival [24]. The therapeutic arsenal currently available for the management of non-small cell lung cancer (NSCLC) comprises surgical intervention, radiotherapeutic modalities, traditional cytotoxic chemotherapy, and an array of targeted molecular therapies. Despite these advances, the clinical utility of existing treatments is often compromised by the development of therapeutic resistance, the occurrence of significant adverse events, and the substantial challenge of effectively treating metastatic disease [25]. Collectively, these limitations accentuate the pressing imperative to identify and develop next-generation therapeutic agents for NSCLC that not only demonstrate superior efficacy and selectivity but also possess more favorable safety and tolerability profiles [26].

Approximately 1.5–4.5% of patients with non-small cell lung cancer (NSCLC) harbor activating BRAF mutations, which engage the MAPK/ERK signaling pathway and modulate tumor-cell proliferation, differentiation, transcriptional regulation, and survival [27]. Within the RAS/RAF/MEK/ERK signaling pathway, the B-Rapidly Accelerated Fibrosarcoma (BRAF) kinase emerges as a critical molecular orchestrator in the initiation and progression of non-small cell lung cancer (NSCLC). This serine/threonine protein kinase, belonging to the RAF family of kinases, integrates upstream RAS signals to modulate a diverse array of cellular processes, including the promotion of cell proliferation, the activation of survival pathways, and the regulation of cellular differentiation, thereby exerting profound influence over tumorigenesis and disease trajectory [28, 29]. BRAF mutations are present in around 6% of cancers, particularly in melanoma, colorectal adenocarcinoma, lung adenocarcinoma, glioblastoma, papillary thyroid carcinoma, and hairy cell leukemia [30].

Development of inhibitors targeting the MAPK cascade was initially advanced in melanoma. Sorafenib, one of the earliest multikinase TKIs evaluated, produced limited benefit in BRAF-mutant disease because clinically achievable exposures were insufficient to suppress mutant BRAF and toxicity-constrained dosing, which drove the design of highly selective agents [31]. Vemurafenib, dabrafenib, and encorafenib are potent,

ATP-competitive BRAF inhibitors engineered to occupy the ATP-binding cleft of the active kinase conformation. Nonetheless, monotherapy commonly succumbs to acquired resistance, most often via reactivation of MAPK signaling. Incorporating downstream MEK inhibition (e.g., trametinib, cobimetinib, binimetinib) more completely abrogates ERK signaling, deepens pathway blockade, and delays resistance. In addition, MEK co-inhibition counteracts RAF inhibitor-induced paradoxical MAPK activation in BRAF-wild-type settings, thereby reducing the characteristic hyperproliferative cutaneous toxicities [32].

In parallel, computational methodologies have become indispensable in the drug discovery process, offering substantial reductions in both time and resource expenditure [33]. In this study, our primary aim is to evaluate the potential of newly synthesized thiosemicarbazone derivatives containing pyrimidine-imidazole as BRAF inhibitors for the treatment of lung cancer. To achieve this, we adopted a comprehensive computational approach that includes density functional theory (DFT) calculations, molecular docking, and ADME analyses. DFT calculations were used to gain molecular-level insights into the electronic properties, conformational stability, and reactivity of the compounds. Molecular docking studies elucidated the interactions of these molecules with the ATP-binding site of BRAF, clarifying their potential inhibitory mechanisms. Furthermore, *in silico* prediction of absorption, distribution, metabolism, and excretion (ADME) parameters was performed to assess the pharmacokinetic profiles and clinical potential of the compounds [34, 35]. By integrating these computational methods, our study aims to contribute to the rational design and optimization of more effective and selective BRAF inhibitors, ultimately supporting the development of new therapeutic agents for lung cancer treatment.

2 Materials and methods

All chemicals, phosphate-buffered saline (PBS) buffer, and trypan blue dye were purchased from Merck or Sigma-Aldrich. NutriCulture Cell Viability Detection Kit-8 (CVDK-8) was purchased from Ecotech Biotechnology® (Erzurum-Turkey). The human lung cancer cell line (H460) used in this study was kindly provided by the Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University (Istanbul, Turkey). Organic elemental analyses were carried out on a Thermo-Scientific Flash 2000 model elemental analyzer. Fourier transform infrared spectra (FT-IR) were recorded on a Shimadzu IR Prestige-21 spectrophotometer at 400 to 4000 cm^{-1} . Proton and carbon nuclear magnetic resonance spectroscopy (^1H and ^{13}C -NMR) were taken with a Bruker Biospin Avance III 400 MHz model instrument. Scanning electron microscopy and energy dispersive X-ray images (SEM-EDX) were performed with a Quanta FEG 250 device. The molar conductivity was measured in dimethyl sulphoxide (DMSO) using a Conductivity 430 Lab.

2.1 Synthesis of thiosemicarbazone derivative containing pyrimidine-imidazole

Novel thiosemicarbazone compound (Pht) was synthesized by condensation reaction of a solution of 4-amino-6-chloropyrimidine-5-carboxaldehyde (4 mmol) dissolved in ethanol (20 mL) and a solution of 4-ethyl-3-thiosemicarbazide (4 mmol) dissolved in ethanol (15 mL) (Fig. 1). The mixture was stirred and heated for five hours under reflux at 90 °C. During reflux, the solution pH was adjusted to 5–5.5 by adding 2 mL of acetic acid. Then, a solution of 2-imidazolecarboxaldehyde (4 mmol) dissolved in ethanol (15 mL) was

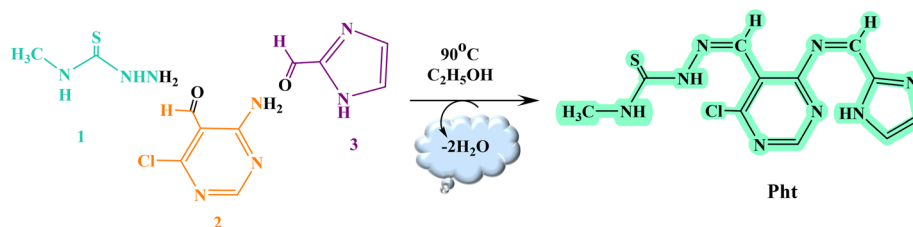


Fig. 1 Synthesis reaction of thiosemicarbazone compound (PhT)

added and refluxed with stirring for a further three hours. Following filtration, purification, and drying, a brick-colored product was obtained.

2.2 Assessment of cell viability and cytotoxic effects in H460 non-small cell lung cancer cells

All biological experiments were conducted using the H-460 cell line, which is representative of non-small cell lung carcinoma. Cells were maintained in RPMI medium (GIBCO), enriched with 10% fetal bovine serum (FBS; GIBCO), and cultured in 25 cm² flask. For routine passaging, cells were rinsed with phosphate-buffered saline (PBS) and detached using a trypsin-EDTA solution (GIBCO). Cultures were incubated at 37 °C under 5% CO₂ in a humidified environment until reaching confluency. During each subculture, cell viability was checked using the trypan blue exclusion assay. After centrifugation, viable and non-viable cells were counted with a Thoma hemocytometer following trypan blue staining. To determine cytotoxicity, the CVDK-8 assay was employed, which is based on the reduction of WST-8 tetrazolium salt. H-460 cells were seeded into 96-well plates and allowed to adhere for 24 h. The cells were then treated with various concentrations of the test compound (dissolved in DMSO, with a final DMSO concentration of 0.1%), specifically at 12.5, 25, 50, 100, and 200 µg/mL. After a 24-hour incubation with the compound, cell viability was assessed according to the manufacturer's instructions for the assay. The protocol involved adding 10 µL of CVDK-8 reagent to each well, followed by a 4-hour incubation. Absorbance was then read at 450 nm using a spectrophotometer to determine the extent of cell viability [36–38].

2.3 In silico study design: DFT, docking, and pharmacokinetic evaluation

Density Functional Theory (DFT) is a widely utilized quantum mechanical method for exploring the electronic structures of atoms and molecules. In pharmaceutical research, DFT plays a crucial role in examining the electronic behavior of drug candidates and their delivery mechanisms. Unlike Molecular Mechanics (MM), DFT provides a higher level of chemical accuracy, particularly in elucidating reaction mechanisms at enzyme binding sites. Its relatively low computational cost, combined with its ability to simulate biologically relevant systems, makes it an advantageous tool. In this study, DFT calculations were performed using the B3LYP functional in combination with the 6-311 + + G(d, p) basis set. All visual analyses, including optimized geometries, HOMO-LUMO orbitals, and Molecular Electrostatic Potential (MEP) maps, were performed using GaussView, following computations completed with the Gaussian 09 W software package [39]. The B3LYP functional, together with the 6-311 + + G(d, p) basis set, was employed for all DFT calculations in this work. This computational approach has been extensively validated in the literature for studying thiosemicarbazone derivatives and related heterocyclic compounds. Researchers demonstrated that B3LYP calculations with 6-31G(d), 6-31G(d, p), and 6-311

+ + G(d, p) basis sets provide accurate vibrational frequencies and molecular properties for acetophenone thiosemicarbazone, with the 6-311 + + G(d, p) level showing excellent agreement with experimental data [40]. Similarly, Ahmad et al. successfully applied B3LYP/6-311 + + G(d, p) calculations to investigate the molecular structure, spectroscopic properties, and quantum descriptors of imidazole derivatives, confirming the reliability of this computational level for heterocyclic systems [41]. The effectiveness of B3LYP for pyrimidine-containing compounds has been further validated by Mayaliwa et al., who used DFT/B3LYP calculations to predict interaction sites and hydrogen bonding patterns in pyrimidone derivatives [42]. Based on this extensive literature evidence, the B3LYP/6-311 + + G(d, p) method was deemed appropriate for the present study, providing reliable predictions of electronic structure, optimized geometries, and reactivity parameters for the synthesized thiosemicarbazone derivative.

In addition, molecular docking analysis and evaluation of the physicochemical properties of the synthesized ligand were conducted using AutoDock software [43] and the Swiss-ADME web tool [44], respectively. 1UWH, the wild-type BRAF kinase domain co-crystallized with sorafenib (BAY 43-9006), was chosen for docking because it provides a high-resolution (2.95 Å), ligand-bound view of the ATP-binding pocket and captures the kinase in an inactive, druggable conformation. This co-crystal provides experimentally validated coordinates for reliable pose prediction and affinity benchmarking in structure-based design. Using the wild-type structure establishes a baseline for inhibitor interactions before modeling oncogenic variants, making 1UWH an appropriate reference for comparative docking studies [45, 46].

2.4 Computational details

All quantum-chemical calculations were carried out using the Gaussian 09 software package [39]. Initial molecular structures were fully optimized, followed by harmonic vibrational frequency analyses, employing the DFT framework with the B3LYP hybrid functional in combination with the 6-311 + + G(d, p) basis set. This computational protocol has been widely reported to provide an appropriate balance between accuracy and computational efficiency for organic and heteroatom-containing systems [33, 47, 48]. Frequency calculations were performed at the same theoretical level to verify that the optimized geometries correspond to true minima on the potential energy surface, as evidenced by the absence of imaginary frequencies. Frontier molecular orbital energies and molecular electrostatic potential (MEP) distributions were generated from the optimized structures and visualized using the GaussView program [49].

2.5 Statistical analysis

All experimental procedures were independently repeated three times, and the resulting data are expressed as the mean value accompanied by the standard deviation (SD). Statistical analyses were performed using GraphPad Prism software (version 8.0.1; GraphPad Software, Inc., San Diego, CA, USA). To compare differences between experimental groups, an unpaired Student's t-test was employed. Prior to conducting parametric analyses, the data distribution was assessed to confirm normality, thereby validating the use of these statistical methods. A p-value of 0.05 or less was considered statistically significant throughout the study.

3 Results and discussion

3.1 Characterization of thiosemicarbazone derivative containing pyrimidine-imidazole

The result of elemental analysis with analytical and physical data of the thiosemicarbazone compound (Pht) is presented in Table 1.

FT-IR spectral data of the thiosemicarbazone compound (Pht) are given in Table 2 and are demonstrated in Fig. 2. The compound showed two strong bands at 1634 cm^{-1} and 1532 cm^{-1} , attributable to $\nu(\text{CH}=\text{N})$. Two absorption bands were observed because of the two unsymmetrical imine groups. The stretching vibrations observed at 1235 cm^{-1} and 856 cm^{-1} , attributable to $\nu(\text{C}=\text{S})$. The aromatic and aliphatic $\nu(\text{CH})$ stretching were determined at 2973 cm^{-1} and 3392 cm^{-1} , respectively. The stretching vibration of the $\nu(\text{C}=\text{C})$ group was observed at 1447 cm^{-1} . The absorption band of $\nu(\text{N-H})$ was detected at 3136 cm^{-1} [11, 12].

$^1\text{H-NMR}$ spectral data of the thiosemicarbazone compound (Pht) are given in Table 3 and are demonstrated in Fig. 3. The peaks belonging to asymmetric azomethine protons ($\text{CH}=\text{N}$) appeared at 8.24 ppm and 8.44 ppm. The peaks attributable to NH protons (Me-NH and N-NH) were observed at 10.47 ppm and 10.05 ppm, respectively. Two signals were observed due to differences in the chemical environments of the NH protons. The aromatic (Ar-H) protons were revealed in the ranges 6.09–7.43 ppm. The peak attributable to the imidazole proton (CH) was revealed at 9.67 ppm. In addition, the methyl proton (HN-CH_3) was observed at 1.18 ppm [50].

Table 1 Analytical and physical data of the thiosemicarbazone compound (Pht)

Molecular Formula ($\text{M}_w, \text{g.mol}^{-1}$)	Color Yield (%)	m.p. ($^{\circ}\text{C}$)	Elemental Analysis Calculated (Found) %			
			C	H	N	S
$\text{C}_{11}\text{H}_{11}\text{N}_8\text{S}$ (322.77)	Dark Green 49	190	41.40 (40.93)	4.00 (3.44)	34.37 (34.72)	9.84 (9.93)

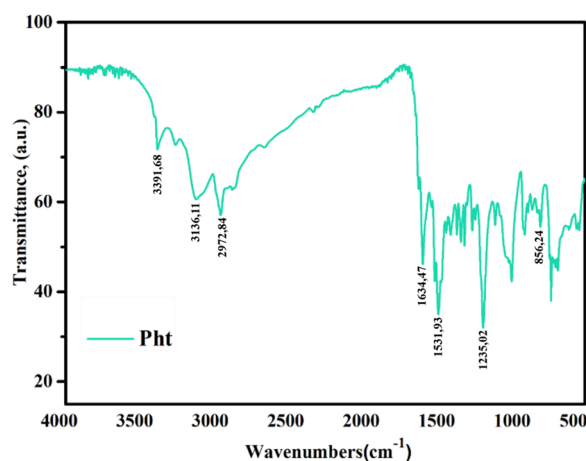


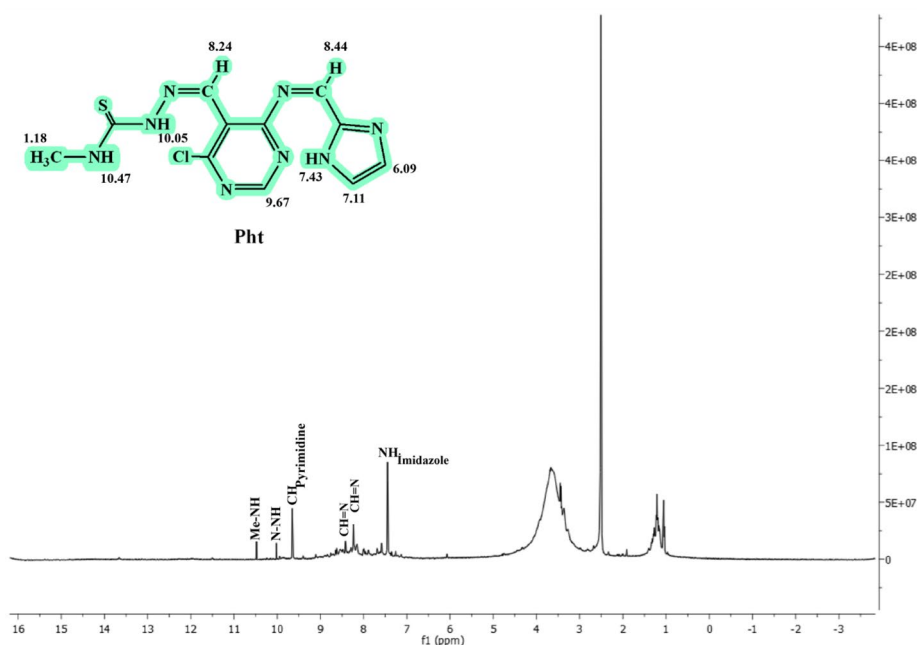
Fig. 2 FT-IR spectrum of thiosemicarbazone compound (Pht)

Table 2 Characteristic FT-IR peaks of thiosemicarbazone compound (Pht)

IR spectral bands (cm^{-1})					
$\nu(\text{N-H})$	$\nu(\text{CH})_{\text{alip.}}$ $\nu(\text{CH})_{\text{arom.}}$	$\nu(\text{CH}=\text{N})$	$\nu(\text{CH}=\text{N})_{\text{tyz.}}$	$\nu(\text{C}=\text{S})$	$\nu(\text{C}=\text{C})$
3136	3392 2973	1634 1532		1235, 856	1447

Table 3 ^1H and ^{13}C -NMR spectra of thiosemicarbazone compound (Pht)

^1H -NMR spectral bands (ppm)					^{13}C -NMR spectral bands (ppm)			
Me-NH	N-NH	CH _{im}	HN-CH ₃	CH=N	Ar-H	N-C(=S)-N _{thio}	CH=N _{arom}	C _{aromatic}
10.47	10.05	9.67	1.18	8.24, 8.44	6.09- 7.43	181.57	169.58, 160.62	157.28- 119.54

**Fig. 3** ^1H -NMR spectrum of thiosemicarbazone compound (Pht)

^{13}C -NMR spectral data of the thiosemicarbazone compound (Pht) are given in Table 3 and are demonstrated in Fig. 4. The peaks of asymmetric azomethine carbons ($\text{CH}=\text{N}$) were observed at 160.62 ppm and 169.58 ppm. The peak attributable to thioamide carbon ($\text{N}-\text{C}(=\text{S})-\text{N}$) was determined at 181.57 ppm. The peaks of aromatic carbons ($\text{Ar}-\text{C}$) aromatic were observed in the range of 119.54–157.28 ppm [51].

SEM-EDS data, which provides information about the structure of the thiosemicarbazone compound, is given in Fig. 5. The SEM image of the thiosemicarbazone compound showed that it has a small-sized, angular particle structure. Based on EDS analysis data, it was determined that the thiosemicarbazone compound contained the elements C, N, O, Cl, and S.

3.2 Evaluation of cytotoxic activity and cell viability in H460 cells

H-460 cells were treated with various concentrations (12.5–200 $\mu\text{g}/\text{mL}$) of the compound for 24 h. While lower concentrations (12.5 and 25 $\mu\text{g}/\text{mL}$) caused a non-toxic metabolic fluctuation above the control level, a dose-dependent reduction in cell viability was observed at higher doses. Statistically significant cytotoxic activity was achieved at the highest concentration of 200 $\mu\text{g}/\text{mL}$ (58.73%, $p=0.003$), identifying it as the effective dose for anticancer evaluation (Table 4).

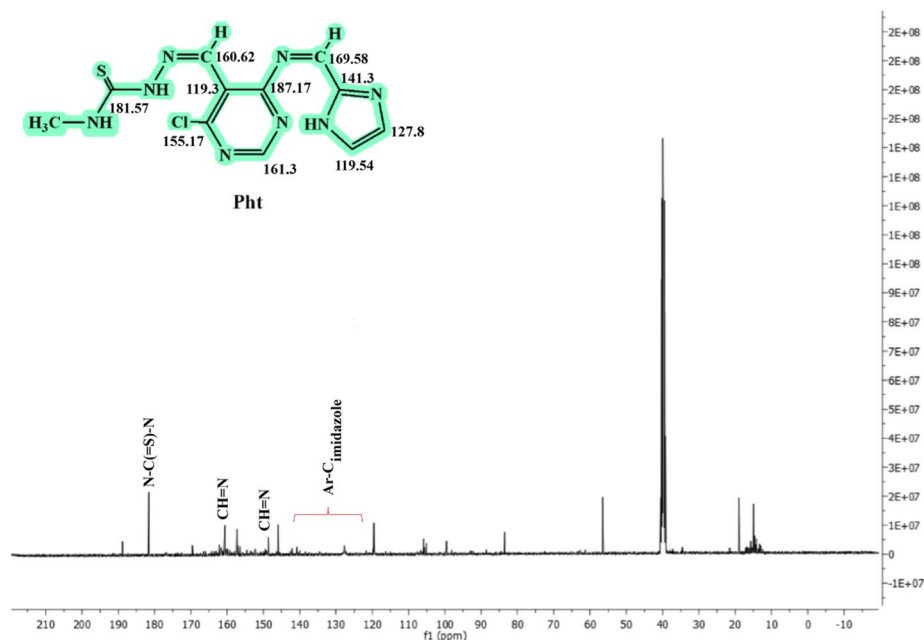


Fig. 4 ¹³C-NMR spectrum of thiosemicarbazone compound (Pht)

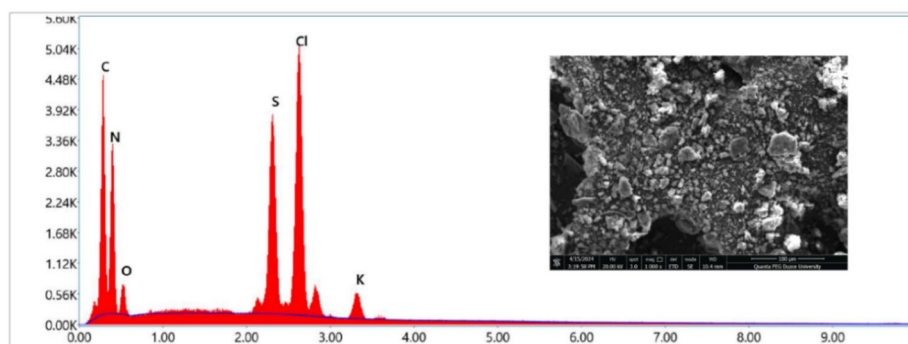


Fig. 5 SEM-EDS analysis of thiosemicarbazone compound (Pht)

3.3 DFT calculations, molecular docking outcomes, and pharmacokinetic predictions

3.3.1 Density functional theory calculations

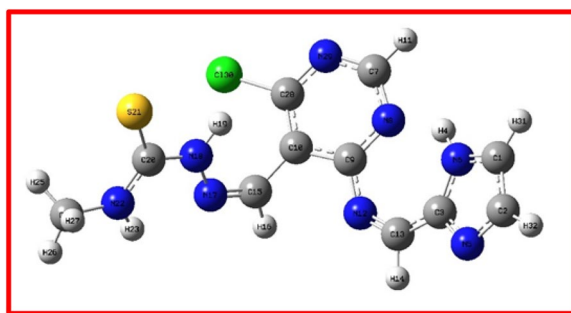
In this study, a combination of computational methods is employed to analyze the fundamental properties of a particular molecule. The ground-state geometries were optimized using the B3LYP/6-311++(d, p) level of theory within the Gaussian 09 W software, which is widely recognized for its accuracy and reliability [52–54]. Following optimization, further analyses were conducted to evaluate key geometric parameters, electronic properties, and Molecular electrostatic potential (MEP) analysis. Additionally, the computed total energies were found to be -1722.438531 Hartree in the gas phase, as illustrated in Fig. 6.

The key geometric parameters of the thiosemicarbazone compound (Pht) are summarized in Table 5. A comparison with literature sources and crystallographic data from the Cambridge Structural Database confirms that the calculated optimized geometry aligns well with typical values for hydrogen bonding, C–C, C–S, and N–N bonds, as well as bond angle values [55]. The optimized molecular structure provides a reliable basis

Table 4 Effects of thiosemicarbazone compound (Pht) on H-460 cell viability at different concentrations (* $p < 0.05$)

Concentrations	Cell viability (%)	Standard deviation	P value
Control	100	0.985	—
200 ug/mL	58.73*	1.448	0.003
100 ug/mL	84.19	1.876	0.077
50 ug/mL	98.74	1.472	0.854
25 ug/mL	123.14	1.328	0.019
12.5 ug/mL	116.93	1.478	0.068

The value 58.73* has been formatted in bold and marked with an asterisk to clearly indicate statistical significance ($p < 0.05$)

**Fig. 6** Optimized structure of thiosemicarbazone compound (Pht) in the gas phase**Table 5** Selected optimized geometrical parameters of the thiosemicarbazone compound (Pht)

Bond length (Å)		Bond angle (°)		Dihedral angle (°)	
N22-C24	1.453	C24-N22-C20	123.77	C2-C1-N6-C3	− 0.43
N18-N17	1.347	N18-C20-S21	119.27	H32-C2-C1-H31	1.25
C15-C10	1.482	N18-N17-C15	120.70	C7-N8-C9-N12	174.07
C20-S21	1.674	C9-C10-C28	115.20	C9-C10-C28-Cl30	176.14
C28-Cl30	1.746	C10-C28-Cl30	120.26	N17-N18-C20-S21	176.60
N18-H19	1.014	C1-C2-N5	110.36	C24-N22-C20-S21	− 0.60
C7-H11	1.085	H27-C24-N22	111.03	C7-N29-C28-Cl30	− 178.07

for interpreting the molecule's chemical reactivity, physical properties, and potential biological activity.

3.3.2 Frontier molecular orbital (FMO) analysis

For the Frontier Molecular Orbital (FMO) analysis, Density Functional Theory (DFT) calculations were carried out using the B3LYP functional with the 6-311++G(d,p) basis set. FMO analysis is a powerful technique for understanding the electronic properties and reactivity of a newly synthesized thiosemicarbazone compound (Pht). In this analysis, the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO) are key descriptors that provide insight into the molecule's chemical stability and potential reactivity. The energy gap (ΔE_{gap}) between these orbitals is a crucial parameter, as it influences the molecular hardness, softness, and charge transfer properties. A smaller energy gap indicates higher reactivity, while a larger gap suggests greater stability. In this study, the HOMO-LUMO energy gap was calculated to evaluate the electronic properties of the studied compound and was found to be 2.8 eV (Fig. 7).

The HOMO is mainly localized over the electron-rich aromatic and sulfur-containing regions, suggesting that these sites are likely to act as electron donors during chemical interactions. Conversely, the LUMO is predominantly distributed over the opposite side of the molecule, particularly around the electron-deficient functional groups, indicating their role as electron acceptors. Several global reactivity parameters were derived from the energies of the HOMO and LUMO orbitals, providing insights into the chemical behavior and stability of the investigated ligand. These descriptors were computed using Koopman's theorem, which relates the orbital energies to key thermodynamic quantities [56]. According to Koopman's approximation, the ionization potential (IP) and electron affinity (EA) can be estimated as the negative of the HOMO and LUMO energies, respectively, $EA = -E_{LUMO}$ and $IP = -E_{HOMO}$. Based on these values, further reactivity indicators such as electronegativity (χ), chemical hardness (η), and chemical potential (μ) were determined using the relations $\chi = -\frac{E_{HOMO} + E_{LUMO}}{2}$, and $\eta = -\frac{E_{HOMO} - E_{LUMO}}{2}$ and $\mu = -\frac{(IP + EA)}{2}$. Additionally, the global softness (σ) was calculated as the inverse of chemical hardness ($\sigma = \frac{1}{\eta}$), and the global electrophilicity index (ω) was obtained from the formula $\omega = \frac{\mu^2}{2\eta}$ [52, 57, 58]. These parameters collectively provide a comprehensive understanding of the molecule's reactivity, electron-donating and electron-accepting abilities, and stability. The calculated values of these global reactivity parameters for the ligand are presented in Table 6.

The ionization potential, representing the minimum energy needed to remove an electron from a molecule, was determined to be 5.932 eV for the ligand. Conversely, its ability to accept an electron, known as the electron affinity, was calculated as 3.132 eV. Electronegativity, which reflects a molecule's tendency to attract electrons, was found to be 4.532 eV, while the chemical potential, which is inversely related to electronegativity,

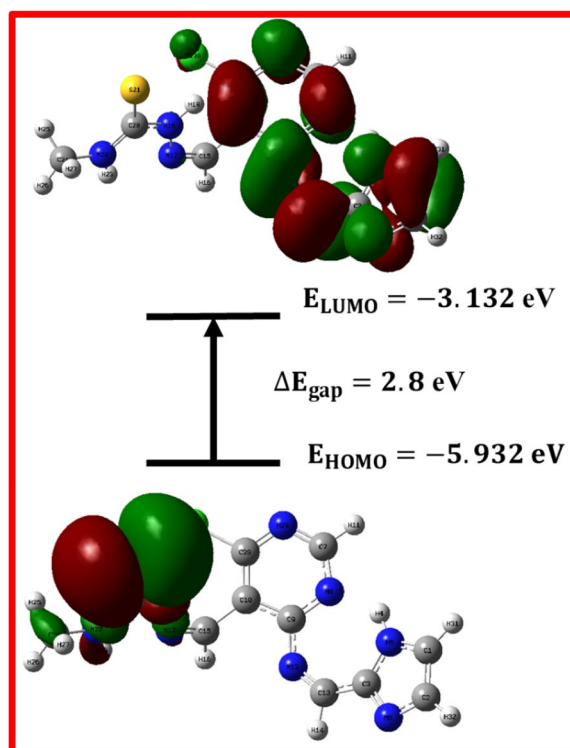


Fig. 7 HOMO–LUMO structures of thiosemicarbazone compound (Pht) in the gas phase

Table 6 Calculated frontier orbital energies and reactivity descriptors for thiosemicarbazone compound (Pht)

Parameters	Values (eV)
E_{HOMO}	− 5.932
E_{LUMO}	− 3.132
Gap	2.8
IP	5.932
EA	3.132
χ	4.532
η	1.4
σ	0.714
μ	− 4.532
ω	7.335

was calculated as − 4.632 eV. The ligand's global electrophilicity index, a measure of its ability to act as an electron acceptor, was determined to be 7.335 eV. Furthermore, the concepts of chemical hardness and softness offer insights into the compound's stability and chemical reactivity. Chemical softness (σ), defined as the opposite of chemical hardness, is an important global reactivity descriptor that indicates a molecular system's ability to change its electron density in reaction to external perturbations. It is directly related to the HOMO-LUMO energy separation and gives useful information about the balance of molecular reactivity and stability. As shown in Table 6, the chemical softness of the investigated molecule was predicted to be 0.714 eV, indicating a moderately soft electronic nature. This softness value indicates that the molecule has enough electronic flexibility to participate in charge-transfer or polarization-driven interactions while avoiding excessive reactivity, which may jeopardize structural stability. Such tendency is especially relevant for heteroatom-rich systems with nitrogen- and sulfur-based functional groups, as seen in the current structure. The presence of numerous conjugated N atoms, together with thione (C=S) activity and aromatic fragments, facilitates delocalization of the border molecular orbitals, increasing electronic flexibility across the molecular framework. The evaluated chemical hardness (1.4 eV) and softness (0.714 eV) values further confirm that the ligand exhibits a balanced reactivity profile, combining sufficient electronic responsiveness with structural stability.

3.3.3 Molecular electrostatic potential (MEP) analysis

MEP (Molecular Electrostatic Potential) maps visualize the distribution of electrostatic potential across the molecular surface, providing important insights into electron density, reactive regions (nucleophilic and electrophilic attacks), and intermolecular interactions [59]. using the DFT at B3LYP/6-311++(d, p) level, the molecular electrostatic potential (MEP) was computed and visualized, as presented in Fig. 8. The color range for the potential surface varies from -5.670×10^{-2} (deepest red) to 5.670×10^{-2} (deepest blue) a.u. The MEP surface was generated at an electron density iso-surface value of 0.0004 a.u. The electrostatic potential distribution is represented through a gradient color scale, generally following the trend: red < green < blue. Regions colored in red signify areas of negative potential, typically associated with electrophilic reactivity. Conversely, blue-colored zones correspond to electron-rich regions and are prone to nucleophilic attack. Neutral electrostatic areas are mostly indicated by green to yellow transitions.

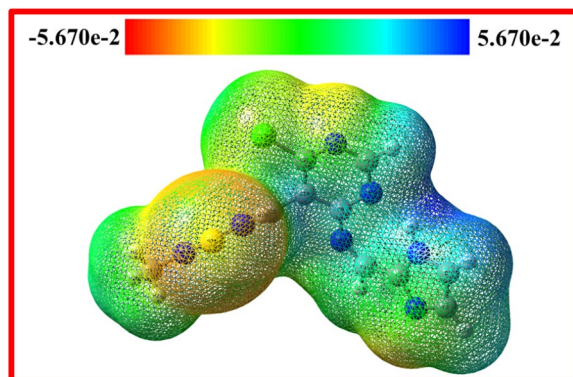


Fig. 8 The MEP surface diagram of the thiosemicarbazone compound (Pht) in the gas phase

3.3.4 Methodological framework for molecular docking

The PDB structure of the newly synthesized thiosemicarbazone compound (Pht) was generated based on Gaussian-optimized geometries. The crystal structure of the BRAF protein (PDB ID: 1UWH) was retrieved from the Protein Data Bank (<http://rscb.org>). Protein preparation was carried out using BIOVIA Discovery Studio [60]. The crystal structure of B-Raf kinase was obtained from the Protein Data Bank (PDB ID: 1UWH). The structure contains two protein chains (A and B), both of which were retained during the docking calculations in order to preserve the native dimeric environment of the binding site. Prior to docking, all crystallographic water molecules, ions, and heteroatoms were removed. Incomplete amino acid residues located outside the active binding region were not reconstructed, as they do not directly influence ligand protein interactions. Polar hydrogens and Kollman charges were added in Auto-Dock Tools. The binding site of the protein was predicted using the POCASA 1.1 server [61]. A docking grid box was then defined, centered at coordinates X: 52.342 Y: 31.424, Z: 74.073 Å, with dimensions of $40 \times 40 \times 40$ Å³ and a grid point spacing of 0.375 Å. The prepared protein was subsequently imported into AutoDock Tools 1.5.6 [34], where hydrogen atoms were added, charges were calculated and assigned, and atom types were specified. Molecular docking simulations were performed using Auto-Dock Vina 1.1.2 [43]. To evaluate the accuracy of the docking results, the ligand was re-docked, and the root mean square deviation (RMSD) between the predicted and reference poses was calculated.

3.3.5 Molecular docking analysis

BRAF represents a highly relevant molecular target in anticancer studies owing to its essential involvement in oncogenic signaling pathways and tumor development. Structural elucidation of the BRAF kinase domain, together with the presence of well defined and drug-accessible binding pockets, enables reliable computational modeling of ligand protein interactions [62]. Moreover, the availability of experimentally characterized inhibitors provides a robust framework for validating docking protocols. Since aberrant activation of BRAF, frequently arising from oncogenic mutations, is a key driver of malignant transformation and disease progression, targeting this protein remains a strategic approach in cancer therapy. In this context, molecular docking serves as an efficient tool for probing binding affinity and interaction patterns of potential anticancer agents, justifying the selection of BRAF (PDB ID: 1UWH) as the docking target in the present study.

Molecular docking simulation is a widely adopted computational technique for elucidating the interactions between a ligand and its macromolecular target—in this case, the BRAF protein kinase. This approach enables the prediction of the most favorable binding orientation of the ligand within the receptor’s active site. The strength and stability of these interactions are typically evaluated by calculating the binding free energy, with more negative values indicating stronger affinities. In the present study, AutoDock version 1.5.6 was used to perform the docking procedure, which included binding site preparation, docking execution, and subsequent analysis [63]. The heterocyclic thiosemicarbazone compound (Pht) was specifically investigated for its potential inhibitory activity against BRAF (PDB ID: 1UWH). The optimal docking pose was selected based on the lowest binding free energy, and the results are summarized in Table 7; Fig. 9. To assess the reliability of the docking outcomes, the ligand was re-docked into the active site, and the root mean square deviation (RMSD) between the initial and re-docked conformations was calculated, yielding a value of 2.630 Å as depicted in Fig. 10.

The key amino acid residues involved in the interaction between Pht compound and BRAF (1UWH) include ILE B:512, HIS B:509, HIS A:584, GLN B:529, GLU B:585, HIS B:584, LYS B:590, LYS A:590, VAL B:510, and LEU A:587, as depicted in Fig. 9. Conventional hydrogen bonds (indicated by green dashed lines) were observed between the ligand and residues such as GLN B:529, GLU A:584, and GLU B:585, which play a crucial role in stabilizing the ligand within the binding pocket. Unfavorable interactions, highlighted in red particularly with LYS A:590 represent electrostatically repulsive (positive–positive) and donor–donor contacts, potentially guiding the ligand toward a more favorable orientation. π -Alkyl interactions (shown as pink lines) with residues like VAL B:510 and LEU A:587 reflect hydrophobic contacts between the aromatic regions of the ligand and the non-polar side chains of the protein. Additionally, a π -cation interaction (orange line) with LYS B:590 demonstrates strong electrostatic attraction between the positively charged side chain and the aromatic ring of the ligand.

3.3.6 ADME analysis

Assessment of Absorption, Distribution, Metabolism, and Excretion (ADME) properties is essential in the early stages of drug discovery, as it enables the prediction of pharmacokinetic behavior and drug-likeness of candidate molecules. Comprehensive ADME profiling is crucial in determining whether a compound possesses the necessary physicochemical properties to qualify as a viable therapeutic agent. In this study, the pharmacokinetic properties and drug-likeness of the newly synthesized thiosemicarbazone

Table 7 Binding energies and inhibition constant of thiosemicarbazone compound with 1UWH

Compound	Protein	Mode	Binding Energy (kcal/mol)	Inhibition Constant (Ki,μM)
Pht	1UWH	1	− 6.85	9.50
		2	− 6.58	–
		3	− 6.00	39.98
		4	− 5.79	56.76
		5	− 5.54	–
		6	− 5.79	–
		7	− 5.58	–
		8	− 5.37	–
		9	− 5.40	110.65
		10	− 4.95	233.57

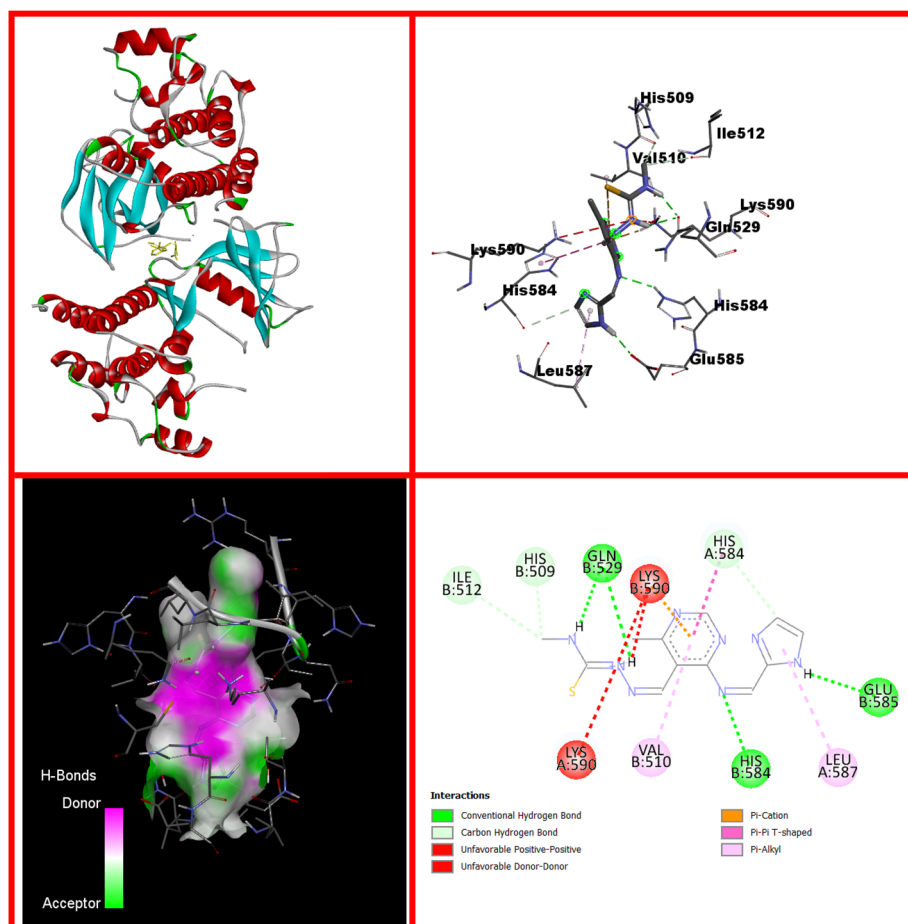


Fig. 9 Showing 3D and 2D interactions of the thiosemicarbazone compound and the standard inhibitor with the active site of BRAF (PDB ID: 1UWH)

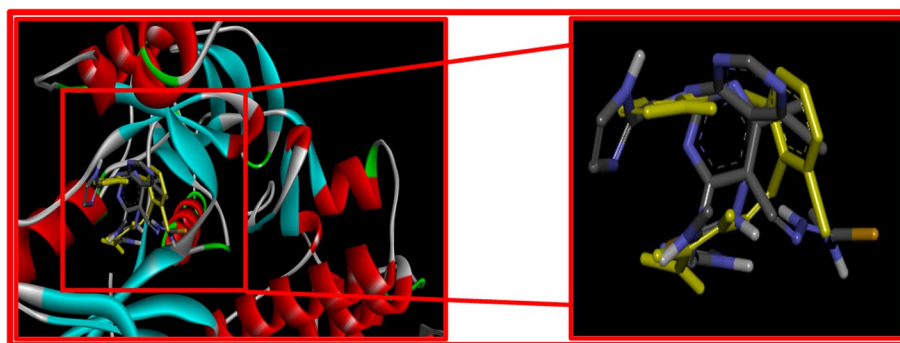


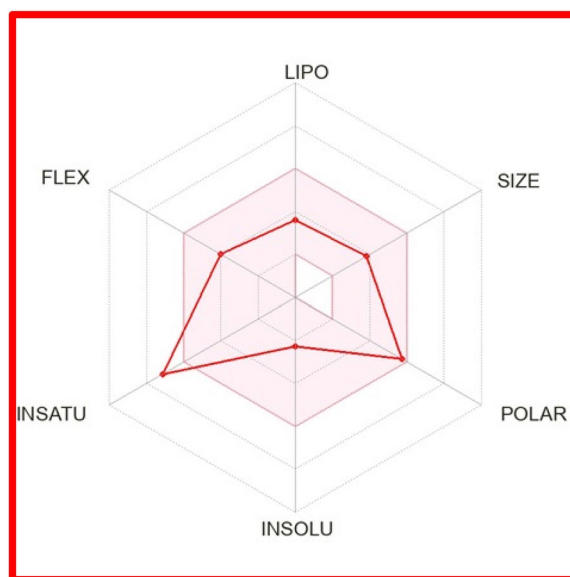
Fig. 10 Re-docking pose with the RMSD value of 2.630 Å

compound (Pht) were evaluated using the freely available SwissADME online tool [44]. The compound Pht was evaluated in light of Lipinski's rule of five [64], which outlines key physicochemical requirements for potential oral drugs. Notably, the ligand fulfilled all evaluated criteria, highlighting its potential as an orally bioavailable drug candidate. These results are summarized in Table 8. With a molecular weight of 309.78 g/mol, the compound is well within the acceptable range defined by Lipinski's rule of five (≤ 500 g/mol). Its Log P value of 3.07 reflects moderate lipophilicity, which is favorable for

Table 8 Selected physicochemical and pharmacokinetic properties of the thiosemicarbazone compound (Pht)

	Mw g/mol	Fraction csp ³	RB	HBA	HBD	MR	TPSA Å ²	Log P	GI absorption	BBB Perm.	Lip. V.	Bio. Sc.
Pht	309.78	0.18	6	4	3	80.86	122.97	3.07	High	No	Yes	0.55

Mw: Molecular Weight, MR: Molar Refractivity

**Fig. 11** Pink zoned oral bioavailability radars for thiosemicarbazone compound (Pht)

membrane permeability. Additionally, the ligand possesses four hydrogen bond acceptors (HBA) and three hydrogen bond donors (HBD), both comfortably within the recommended limits ($\text{HBA} \leq 10$, $\text{HBD} \leq 5$) [65]. The topological polar surface area (TPSA) of $122.97 \text{ \AA}^2$ indicates that the molecule is likely to possess good oral bioavailability, although values slightly exceeding $120 \text{ \AA}^2$ can occasionally limit absorption. Notably, the compound exhibits high gastrointestinal absorption, which compensates for its borderline TPSA. With six rotatable bonds, the ligand exhibits moderate molecular flexibility, which is well within the acceptable range for orally administered drugs (≤ 10). The molar refractivity (MR) of 80.86 further supports favorable electronic polarizability and molecular size. Importantly, the compound is not predicted to cross the blood-brain barrier, which may be advantageous in minimizing potential central nervous system side effects, depending on the therapeutic context. The ligand fully complies with Lipinski's rule of five and achieves a satisfactory bioavailability score (0.55), collectively supporting its potential as a promising candidate for further drug development.

The bioavailability profile of the thiosemicarbazone compound (Pht) is illustrated in Fig. 11 using radar plots shaded in pink, which represent key physicochemical parameters: lipophilicity (LIPO), molecular weight and size (SIZE), polarity (POLAR), solubility (INSOLU), degree of saturation (INSATU), and molecular flexibility (FLEX). This visualization provides a comprehensive overview of how these structural features impact the ligand's drug-likeness and absorption potential. The analysis shows that the red line closely aligns with the optimal (pink) area, indicating a well-balanced lipophilicity that favors membrane permeability. The molecular size is within the desirable range,

supporting favorable bioavailability. Polarity, which affects hydrogen bonding and solubility, is also within acceptable limits, though slightly low, which may suggest limited water solubility. The degree of unsaturation is optimal, enhancing metabolic stability. Additionally, the ligand exhibits suitable molecular flexibility, avoiding excessive flexibility that could compromise binding specificity, thereby supporting its potential for selective interaction with biological targets.

In the therapeutic management of non-small cell lung cancer (NSCLC), BRAF has emerged as a clinically actionable target within a molecularly defined subset. Building on experience in melanoma, selective BRAF inhibitors (BRAFi) and downstream MEK inhibitors (MEKi) have been translated into NSCLC, where combined dabrafenib plus trametinib constitutes a preferred first-line regimen for BRAF V600-mutant disease and yields meaningful response rates and progression-free survival, albeit with outcomes that remain inferior to those achieved for other oncogenic drivers [28]. As with other kinase-directed therapies, acquired resistance is common and is most frequently mediated by reactivation of the MAPK pathway through mechanisms such as BRAF amplification or splice variants, alterations in MEK1/2, upstream RAS/RTK activation, and adaptive rewiring. Moreover, first-generation, monomer-selective BRAFi display limited efficacy against non-V600 class II/III BRAF alterations and can induce paradoxical pathway activation in BRAF-wild-type contexts; next-generation pan-/dimer-selective RAF inhibitors and rational combinations (e.g., with MEKi or immune checkpoint inhibitors) are under active clinical investigation to broaden coverage and delay resistance. Against this backdrop, heterocyclic thiosemicarbazone scaffolds have attracted interest due to their rich coordination chemistry and multifunctional anticancer mechanisms. Incorporating pyrimidine and imidazole moieties into the thiosemicarbazone framework can enhance kinase engagement potential, optimize physicochemical properties, and improve cytotoxic selectivity across various cancer models. Pyrimidine-based thiosemicarbazones have been associated with stronger nucleic-acid interactions and inhibition of ribonucleotide reductase, whereas imidazole-containing analogs can modulate kinase activity and cellular redox balance, suggesting complementary modes of action [27, 29, 30, 66]. In this study, we leverage these features to pursue BRAF as the primary target, supported by structure-based design and docking, with the goal of achieving potent MAPK pathway suppression while maintaining selectivity. Further validation in biochemical BRAF enzymatic assays, pathway readouts (p-ERK), and kinome-wide profiling will be essential to substantiate on-target activity and therapeutic potential. It was rationally designed, synthesized, and evaluated a pyrimidine-imidazole thiosemicarbazone (Pht) as a candidate BRAF inhibitor relevant to NSCLC. The pyrimidine and imidazole rings were incorporated to enhance target engagement and pharmacological properties, consistent with reports that heterocyclic thiosemicarbazones outperform aliphatic analogs. DFT calculations yielded a HOMO–LUMO gap of 2.8 eV, and molecular electrostatic potential mapping revealed complementary nucleophilic and electrophilic regions, which are compatible with the occupancy of the BRAF ATP site. Docking to the sorafenib-bound BRAF structure (PDB 1UWH) predicted favorable binding for Pht, with a best score of -6.85 kcal/mol and an estimated micromolar affinity, accompanied by canonical hydrogen-bonding and hydrophobic interactions in the active site. Collectively, these findings provide a structure-based rationale for BRAF engagement while indicating the need for further affinity optimization.

While the present study provides a mechanistically grounded rationale for the BRAF-inhibitory potential of the Pht compound, several limitations warrant acknowledgment. The observed anticancer activity was assessed at relatively elevated concentrations under in vitro conditions, and the current investigation does not encompass direct kinase inhibition assays or in vivo validation. Consequently, future studies should prioritize systematic structural optimization to improve potency and selectivity, alongside rigorous evaluation in animal models, to comprehensively substantiate the compound's therapeutic efficacy and translational relevance.

4 Conclusions

In this study, a novel pyrimidine–imidazole-based thiosemicarbazone derivative (Pht) was successfully synthesized via a condensation reaction and thoroughly characterized using elemental analysis, FT-IR, ^1H -NMR, ^{13}C -NMR, and SEM-EDS techniques. DFT calculations at the B3LYP/6-311 + + G(d, p) level provided critical insights into the electronic structure, revealing a HOMO-LUMO gap of 2.8 eV and a global softness of 0.714 eV, which together confirm the molecule's favorable reactivity and structural stability. Molecular docking simulations against BRAF kinase (PDB ID: 1UWH) demonstrated a high binding affinity of -6.85 kcal/mol, stabilized by key hydrogen bonds with GLN529 and GLU585, along with favorable hydrophobic interactions. In vitro cytotoxicity assays against the H460 non-small cell lung cancer (NSCLC) cell line revealed the anticancer potential of the Pht compound, showing a dose-dependent reduction in cell viability, with a statistically significant inhibitory effect observed at 200 $\mu\text{g/mL}$. This cytotoxic activity, combined with its high binding affinity toward BRAF kinase, points toward its potential as a promising antiproliferative agent within the context of this preliminary study. Furthermore, in silico ADME profiling confirmed that the compound strictly adheres to Lipinski's rule of five and exhibits high gastrointestinal absorption, supporting its potential for oral administration. Collectively, these findings highlight the pyrimidine–imidazole thiosemicarbazone scaffold as a promising lead for the development of targeted BRAF inhibitors. Future investigations will focus on molecular dynamics simulations to assess long-term complex stability and structural optimizations to enhance the cytotoxic potency of the derivative for clinical translation.

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Author contributions

E.G. synthesized and characterized the compound. E.H.Ö. provided characterization, data analysis, and writing & editing support. D.N. provided supervision, project administration, funding acquisition for the chemical, and the overall study. Y.E. conducted all in silico analyses, including molecular docking, DFT, and ADME calculations. H.E.K. designed and executed the in vitro cytotoxicity studies. The manuscript was drafted and edited by D.N., H.E.K., E.H.Ö., and Y.E. All authors have read, reviewed, and approved the final version of the manuscript. All authors accept equal and full accountability for the accuracy and integrity of all aspects of the published work, regardless of their corresponding author status.

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Data availability

The data that support the findings of this study are available from the authors upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable for studies not involving humans or animals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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