Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 15, Issue 2, 2025, 23
https://doi.org/10.33263/BRIAC152.023

Functionalized Thioureas in Medicinal Chemistry: Binding Ability of Thiourea Derivatives to Biological Targets, Focused on Their Anticancer Activity

Yurii Zborovskii [1], Viktor Orysyk [1,*], Svitlana Orysyk [2], Myhailo Vovk [4]

[1] IOCh-NASU, Institute of Organic Chemistry of the National Academy of Science of Ukraine, Akademika Kuharya Str. 5, 02094 Kyiv, Ukraine; yu.zborovsky@gmail.com (Y.Z.);
[2] IGIC-NASU, V.I. Vernadsky Institute of General and Inorganic Chemistry of the National Academy of Science of Ukraine, Palladina Ave., 32/34, 03142 Kyiv, Ukraine; s.oryslend@gmail.com (S.O.);
* Correspondence: vis.viktorys@gmail.com;

Scopus Author ID 7801671933
Received: 11.11.2024; Accepted: 7.04.2025; Published: 14.04.2025

Abstract: Thiourea and its derivatives have been recognized for their broad chemical versatility and biological activity, which make them promising candidates for pharmaceutical applications, including cancer therapy. The thiourea scaffold contains three reactive centers: a thionic group and two amino groups capable of forming donor-acceptor bonds with metal cations and non-covalent bonds with various functional groups of organic compounds. The article reviews recent advancements in the design and synthesis of thiourea-based compounds, focusing on their interactions with key biological targets such as DNA, enzymes, and cell receptors through hydrogen bonds and π-π-interactions. Molecular docking studies, along with in vitro and in vivo tests, are discussed as tools for evaluating the antitumor potential of these compounds. Modified thiourea derivatives exhibit potent anticancer properties by inhibiting various enzymes involved in carcinogenesis. Their multi-targeted action makes them attractive candidates for overcoming challenges such as drug resistance and side effects associated with traditional cancer therapies. Thiourea derivatives hold great promise as next-generation anticancer agents. The review highlights the role of molecular modifications, including functional groups and lipophilic moieties, in improving their bioactivity, selectivity, and pharmacokinetic profiles. Further research is needed to optimize these compounds for clinical use.

Keywords: anticancer chemotherapeutics; non-covalent interaction; drug discovery; drug targets; enzyme inhibitors; molecular docking; thiourea.

1. Introduction

Thiourea and its derivatives represent an essential class of organic compounds with diverse chemical properties and broad applications in organic synthesis. These compounds are widely used as reagents in analytical chemistry, catalysts in organic reactions, and selective agents for metal cation separation in hydrometallurgical processes. In addition to these industrial uses, thioureas are also valued for their biological activities, positioning them as promising candidates for pharmaceutical and pesticide development. Their two amino groups enable thiourea derivatives to bind with a variety of organic and inorganic entities, such as carboxylic acid anions [1–4], carbonyl compounds [5–8], and inorganic anions like fluoride (F^-) [9-14], chloride (Cl^-) [15], cyanide (CN^-) [13,16,17], sulfate (SO_4^{2-}) [18–20], and dihydrogen phosphate ($H_2PO_4^-$) [9,10,14,21,22], through hydrogen bonding networks (Figure

1). This versatility makes them particularly attractive for the design of molecules that can interact with different biological targets.

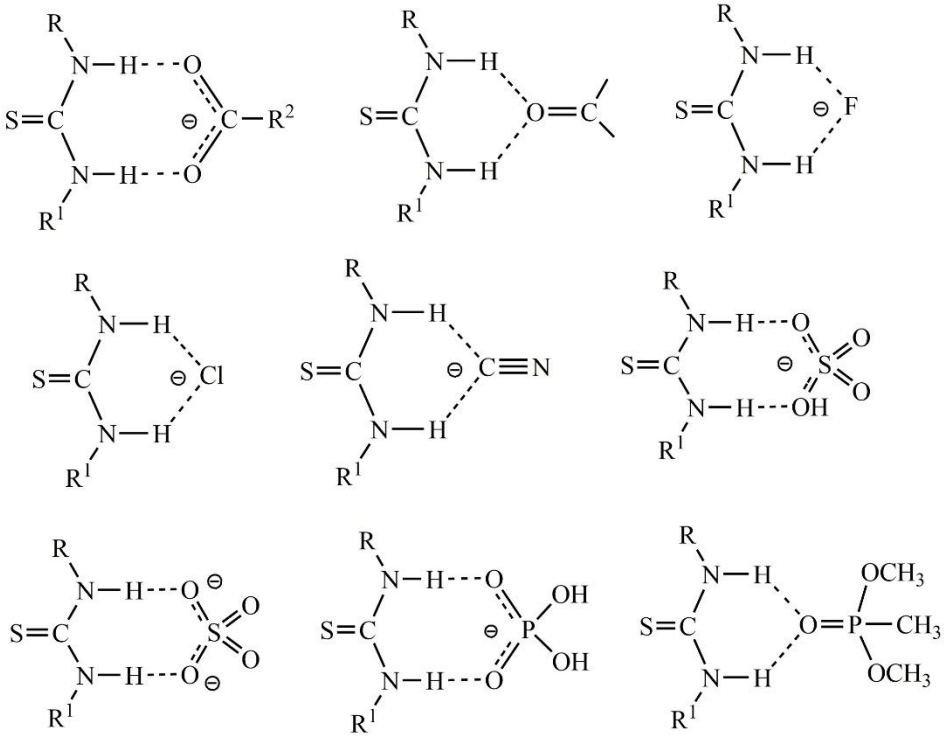

Figure 1. Hydrogen bonding interactions of thioureas with carboxylates, carbonyl compounds, chloride, fluoride, cyan, hydrogen sulfate, sulfate, dihydrogen phosphate anions, and dimethyl methylphosphonate.

The versatility of thiourea derivatives has been demonstrated in numerous experimental studies [2,3,9–17, 20] and two reviews [23,24], which have shown their potential as chemosensors for detecting organic and inorganic anions [13,14,16,17,25], as well as hazardous chemical substances in the environment, including chemical warfare agents [26–29]. Moreover, thioureas are effective catalysts for various organic reactions, including asymmetric catalysis, due to their binding ability to functional groups of organic compounds [30–41]. Thiourea's ability to form hydrogen or donor-acceptor bonds with biological targets –including enzymes, proteins, DNA, and cell receptors – alongside its capacity to transport anions through cell membranes explains its extensive biological activity. In addition, these properties make the thiourea moiety a key pharmacophore for designing promising drug prototypes [42–46].

Several drugs, including antituberculosis agents such as Thiocarlide and Thioacetazone [47,48], antiseptic Noxythiolin [49], and histamine receptor antagonists like Burimamide [50], Metiamide [51], and Thioperamide [52], incorporate thiourea into their structures. Additionally, thiourea derivatives are being studied in advanced research stages for potential anticancer activity, with compounds like Tenovin-1, Tenovin-6, SHetA2, and Triapine (3-AP) showing promise as novel cancer therapies (Figure 2) [53–58]. Despite being sulfur-containing analogs of urea as metabolites, only unsubstituted thiourea has been identified as a natural substance in the seeds of Laburnum anagyroides [59], and thioureide structures are also present in Zapotidine, a sulfur-containing alkaloid from *Casimiroa edulis* [60].

Beyond their significant anticancer properties, many thiourea derivatives display a wide spectrum of biological activities, including antiviral [61,62], antibacterial [63,64], antifungal [65], antiparasitic [66], insecticidal [67], antioxidant [68], herbicidal [69], and plant growth regulatory effects [70]. Their anticancer activity may result from their ability to inhibit various

enzymes, such as DNA topoisomerase-II [71], sirtuins [72,73], vascular endothelial growth factor receptor-2 (VEGFR-2), epidermal growth factor receptor (EGFR), and lysine-specific demethylase 1 (LSD1) [74–76].

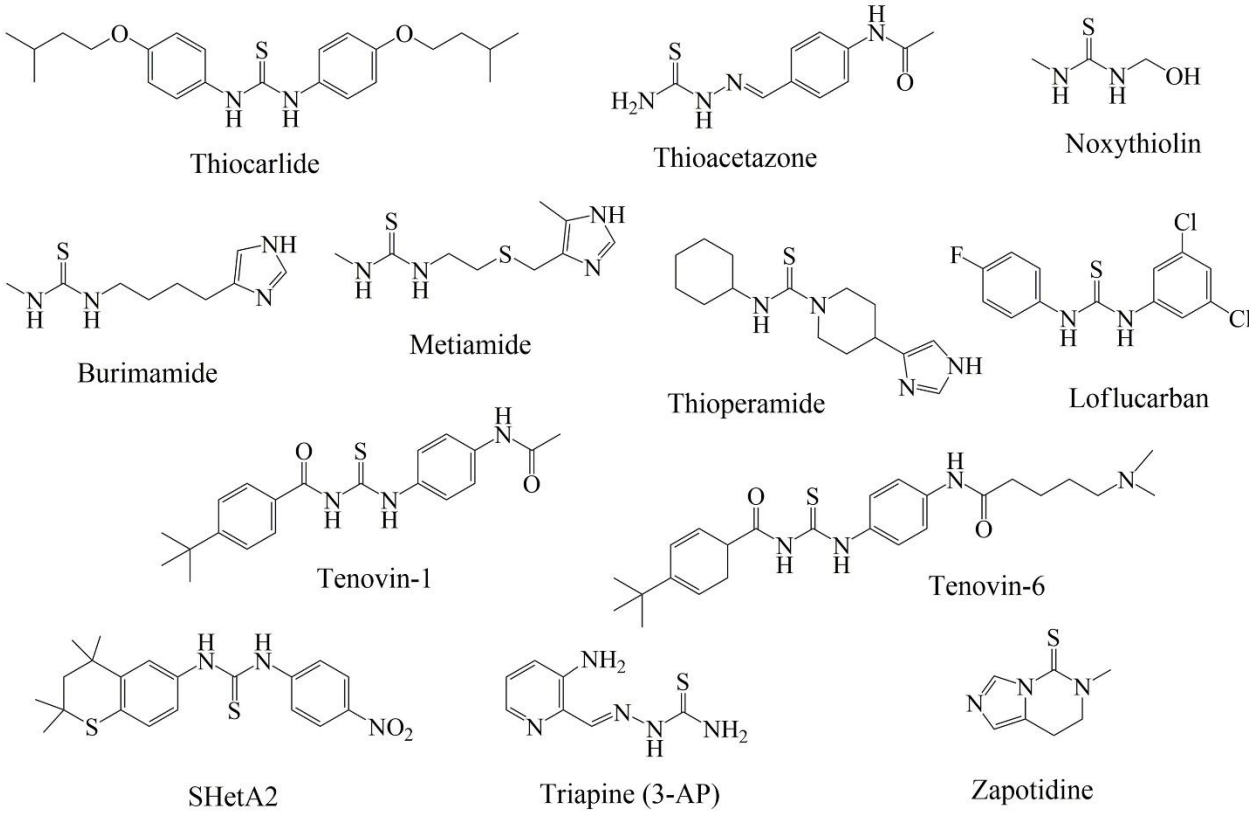

Figure 2. Chemical structures of promising medications and natural thiourea derivatives.

The last comprehensive review on thiourea derivatives as anticancer agents was published in 2015 [77], and since then, numerous studies have expanded our understanding of their potential in cancer therapy. Cancer remains one of the leading causes of death globally, second only to cardiovascular diseases [78, 79]. A critical challenge in cancer treatment is the cytotoxicity of many antiproliferative agents to normal cells, which increases the risk of secondary cancers. Moreover, the emergence of multidrug resistance (MDR) and severe side effects limit the effectiveness of many current therapies, making single-target treatments less effective or insufficient.

As a result, the development of multi-target anticancer agents has become increasingly important. One promising strategy involves molecular hybridization, which combines multiple pharmacophoric units in a single molecule. This approach has the potential to improve selectivity, reduce side effects, minimize drug resistance, and enhance treatment efficacy [80, 81]. This review focuses on recent advancements in the design of antitumor agents based on functionalized thioureas, highlighting their interactions with various biological targets and molecular docking results, as well as in vitro and in vivo studies over the past eight years.

Numerous experimental studies demonstrated that the antitumor activity of thiourea derivatives arises from their ability to target multiple pathways involved in carcinogenesis. The thiourea scaffold is a valuable building block for designing new drug candidates with diverse therapeutic applications. Researchers have modified the thiourea structure by adding functional groups and structural fragments to improve ligand binding to biological targets. Combined with high-throughput screening and molecular docking, this approach has developed novel and potent antitumor agents.

This review also highlights the ability of thiourea and its derivatives to form stable donor-acceptor or non-covalent bonds with important biological metabolites. This property underpins their high biological activity. By analyzing recent experimental studies, including in vitro and in vivo results, as well as molecular docking simulations, we examine how different substituents in thiourea derivatives influence their anticancer activity. Moreover, we emphasize the role of lipophilicity — an essential physicochemical property in drug design — in facilitating the penetration of compounds through cell membranes and their interaction with lipophilic binding pockets in enzymes, proteins, and other receptors. At the same time, we focus on the fact that incorporating lipophilic groups such as hydrocarbon substituents, aromatic and heteroaromatic rings, and benzyl or aroyl moieties significantly enhances the bioactivity of thiourea derivatives.

In conclusion, thiourea derivatives hold significant promise as multitarget anticancer agents. Their ability to form stable interactions with key biological molecules, along with structural modifications to enhance selectivity, reduce toxicity, and overcome drug resistance, positions them as leading candidates in the search for new and effective cancer therapies.

2. Materials and Methods

A comprehensive literature search was performed using the following databases: Reaxys, ScienceDirect, Web of Science, PubMed, Scopus, Google Scholar, and ScienceDirect for articles published mainly from 2014 to 2024. The literature search was done without author bias, and keywords were chosen.

The chemical structures of the identified compounds in this review were drawn using ChemDraw 12.0 software.

3. Results and Discussion

3.1. N-Aryl and N, N′-diaryl substituted thioures.

Recent investigations have demonstrated that *N*-aryl and *N*, *N*′-diarylthioureas are promising anticancer agents capable of effectively inhibiting tumor cell proliferation. El-Atawy *et al*. [82] synthesized two series of urea and thiourea derivatives as potential antitumor agents. *In vitro* testing of the synthesized compounds revealed moderate antitumor activity of *N*, *N*′-diphenylthioureas. Cytotoxicity results showed that compound **1** (Figure 3) was the most effective in suppressing human MCF-7 cancer cell growth with an IC_{50} value of 338 μM after a 24-hour incubation period, indicating very low or even lack of antiproliferative activity. This indicates that using diarylthiourea compound **1** is safe for living tissues.

It has been demonstrated that incorporating electron-withdrawing substituents, like nitro or trifluoromethyl groups, into the benzene rings of N, N′-diphenylthioureas increases the acidity of the NH groups. This change enhances their hydrogen bonding with anionic guests [5, 20], thereby boosting the biological activity of these thiourea derivatives [83]. Yuan Zhang's team [84] developed a series of novel N, N′-diarylsubstituted thioureas as strong inhibitors targeting the mutant K-Ras protein, a critical regulator of cell proliferation, differentiation, and tumor survival. Biological tests showed that 1,3-bis(4-(trifluoromethyl)phenyl)thiourea (**2**) was the most effective compound, significantly reducing the proliferation of the A549 lung cancer cell line with an IC_{50} value of 0.2 μM. In comparison, the corresponding urea derivative **3** displayed much lower activity, with an IC_{50} of 22.8 μM. Docking studies indicated that this difference may stem from compound **2**'s strong binding

affinity for the hydrophobic pocket of K-Ras and its ability to form a hydrogen bond with the Glu37 residue (Figure 3).

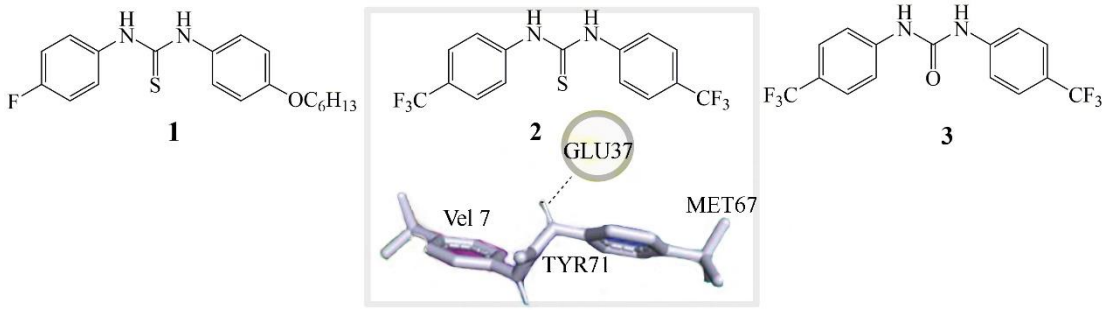

Figure 3. Line drawings of compounds **1-3** and moiety of molecular docking of compound **2** and K-RasG12V protein.

In an effort to develop new antitumor agents, Bielenica *et al*. [85] synthesized a series of 3-(trifluoromethyl)phenylthiourea derivatives (**4a-l**, Figure 4). Their cytotoxicity was evaluated *in vitro* against a panel of cell lines.

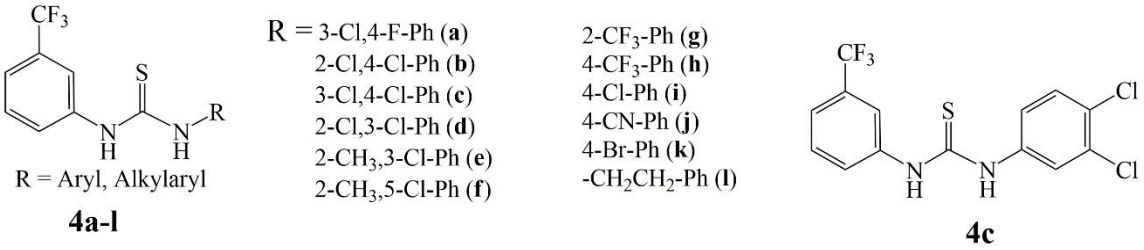

Figure 4. Line drawings of compounds **1-4**.

Among the compounds evaluated, 1-(3,4-dichlorophenyl)-3-[3-(trifluoromethyl)phenyl]thiourea (**4c**) was identified as the most promising, showing the strongest cytotoxic effect with IC_{50} values of 9.0, 1.5, and 6.3 µM against human primary colon cancer (SW480), metastatic colon cancer (SW620), and chronic myelogenous leukemia (K562) cell lines, respectively. Remarkably, it also exhibited favorable selectivity compared to the normal human keratinocyte cell line from adult skin (HaCaT), with an IC_{50} of 24.7 µM [85]. This study highlighted that the most effective thiourea derivatives inhibit interleukin-6, a cytokine known to drive inflammatory and autoimmune responses in diseases, including pancreas, prostate, and colon cancers.

Introducing electron-withdrawing substituents like 4-nitrophenyl or perfluorophenyl into the thiourea structure further increases the NH groups' acidity, facilitating their interactions with acceptors by hydrogen bond and enhancing the biological activity of these derivatives. H. Zou and colleagues [86] demonstrated that 1-(4-chloro-3-methylphenyl)-3-(4-nitrophenyl)thiourea (**5**) suppresses the growth of eight distinct breast cancer cell lines with IC_{50} values between 2.2 and 5.5 µM (Figure 5).

Liu *et al*. [87] designed and synthesized a series of *p*-nitrodiaryl-substituted thioureas **6-15** as analogs of SHetA2 (Figure 5), which is known to be an effective antitumor agent that functions without retinoid receptor activation, consequently avoiding retinoid toxicity when tested in animal models.

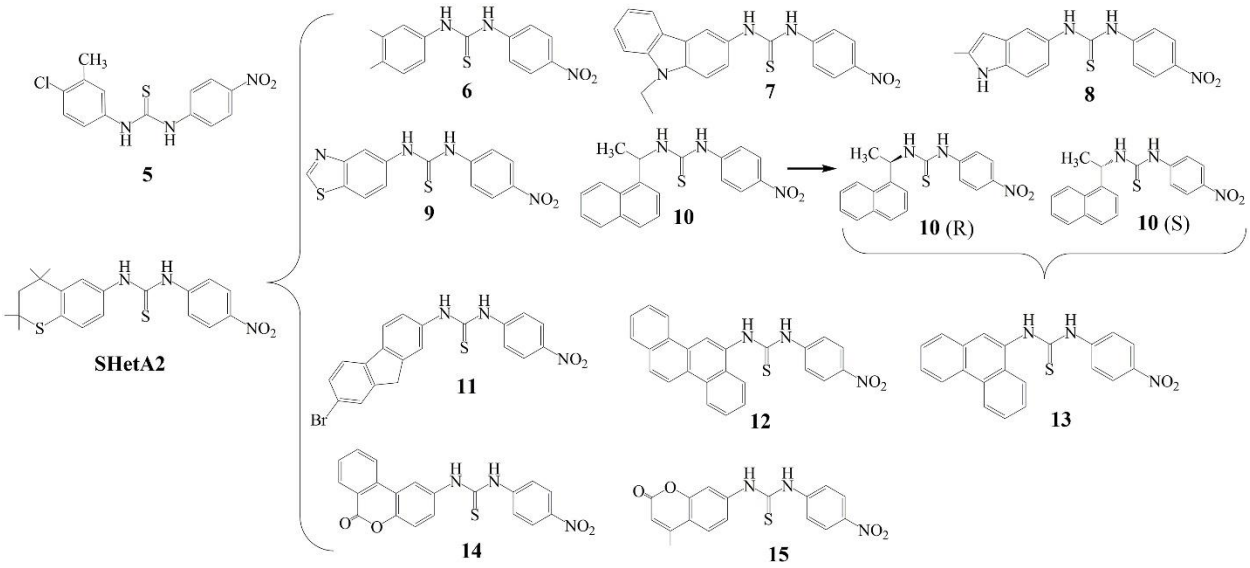

Figure 5. Line drawings of SHetA2 and compounds **5-15**.

SHetA2 also exerts selective anticancer activity by regulating apoptosis, cell growth, differentiation, and angiogenesis. However, it has two main drawbacks: excessive lipophilicity and a complex, six-step synthesis. To address these issues, the authors modified the SHetA2 structure, drawing on prior structure-activity relationships. They substituted the thiochromane ring with an alternative ring structure that adhered to Lipinski's rule, retained drug-like characteristics, and avoided producing reactive metabolites. Compounds **6-15** generally resemble SHetA2 but vary in size and shape. These new compounds were evaluated for their capacity to inhibit the growth of breast cancer cell lines (MCF-7, T-47D, and MDA-MB-453) and prostate cancer cell lines (DU-145, LNCaP, and PC-3). Compounds **10** and **12** proved most effective in this series, with GI_{50} values as follows: 6.2/3.2 µM (MCF-7); 2.9/2.5 µM (T-47D); 6.3/4.8 µM (MDA-MB-453); 13.7/21.8 µM (DU-145); 13.9/20.0 µM (PC-3); 6.1/3.5 µM (LNCaP).

Ginn *et al*. [88] synthesized 1-(1-(naphthalen-1-yl)ethyl)-3-(4-nitrophenyl)thiourea (**10**) in two enantiomeric forms, **10(R)** and **10(S)**, to evaluate their distinct anticancer properties (Fig. 5). The findings reveal that the **10(S)** enantiomer exhibits a stronger growth inhibitory effect (IC_{50} 3.0–5.5 µM) compared to the **10(R)** enantiomer (IC_{50} 5–13 µM) against MCF7, T47D, and MDA-MB-453 breast cancer cells. The authors attribute this increased activity of the **S** enantiomer to its capacity to arrest cell cycle progression by suppressing the expression of key cell cycle regulators.

N. Arshad's team synthesized a new adamantane-naphthyl thiourea conjugate, 1-(adamantane-1-carbonyl)-3-(1-naphthyl) thiourea (compound **16**), as a potential anticancer agent [89]. The authors used molecular docking to explore the molecular mechanism and strength of **16**'s interactions with DNA, discovering that it interacts spontaneously through a mixed binding mode. The planar naphthyl group intercalates between DNA base pairs DA (A6) and DC (B23), while the asymmetric structure shifts to interact with the minor grooves of DNA around base pairs DA (A5) and DG (B24) (Figure 6a). Hydrophobic interactions between 16 and DNA are depicted with dotted lines in Figure 6a. Molecular docking also simulated potential interactions of this thiourea with the enzyme urease, showing that compound **16** fits well into the urease binding pocket, where it establishes interactions and inhibits the enzyme's active site. The Ligand-protein interaction diagrams (Lig plot) present two types of interactions in the urease pocket: (1) hydrophobic contacts between **16** and urease's amino acid residues

and (2) arene-arene interactions between Methionine (Met 475) and the aromatic ring of **16** (Figure 6b).

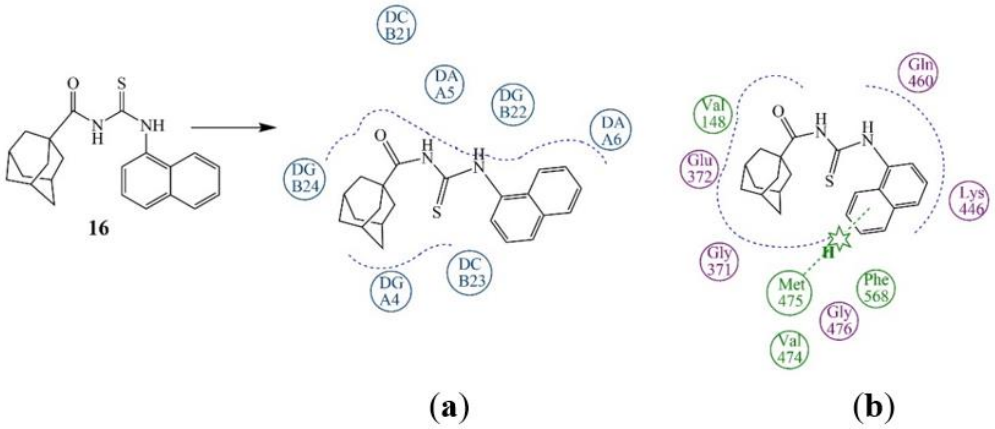

Figure 6. Line drawing of compound **16** and its interaction with residues of urease enzyme according to Lig plot calculated at PM3 semi-empirical level of theory.

Further evaluation of compound **16** was conducted on the hepatocellular carcinoma cell line (Huh-7) and normal human embryonic kidney cells (HEK-293). Compound **16** demonstrated considerable cytotoxicity against Huh-7 cells compared to normal HEK-293 cells, which may indicate selective cytotoxicity toward cancer cells [89], despite the fact that the selective index of this compound was not determined by the authors.

In a recent study by Yeşilkaynak *et al*. [90], two N-allylthiourea derivatives, **17** and **18**, were tested *in vitro* for their anticancer activity against MCF-7 breast cancer cells. *N*-(Allylcarbamothioyl)-2-chlorobenzamide (**17**) demonstrated superior cytotoxic activity (IC_{50} of 2.6 µM) compared to N-(allylcarbamothioyl)-2-methylbenzamide (**18**) (IC_{50} of 7 µM). Molecular docking experiments were carried out to assess the interactions between the synthesized compounds and BRAF (V600E) protein kinase. The results indicated that the compounds demonstrated a strong binding affinity and an inhibitory effect on BRAF (V600E) protein kinase. BRAF, a serine-threonine RAF kinase family member, is part of the RAS-RAF-MAPK signaling pathway, which is critical for cell survival, proliferation, and differentiation. Activating mutations in BRAF cause unchecked stimulation of the MEK-ERK pathway, leading to neoplastic transformation.

The study confirmed that both compounds formed stable complexes through strong interactions at the target protein's active site. Analysis of the compounds' interactions within the active pocket of BRAF showed that hydrophobic interactions mainly facilitated binding. Both compounds interacted with key BRAF residues — PHE595, ALA481, THR529, and CYS532 — essential for stable binding to the ATP site (Figure 7). Since ATP's adenine binds here via Van der Waals forces, the excellent compatibility of these compounds within the hydrophobic pocket (CYS532and ALA481) that binds to ATP improves the binding affinity of the compounds.

Thus, the antitumor activity of aryl-substituted thioureas can be linked to their hydrophobic nature and capacity to participate in π-π interactions with proteins. Furthermore, the aryl substituent affects thioureas' lipophilicity and membrane permeability, while its planar structure facilitates DNA intercalation or binding to specific proteins.

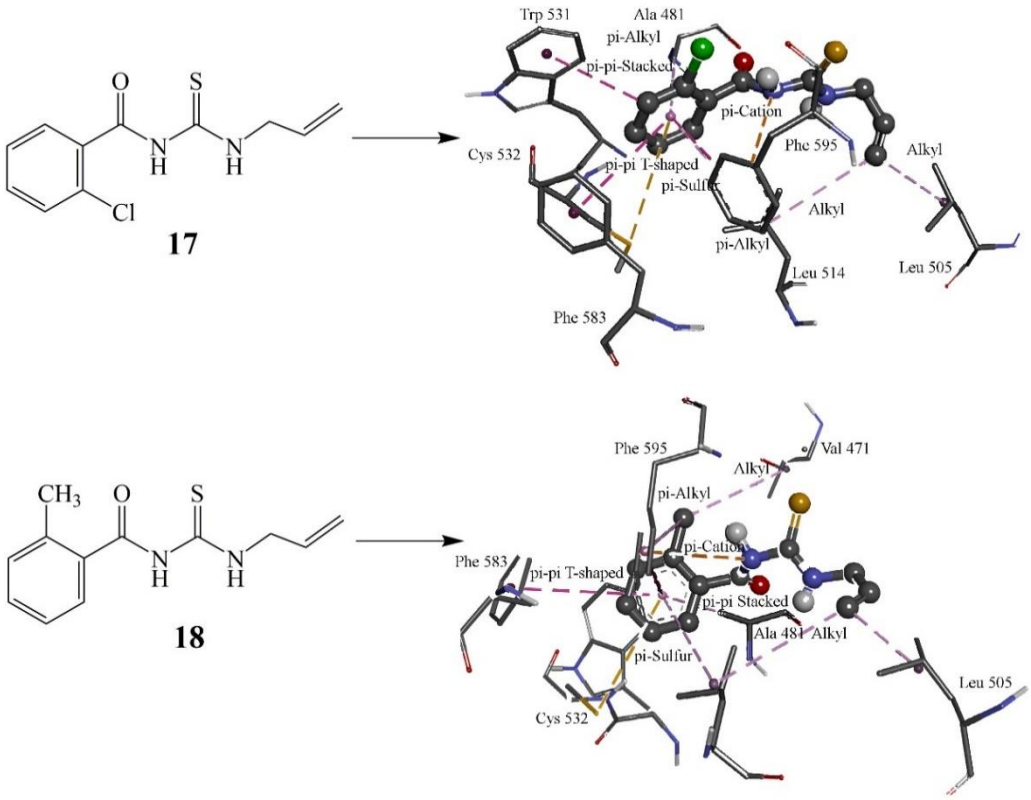

Figure 7. N-allythioureas **17** and **18** and their interactions with BRAF.

Studies on *N*-aryl and *N,N'*-diaryl substituted thioureas highlight their considerable potential as anticancer agents. Their effectiveness is attributed to their interactions with key proteins and DNA *via* hydrophobic and π-π interactions, along with their ability to impact critical signaling pathways like RAS-RAF-MAPK. Structural modifications, especially the addition of electron-withdrawing substituents, enhance these compounds' biological activity and specificity toward cancer cells, positioning them as promising candidates for further development as anticancer drugs. To advance these compounds from laboratory studies to clinical use, extensive preclinical studies are necessary, including detailed toxicity assessments and investigations of their mechanisms of action. Additionally, exploring these compounds in combination therapies could open new pathways to overcoming resistance and improving outcomes in cancer treatment.

3.2. N-Aryl-N'-heterocyclic substituted thioureas.

Introducing a heterocyclic substituent into the structure of thioureas enhances their specificity in interacting with target proteins. Additionally, the heterocyclic substituent affects the electron density distribution in the molecule and can participate in additional hydrogen bonds with biological targets.

S.A. Elseginy and co-workers [91] have designed and synthesized a novel series of 1-aryl-3-(pyridin-2-yl) substituted urea and thiourea derivatives as potent anticancer agents (Figure. 8). Structural modification and subsequent testing revealed that urea derivatives are less active compared to their corresponding thiourea derivatives (compounds **19-21**). *In vitro* evaluation demonstrated that thiourea derivative **20** exhibited the highest antitumor activity, with IC_{50} values of 1.3 and 0.7 µM against MCF-7 and SkBR3 breast cancer cells, respectively.

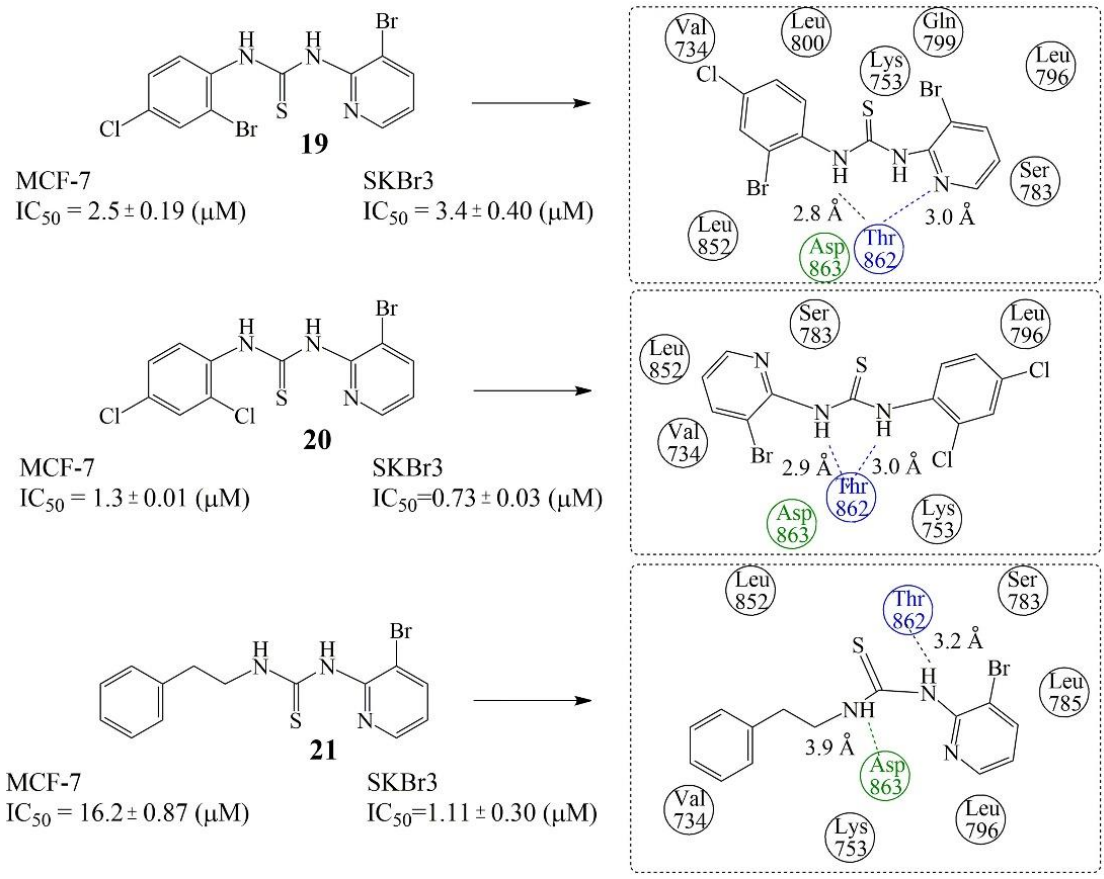

Figure 8. Line drawings of compounds **19-21** and their interaction with HER2 protein.

The findings indicate that the potent activity of these thiourea derivatives is attributed to their ability to inhibit the human epidermal growth factor receptor (HER2), a key family of multidomain proteins involved in the progression of various cancers. The activity of compounds **19-21** is linked to their capacity to form hydrogen bonds with the critical residue Thr862, as well as an additional hydrogen bond with Asp863 (for compound **21**) (Figure 8). These compounds also engage in π-π T-shaped interactions with Phe864 and hydrophobic interactions with several important hydrophobic residues, including Leu726, Val734, Ala751, Lys753, Leu785, Leu796, Leu800, and Leu852. These observations suggest that the short linker and the presence of the bromopyridine group, along with the 2,4-substituted phenyl rings, are crucial for their anticancer efficacy.

Ragab *et al*. [92] synthesized a series of 1,3,4-thiadiazinethiourea derivatives and evaluated their *in vitro* cytotoxic activity against the A549 lung cancer cell line using the MTT bioassay. Compounds **22**, **23**, and **24** demonstrated the highest cytotoxic activity, with IC_{50} values of 0.27, 0.30, and 0.32 μM, respectively, compared to Sorafenib (IC_{50} 3.85 μM) (Figure 9). These compounds were selected for further testing against key biological targets involved in tumor cell survival and proliferation, including vascular endothelial growth factor receptor 2 (VEGFR2), B-RAF, and matrix metalloproteinase 9 (MMP9). All three compounds showed promising inhibitory activity. Compound **24**, in particular, demonstrated potent VEGFR2 inhibition (IC_{50} 0.11 μM), similar to Sorafenib, through hydrogen bonding with two key amino acids in the active site (Figure 9a). The thiourea N-H group forms an H-bond with Asp1046 (2.7 Å), and the aniline N-H group interacts with Glu885 (2.6 Å) in the αC helix. Additionally, the chlorophenyl group fits into a hydrophobic pocket formed by Ile888, Leu889, Val899, Cys1024, and Ile1025 [92]. Compound **24** also exhibited strong inhibition of B-RAF (IC_{50} 0.178 μM) and MMP9 (IC_{50} 0.08 μM) (Figure 9b). B-RAF inhibition is attributed to π-π

interactions between the phenyl group and His574, while MMP9 inhibition results from hydrogen bonding with Glu402 and coordination of the catalytic zinc ion by His401, His405, and His411, with the thiadiazine nitrogen atoms completing the coordination (Figure 9c).

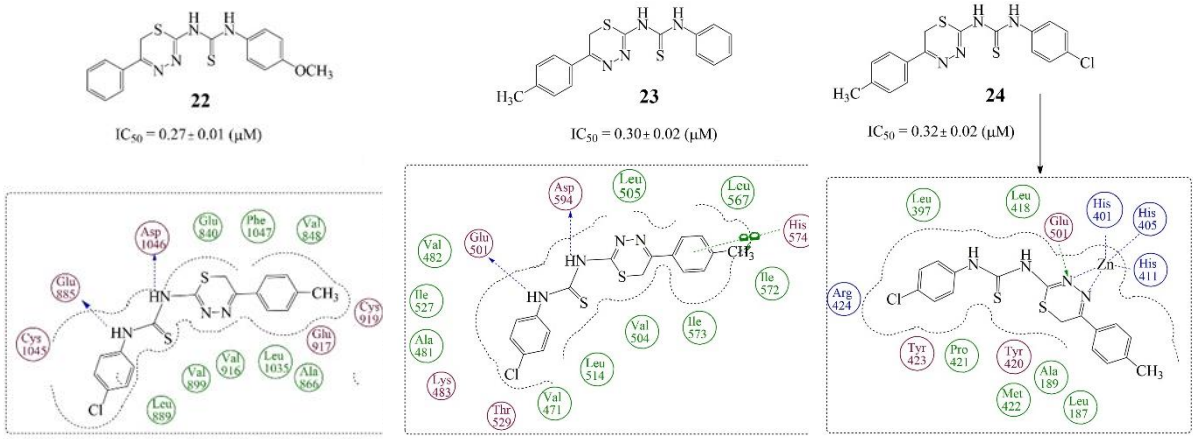

Figure 9. Line drawings of compounds **22-24** and the best docking pose of **24** in the active site of (**a**) VEGFR-2; **(b)** B-RAF; **(c)** MMP9.

In general, compounds **22-24** exhibited significantly higher selectivity for A549 cancer cells compared to the normal human fetal lung fibroblast cell line WI-38, with higher selectivity indices compared to Sorafenib (**22**: IC_{50} 137 μM, SI = 507; **23**: IC_{50} 89 μM, SI = 297; **24**: IC_{50} 80 μM, SI = 249; Sorafenib: IC_{50} 30 μM, SI = 8). Thus, compounds **22-24**, especially **24**, are promising anticancer agents that warrant further preclinical and clinical trials.

Among receptor protein tyrosine kinases (RPTKs), the epidermal growth factor receptor (EGFR) plays a crucial role in cancer progression, and its dysregulation leads to the development of various cancers. Therefore, designing selective HER-2 inhibitors with minimal toxicity is essential. Yang *et al*. [93] synthesized a series of dioxin-containing pyrazoline derivatives with thiourea scaffolds **I** and **II** (Figure 10) to enhance HER-2 inhibition while minimizing EGFR activity. The introduction of the oxygen heterocyclic ring was based on its reported ability to inhibit HER-2.

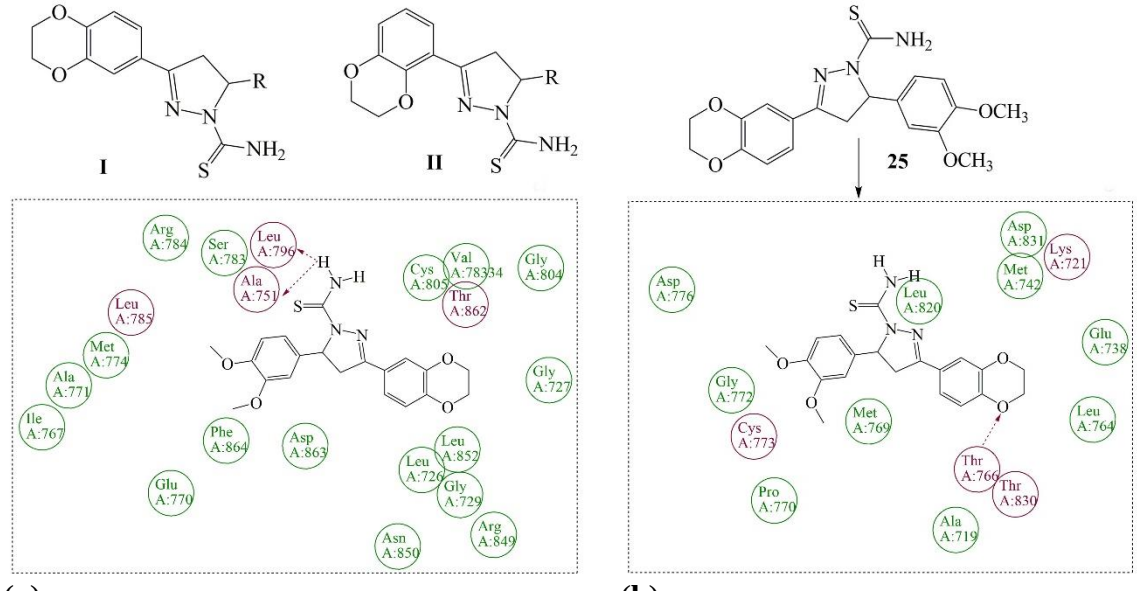

Figure 10. Compounds **25 I, II** and their binding with corresponding targets: **(a)** 3PP0; (b) 1M17.

Compound **25** (3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide) exhibited potent HER-2 inhibition (IC_{50} 0.03 μM) and demonstrated activity against the MDA-MB-453 breast cancer cell line (GI_{50} 0.15 μM). Its potency was comparable to Lapatinib (IC_{50} 0.01 μM, GI_{50} 0.03 μM) and slightly more effective than Erlotinib (IC_{50} 0.16 μM, GI_{50} 1.56 μM). Molecular docking studies revealed that the compound binds to key residues in HER-2 (25 with 3PP0 and 1M17) through hydrogen bonds formed by the carbothioamide group with ALA751 (N-H…O: 2.235 Å) and LEU796 (N-H…O: 2.242 Å), likely explaining its high activity (Figure 10a).

These findings suggest that the interactions between both rings of compound **25** and surrounding residues contribute to its strong binding to HER-2. Additionally, compound **25** binds to 1M17 *via* a hydrogen bond between the dioxin ring and THR766 (O…H-O: 2.109 Å), indicating its selectivity for HER-2 over EGFR (Figure 10b). Notably, compound **25** showed over 900 times greater potency for HER-2 compared to EGFR, with IC_{50} values of 0.19 μM for Erlotinib and 1.00 μM for Lapatinib.

In conclusion, introducing heterocyclic substituents into thiourea derivatives significantly enhances their specificity and interactions with target proteins, making them potent anticancer agents. Thiourea compounds containing pyridine or thiadiazine rings show strong anticancer activity, often surpassing traditional drugs like Sorafenib in potency and selectivity. These compounds effectively interact with key cancer-related targets such as HER2, VEGFR2, and B-RAF, modulating their pathways through hydrogen bonding and hydrophobic interactions with a favorable safety profile for further development.

3.3. Hybrid thiourea derivatives.

D.R. Parmar and colleagues [74] applied molecular hybridization to create potent antitumor agents by combining pharmacophore elements from clinically used drugs like Sorafenib and Sunitinib with thiourea. This strategy aimed to improve HER-2 inhibition while minimizing EGFR activity. The design included: 1) a planar heteroaromatic ring with a hydrogen bond acceptor (preferably nitrogen) for interactions with Cys917 in the ATP-binding site (Figure 11) [74]; 2) a central aromatic ring as a spacer between the ATP-binding and DFG domains; 3) a pharmacophore with both hydrogen bond donor and acceptor groups (e.g., amide or urea), interacting with Glu883 and Asp1044 in the DFG motif; 4) a hydrophobic group filling the allosteric pocket. Using this approach, they synthesized several potent VEGFR-2 inhibitors based on 3-(4-methoxyphenyl)azetidine-containing thiourea derivatives **26** and **27** (Figure 11), tested against human cancer cell lines. The synthesized compounds were then assessed for their in vitro inhibitory activity against a range of human cancer cell lines.

Among the compounds synthesized, 3-(4-methoxy-3-(2-methoxypyridin-4-yl)phenyl)-N-(4-methoxyphenyl)azetidine-1-carbothioamide (**27c**) emerged as the most potent inhibitor against prostate PC3, U251, skin A431, and kidney 786-O cancer cell lines, with EC_{50} values of 0.25, 0.60, 0.03, and 0.03 μM, respectively, surpassing Doxorubicin in potency. It bound strongly to VEGFR-2, with a ΔG of -22.84 kcal/mol, forming hydrogen bonds with Glu883 and Asp1044. The linker formed hydrophobic interactions with key residues, and the 2-methoxypyridine moiety occupied the hinge region (Figure 12).

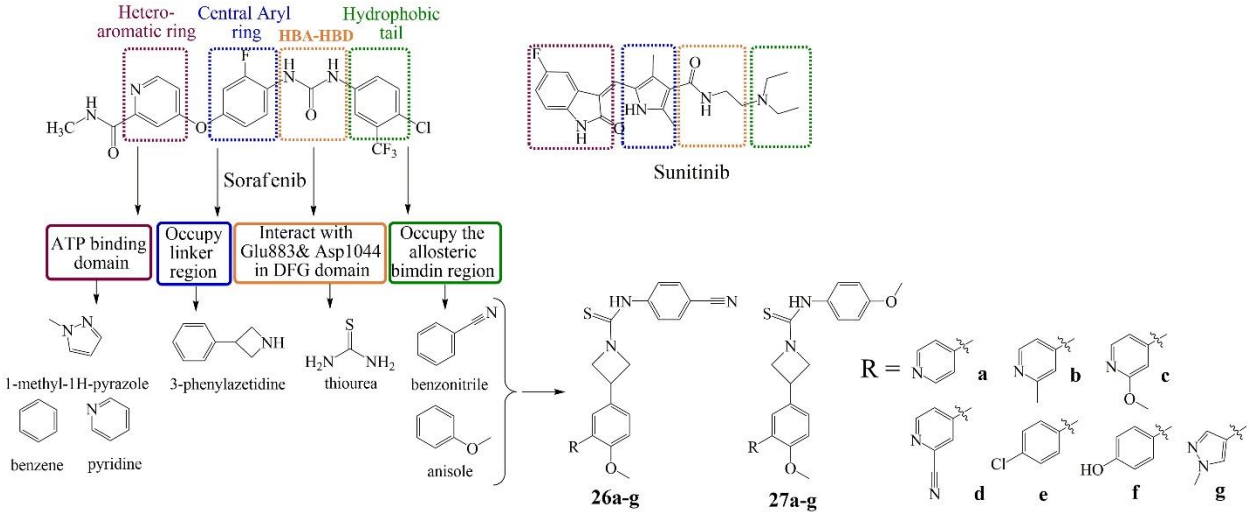

Figure 11. Schematic rationale for the design of 3-(4-methoxyphenyl)azetidine-containing thiourea derivatives **26**, **27**.

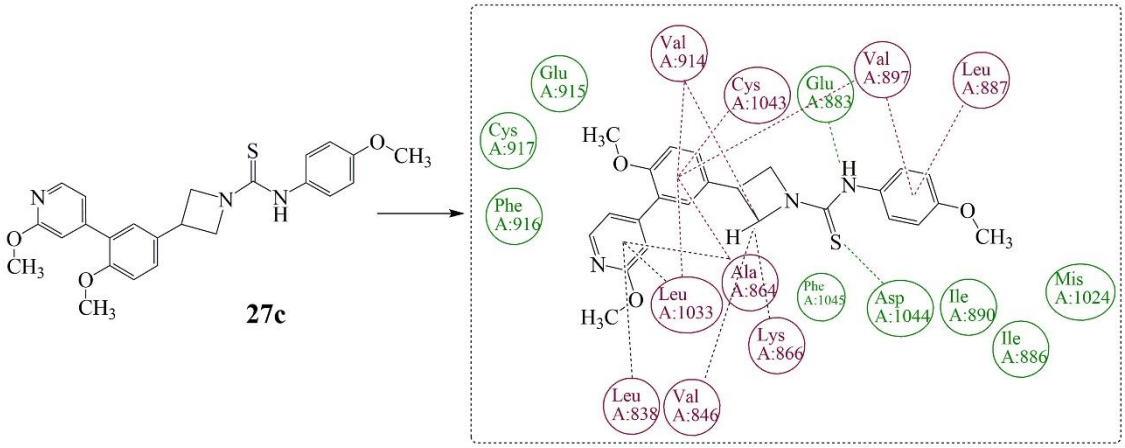

Figure 12. 3-(4-Methoxy-3-(2-methoxypyridin-4-yl)phenyl)-*N*-(4-methoxyphenyl)-azetidine-1-carbothioamide docked into the active site VEGFR-2.

In a related study, V.R. Solomon and colleagues [94] used molecular hybridization to combine Chloroquine (CQ), an antimalarial drug, with a piperazine ring, resulting in several 4-aminoquinoline derivatives (Figure 13, compound **A**). These analogs exhibited strong cytotoxicity against breast cancer cells, selectively targeting cancer cells. The authors further enhanced their anticancer activity by adding an isatin moiety to the 4-piperazinylquinoline ring, creating more potent analogs (compounds **B** and **C**).

They then merged the 4-piperazinylquinoline fragment with urea and thiourea pharmacophores (compounds **D**, **E**), which are known inhibitors of human DNA topoisomerase (Figure 13). These compounds showed greater antiproliferative activity than urea derivatives. Among them, 4-(7-chloroquinolin-4-yl)-N-(2-morpholinoethyl)piperazine-1-carbothioamide (**28**) demonstrated the most potent effects, with GI_{50} values of 3.0, 4.6, and 4.5 μM against MDA-MB231, MDA-MB468, and MCF7 breast cancer cell lines, respectively. The anticancer activity of this compound was 7–11 times higher in cancer cells than in normal cells, suggesting it could be a promising lead for breast cancer therapy.

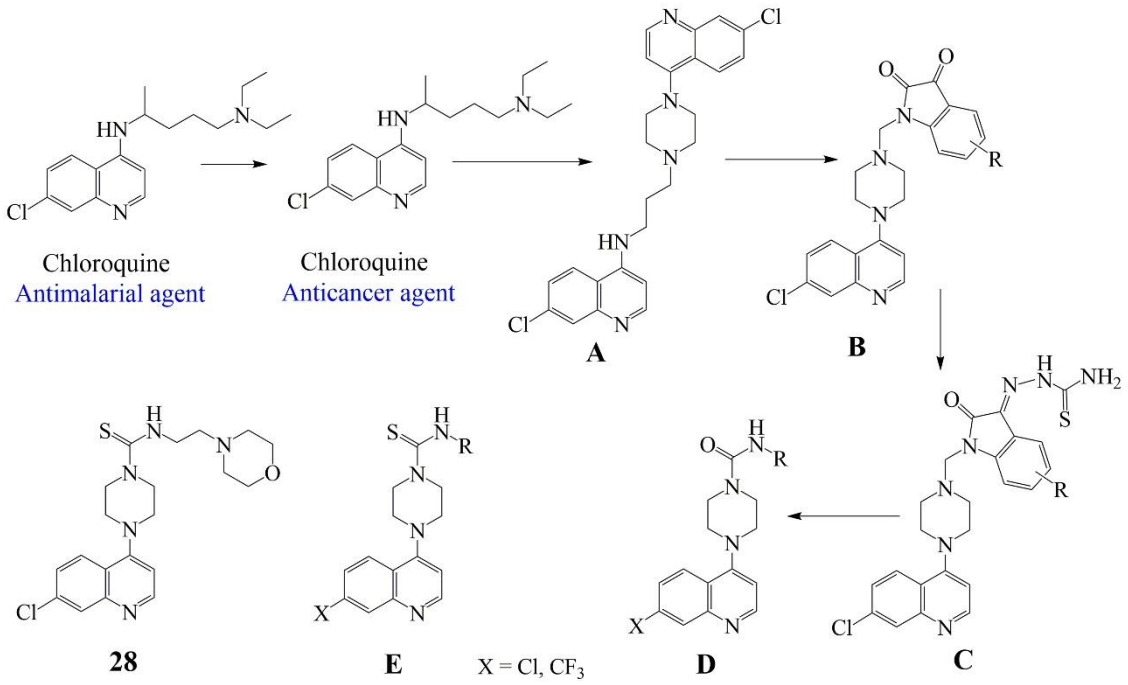

Figure 13. Line drawings of 4-aminoquinolines, obtained by a pharmacophore hybridization approach.

Thus, the bioactivity of thiourea derivatives is linked to three key features: nitrogen as a hydrogen-bond donor, sulfur for complementary binding, and additional substituents that enhance binding. The sulfur- and nitrogen-containing core structure allows the formation of both hydrogen bond acceptors and donors, promoting interactions with various enzymes. While thiourea derivatives can lack selectivity and act as PAINS (pan-assay interference compounds), this is not always the case, and some may show targeted activity.

Future research should focus on optimizing the structural components of hybrid thiourea derivatives to enhance their specificity and efficacy. Investigations into different heterocyclic fragments and their effects on biological activity could lead to the development of even more effective agents. Additionally, exploring these compounds' pharmacokinetics, toxicity profiles, and potential resistance mechanisms will be crucial for their successful clinical translation. Combining these hybrids with other therapeutic modalities may improve their therapeutic outcomes and provide novel strategies for overcoming cancer resistance. This chapter also demonstrated that molecular hybridization, which consists of combining two or more pharmacophoric moieties in a single molecule to obtain the synergistic effect or to obtain antitumor agents that have a novel mode of action, has become one of the most important and successful strategies applied to the design of new drugs.

*3.4. Hybrids benzo[*d*][1,3]dioxol-5-yl thiourea derivatives.*

The results presented above emphasize that combining two or more pharmacophoric groups into one molecule is a promising strategy for drug discovery, potentially leading to synergistic effects and the development of antitumor agents with novel mechanisms. S.Y. Abbas and colleagues [75] applied this approach by integrating thiourea and benzodioxole moieties into a single molecule, creating a series of 1,2-disubstituted thioureas (**29-31**) as potential antitumor agents (Figure 14). Most of these compounds showed superior cytotoxicity compared to doxorubicin and inhibited the epidermal growth factor receptor (EGFR), a key protein in the development of solid tumors. Molecular docking studies revealed that compounds **29-31** bind to EGFR in a manner similar to Erlotinib, with binding affinities of -

21.96, -24.99, and -19.70 kcal/mol, respectively. The benzodioxole core occupies a hydrophobic pocket, interacting with Val702, Ala719, and Leu820, which enhances their binding profile.

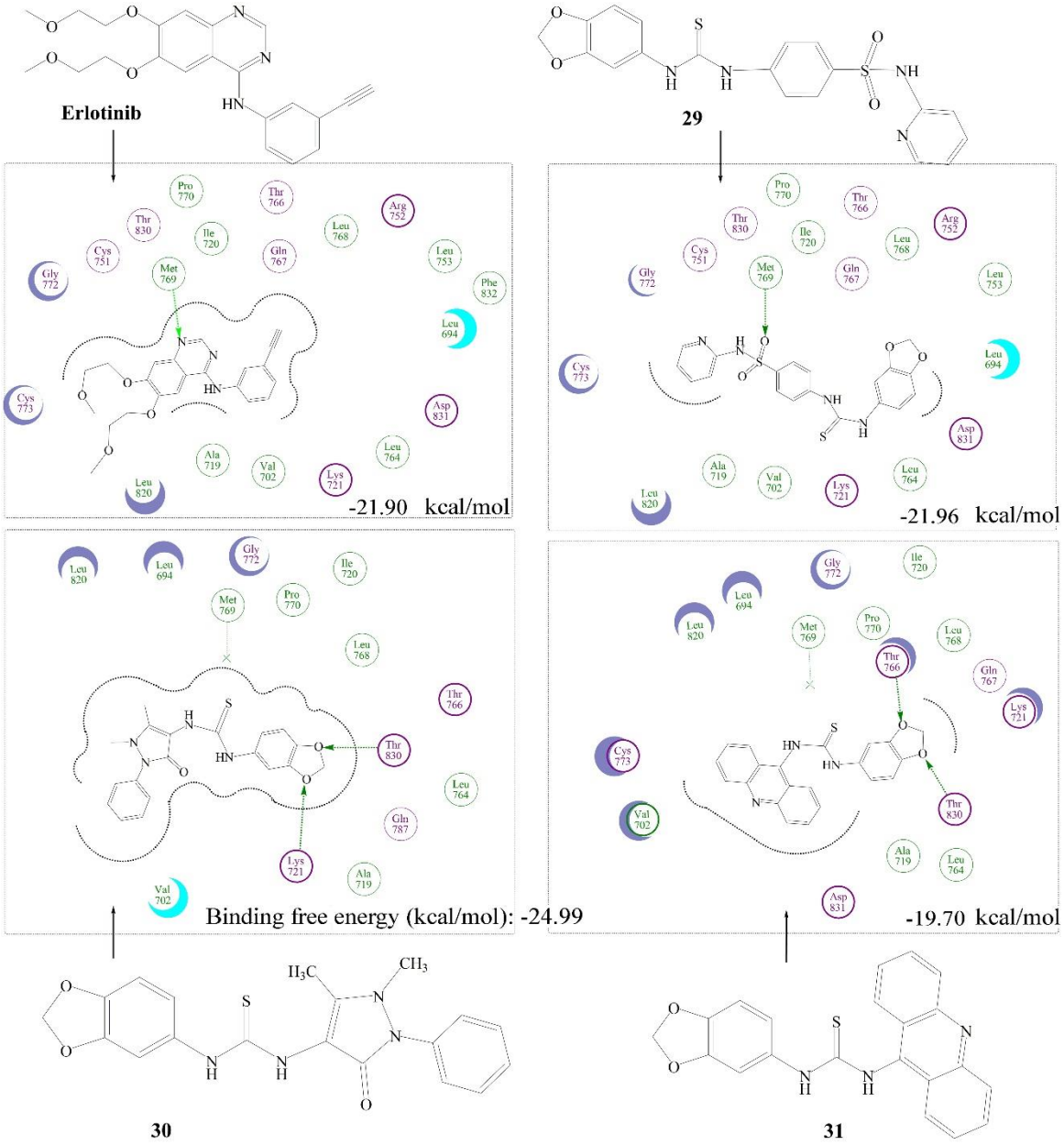

Figure 14. Erlotinib and 1,2-disubstituted thioureas **29-31**, and their interaction with EGFR.

Further studies showed that these compounds were non-toxic to normal human WI-38 cells and exhibited superior cytotoxic activity against HCT116, HepG2, and MCF7 cancer cell lines compared to doxorubicin. For example, compounds **29-31** showed antiproliferative activities with IC_{50} values of 4.78/3.16/1.71, 7.03/3.79/2.14, and 13.41/16.96/4.63 μM/L, respectively, whereas doxorubicin, lapatinib, and erlotinib showed higher IC_{50} values. Compounds **30** and **31** also demonstrated significant inhibitory activity against Wt-EGFR and induced apoptosis in the HepG2 cell line.

The presence of the benzodioxole ring likely plays a crucial role in the antitumor activity of these disubstituted thioureas. Compounds containing a 1,3-benzodioxole group are known to exhibit various biological activities, including anti-inflammatory, anticonvulsant, antidepressant, and anticancer effects. Safrole (5-(2-propenyl)-1,3-benzodioxole) (Figure 15) is a natural product that has shown versatile uses as an efficient natural synthon [95].

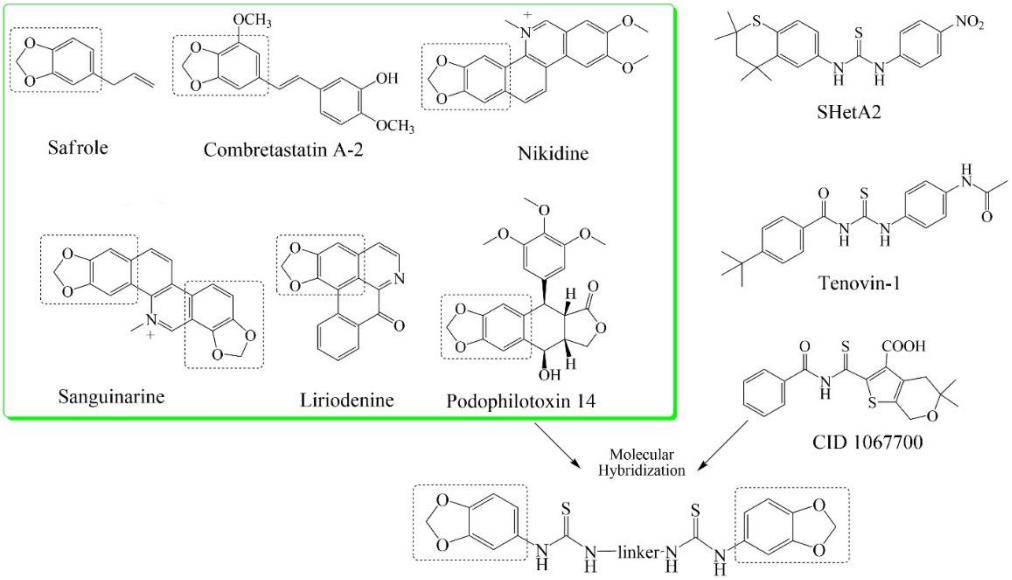

Figure 15. Hybrids benzo[*d*][1,3]dioxol-5-yl thiourea derivatives as antitumor agents.

The effectiveness of the 1,3-benzodioxole derivatives as antitumor agents is well-documented. It is found in numerous bioactive natural products derived from plants and marine organisms, such as nitidine chloride, liriodenine, berberine, sanguinarine, harringtonine, podophyllotoxin, trabectedin, and homoharringtonine, all known for their potent antitumor properties. Additionally, benzodioxole derivatives have been explored as potential anticancer agents due to their favorable bioavailability and low toxicity.

Building on this, R.A.K. Al-Harbi and colleagues synthesized bis-thiourea derivatives, each incorporating two benzo[d][1,3]dioxol-5-yl groups connected by various spacers between the two nitrogen atoms. These compounds showed strong antiproliferative effects against tumor cells (Figure 16) [95].

The antitumor activity of these bis-benzo[d][1,3]dioxol-5-yl thioureas was tested on HepG2, HCT116, and MCF-7 cancer cell lines, with Doxorubicin as a control. Most compounds demonstrated significant anticancer activity, with greater selectivity for HepG2 and HCT116 cells compared to MCF-7. The structure-activity relationship indicated that the linker type between thiourea moieties significantly affected cytotoxicity. For example, compounds without a linker (**32**) had lower activity, while those with ethylene (**33**) or thiourea linkers (**34**) showed improved cytotoxicity. Compound **34** exhibited strong activity, with IC_{50} values of 6.7 µM for HepG2, 3.2 µM for HCT116, and 12.4 µM for MCF-7, outperforming Doxorubicin (7.5, 8.3, and 4.6 µM, respectively).

In compound **35**, the substituents on the ortho-phenylene linker were crucial in modulating anticancer activity. Different C-3 position substituents resulted in significant variations in cytotoxicity. A hydrogen atom (**35a**) produced moderate activity (IC_{50} 109–118 µM). A methyl group (**35b**) slightly enhanced activity (IC_{50} 71–89 µM). However, a nitro group (**35c**) did not significantly improve activity (IC_{50} 132–141 µM). Introducing a benzoyl group (**35d**) at the C-3 position markedly increased cytotoxicity. Compound **35d** exhibited potent activity against HepG2, HCT116, and MCF-7 cell lines, with IC_{50} values of 10.6 nM, 7.3 µM, and 13.2 µM, respectively. This suggests that the benzoyl group enhances hydrophobic interactions, improving the compound's ability to interact with target proteins and penetrate the cell membrane, highlighting the importance of substituent size and hydrophobicity in enhancing anticancer potency [95].

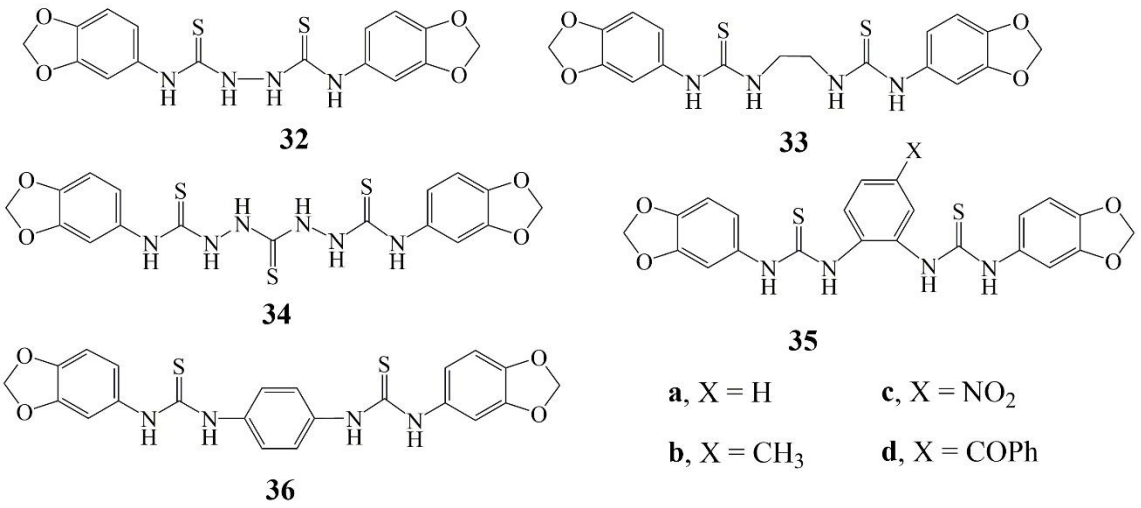

Figure 16. Line drawings of bisbenzo[d][1,3]dioxol-5-yl thiourea derivatives **32-36**.

The introduction of a *para*-phenylene linker in *bis*-benzo[d][1,3]dioxol-5-yl thiourea compound **36** significantly improved its antitumor activity, making it one of the most cytotoxic compounds in this study. It demonstrated stronger efficacy than doxorubicin, with IC_{50} values of 2.4 µM for HepG2, 1.5 µM for HCT116, and 4.5 µM for MCF7, compared to doxorubicin's IC_{50} of 7.5, 8.3, and 4.6 µM, respectively. This highlights the key role of the *para*-phenylene linker in enhancing cytotoxicity.

Most compounds tested, including compounds **33**, **34**, and **36,** exhibited minimal cytotoxicity against normal WI-38 cells (IC_{50} > 150 µM), indicating selective toxicity toward cancer cells. The active compounds showed IC_{50} values below 9.3 µM for HepG2 and HCT116 cells, supporting their preferential anticancer effects. Additionally, compounds **33**, **34**, and **36** inhibited wild-type EGFR (Wt-EGFR) and exhibited superior activity against EGFR mutants (EGFRL858R and EGFRT790M), particularly compound **34**, which was more effective than lapatinib. Molecular docking studies revealed that the thiourea group formed strong hydrogen bonds with Met769 and Arg819, while the benzo[d][1,3]dioxole rings interacted with hydrophobic pockets Leu694 and Cys773. The binding energies ranged from -21.6 to -24.5 kcal/mol, indicating robust binding with their targets [96].

In conclusion, *bis*-benzo[d][1,3]dioxol-5-yl thiourea derivatives, especially those with a para-phenylene linker, display potent antitumor activity and outperform doxorubicin, highlighting the importance of linker selection in optimizing cytotoxic effects. These studies highlight the potential for further optimization of benzo[d][1,3]dioxol-5-yl thiourea derivatives to improve selectivity and reduce side effects. Investigating structure-activity relationships and exploring different substituents on the benzo[d][1,3]dioxol-5-yl ring could lead to obtaining more potent compounds. Additionally, detailed studies on pharmacokinetics, bioavailability, and long-term safety are essential for advancing these derivatives toward clinical use. Combining these hybrids with other therapeutic agents may also enhance their effectiveness and provide new strategies for cancer treatment.

3.5. Bis-thiourea derivatives.

After exploring the impact of substituents in thiourea molecules on their binding to biological targets, researchers began investigating the effect of an additional thiourea pharmacophore on biological activity. Bis-thiourea compounds containing two thiourea units were identified as having notable pharmacological potential. Shing *et al.* [96] focused on

developing antitumor agents to overcome resistance mechanisms common to traditional tubulin-binding drugs. Their compound, 1,1′-[1,3-phenylene]bis[3-(3,5-dimethylphenyl)thiourea] (**37**), exhibited high cytotoxicity against various cancer and drug-resistant cell lines, inducing apoptosis (Figure 17). Compound **37** inhibited the viability of twelve tumor and drug-resistant cell lines with IC_{50} values ranging from 161 to 2640 nM. It also arrested cell growth in glioblastoma multiforme xenografts in mice at tolerable doses. It overcame resistance due to β-tubulin mutations and P-glycoprotein overexpression, positioning it as a promising anticancer agent.

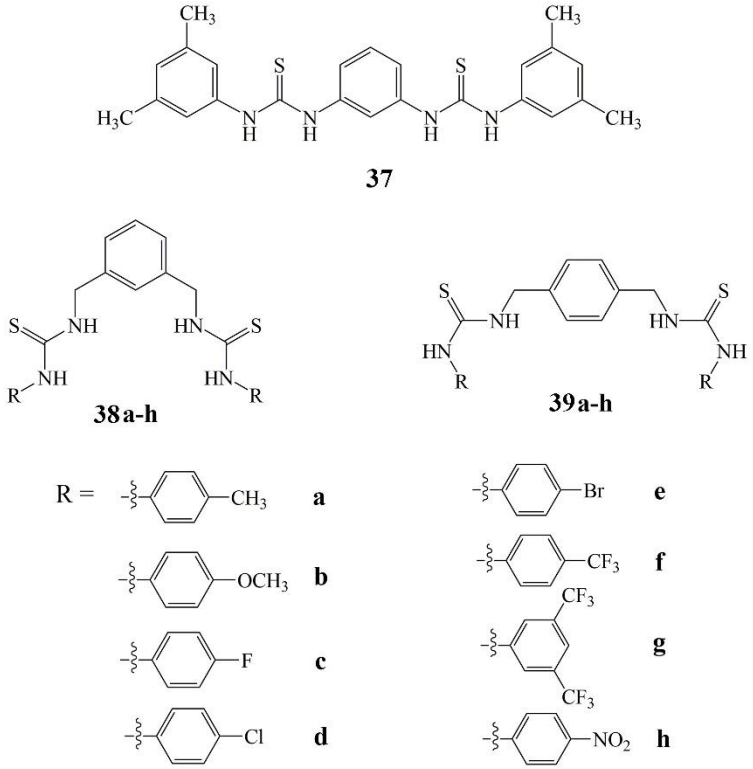

Figure 17. Line drawings of compounds **37-39**.

In a related study, R. Pingaew and colleagues synthesized two series of bis-thiourea derivatives, **38** and **39** [97], and evaluated their anticancer activity against six cancer cell lines, including HuCCA-1 (cholangiocarcinoma), HepG2 (hepatocellular carcinoma), A549 (lung carcinoma), MOLT-3 (leukemia), MDA-MB-231 (hormone-independent breast cancer), and T47D (hormone-dependent breast cancer). The *meta*-substituted *bis*-thioureas (**38**) were more active than the *para*-isomers (**39**), with compounds containing electron-withdrawing groups showing the highest potency. For example, **38f** had an IC_{50} of 1.20 μM against MOLT-3 cells, and 38g had an IC_{50} of 1.50 μM against HepG2 cells. QSAR analysis revealed key factors, such as molecular mass, polarizability, electronegativity, and specific bond types (C–N, F–F, and N–N and van der Waals volume), as critical predictors of anticancer efficacy.

Another crucial component in the structure of thioureas is the presence of an alkyl chain, which influences their lipophilicity, spatial orientation, metabolic stability, binding specificity, and solubility. Optimizing the alkyl chain is crucial for developing biologically active thioureas, particularly those with antitumor properties. For example, a polyamine analog of alkylated bis-thiourea (**40**) showed antitumor activity as a lysine-specific demethylase inhibitor [98].

Building on these findings, the authors of the тnext study [42] synthesized nitro-phenylene derivatives of symmetrical bis-thioureas (**41-43**) and evaluated their DNA binding,

urease inhibition, and anticancer potential (Figure 18). Molecular docking revealed that all compounds interacted with DNA via partial intercalation and groove binding, with compound **43** showing the strongest interactions, including hydrogen bonding with DNA base pairs and a higher binding affinity, indicated by binding free energy (ΔG) and binding constant (Kb) values.

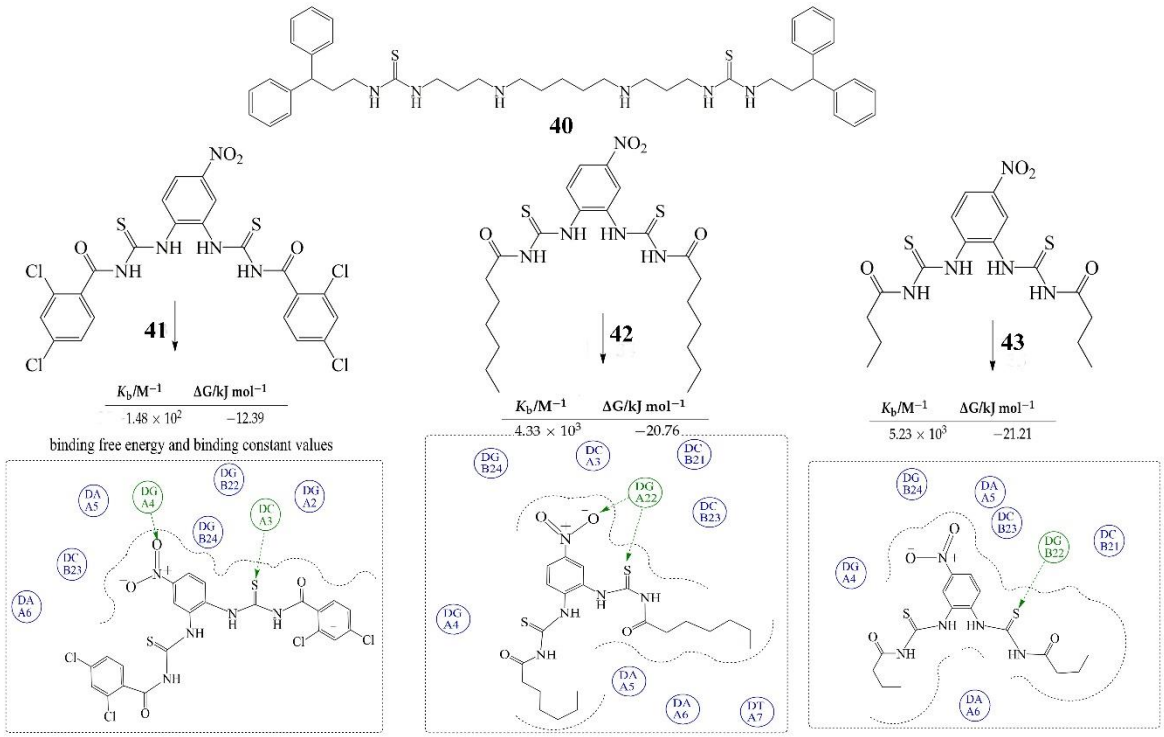

Figure 18. *Bis*-thiourea **40-43** and their molecular docking with DNA.

In vitro experiments, these compounds displayed dose-dependent cytotoxicity against the glioblastoma U87-MG cell line, with IC_{50} values of 2.5, 2.7, and 2.5 μM, respectively. Compounds **41** and **43** exhibited higher cytotoxicity against both tumor (U87-MG) and normal (HEK-293) cell lines, while compound **42** had reduced effects on tumor cells at concentrations above 200 μM and minimal effects on normal cells. These results suggest *bis*-thiourea derivatives as promising drug candidates for further investigation.

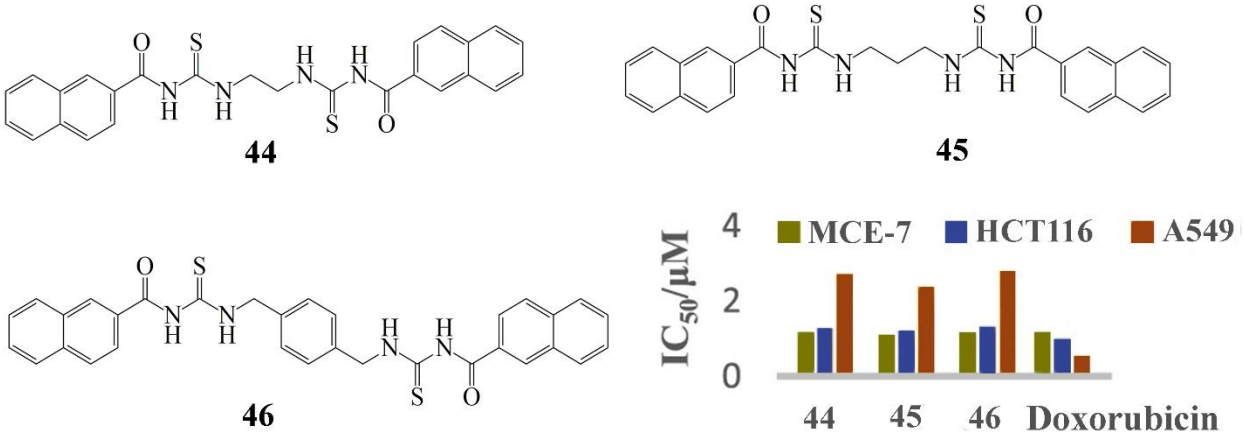

Figure 19. Line drawings and *in vitro* antiproliferative activities of compounds **44-46** toward MCF-7, HCT116, and A549 cancer cells.

Arafa *et al*. [99] expanded the N-naphthoyl thiourea library by synthesizing compounds **44-46** (Figure 19), which could serve as scaffolds for designing biologically active heterocycles. The antiproliferative effects of these compounds were tested on MCF-7,

HCT116, and A549 cancer cell lines. Most of the thiourea derivatives exhibited significant cytotoxicity, some surpassing Doxorubicin in activity. The *bis*-thioureas showed notable antitumor effects, with IC_{50} values of 1.2, 1.3, 2.7, and 86.1 µM for compound **44**; 1.1, 1.2, 2.4, and 90.2 µM for compound **45**; and 1.2, 1.4, 2.8, and 91.4 µM for compound **46**, across cancer and normal cell lines.

Future research should focus on optimizing these derivatives by adjusting linker lengths and substituents to enhance specificity and reduce off-target effects. Further studies on their mechanisms of action and in vivo evaluations are essential to explore their full therapeutic potential. Combining them with other agents and developing targeted delivery systems could improve their clinical efficacy and safety.

3.6. Sulfurarotinoid-containing thiourea derivatives.

By incorporating synthetic heteroarotinoid fragment into the thiourea scaffold, the hybrid anticancer agent [(4-nitrophenyl)amino][2,2,4,4-tetramethylthiochroman-6-yl]amino]methane-thione (SHetA2) was developed (Figure 20) [56, 100–102]. SHetA2 is the lead compound in a flexible heteroarotinoids (Flex-Hets) class that induce apoptosis in cancer cells independently of retinoic acid receptors while sparing normal cells. It causes G1 arrest and apoptosis in ovarian cancer cells at IC_{50} concentrations of 0.4–4.6 µM across 60 cancer cell lines and shows effectiveness *in vivo*. In this regard, SHetA2, SHetA3, and SHetA4 induce differentiation and apoptosis in cancer cell lines and primary cultures, with SHetA2 being the most effective in many cancer types, including ovarian, lung, and kidney cancers [101, 102]. SHetA2 has low toxicity (maximum tolerated dose > 1500 mg/kg/day) and selectively targets mitochondria in cancer cells, reducing Bcl-2 and Bcl-xL proteins to trigger apoptosis. Normal cells only undergo G1 arrest without apoptosis.

Unlike retinoids, Flex-Hets, including SHetA2, induce apoptosis through the intrinsic mitochondrial pathway, involving mitochondrial membrane disruption, ROS generation, cytochrome c release, and caspase-3 activation, without relying on retinoic acid receptors or being inhibited by receptor antagonists [101]. SHetA2 was the most potent, showing no apoptosis or necrosis in normal human ovarian surface epithelial (HOSE) or endometrial (D1) cells after 24 hours of treatment.

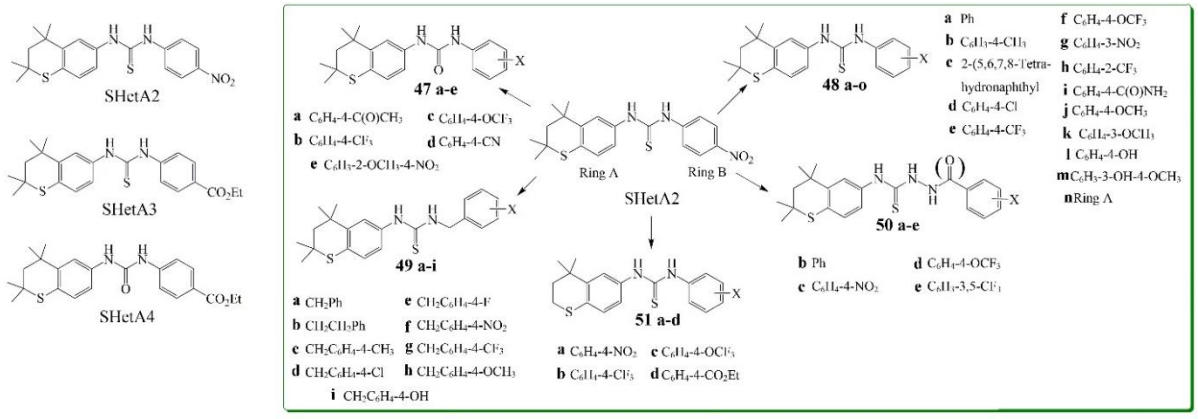

Figure 20. The SHetA2 and its modified analogues **47**-**51**.

Nammalwar *et al*. [102] synthesized a series of SHetA2 analogs (**47-51**) and evaluated their activity against the A2780 ovarian cancer cell line. The analogs were developed by modifying the pK/pD properties, and their activity was assessed using structure-activity relationship (SAR) and diversity-oriented synthesis (DOS). SHetA2 interacts with the heat

shock protein mortalin, and various analogs with different functional group substitutions on Rings A and B were created to explore binding properties. Urea (**47a-e**) and thiourea (**48a-n**) derivatives with functional groups (chloro, fluoro, methyl, methoxy, cyano, trifluoromethyl, etc.) introduced at C4′ of Ring B were synthesized to assess their binding affinity and biological potency (Figure 20).

A series of N-benzylthiourea (**49a-i**) was developed to examine the impact of linker flexibility on binding affinity [102]. To probe the structural features, hydrazines and hydrazide derivatives (**50a-e**) were synthesized, and analogs with the C2 gem-dimethyl groups removed (**51a-d**) were explored to assess their effect on drug scaffold and improve drug-like features(Figure 20). Biological testing revealed that several analogs had activity similar to SHetA2, with improved logP values. Notably, CF_3 (**47b**, IC_{50} 3.8 µM) and OCF_3 (**47c**, IC_{50} 1.9 µM) outperformed SHetA2, with **47c** being the most potent against A2780 cells. C2′ and C3′ modifications reduced activity, while C4′ substitutions enhanced binding to mortalin. Among N-benzyl derivatives, 4′-CF_3 (**49g**) showed promising activity (IC_{50} 3.3 µM). Urea and thiourea analogs with electron-withdrawing groups at C4′ exhibited strong activity, with compounds **47b** and **47c** selectively targeting cancer cells due to interaction with mortalin.

Thus, integrating sulfur-containing heteroarotinoids into thiourea scaffolds significantly enhances their interaction with biological macromolecules, potentially offering more effective and selective anticancer agents. Future research should prioritize the optimization of the chemical structure of these sulfur heteroarotinoid thiourea derivatives, focusing on improving their pharmacokinetic and pharmacodynamic profiles. Comprehensive studies on their mechanisms of action, including iidentifying specific molecular targets and the pathways involved, are crucial for understanding their full therapeutic potential. Further investigation should also explore the synergy of these compounds with existing cancer therapies to enhance their efficacy, as well as evaluate their safety and efficacy through in vivo models. Tailoring these derivatives to target specific cancer types and incorporating advanced drug delivery systems will likely improve their clinical outcomes, making them more effective as part of personalized cancer treatment strategies.

3.7. Pyrimidine and quinazoline-containing thioureas.

Pyrimidine and quinazoline are some of the most important pharmacophores in medicinal chemistry, featured in many commercial drugs, including anticancer agents. M.B. Alshammari and colleagues developed a method for synthesizing hybrid thiourea derivatives (**52a-f**) containing a uracil moiety (Figure 21) [103]. These compounds were evaluated for in vitro antiproliferative activity against four cancer cell lines, with compound **52c** being the most potent. It showed GI_{50} values of 1.8, 1.4, 2.1, and 2.1 µM against cancer cell lines A-549 (epithelial), MCF-7 (breast), Panc-1 (pancreatic), and HT-29 (colon), respectively. The GI_{50} values for the other derivatives ranged from 3.7 to 9.1 µM.

Koca *et al*. [104] synthesized a series of pyrimidinyl acyl thiourea derivatives (**53a-j**) to selectively inhibit oncogenic heat shock protein 90 (Hsp90) in MCF-7 (breast) and Saos-2 (osteosarcoma) cell lines (Figure 21). All compounds inhibited cell proliferation, with compound **53h** being the most potent, showing IC_{50} values of 5.2 µM (MCF-7) and 1.3 µM (Saos-2). The presence and positioning of side groups, particularly the alpha-naphthyl group, were crucial for enhancing anticancer activity, as it mimicked adenine in the ATP-binding site. The addition of a phenyl ring and methyl groups, as well as altering the methyl group's position from *para* to *ortho* on the phenyl ring, reduced the anticancer activity.

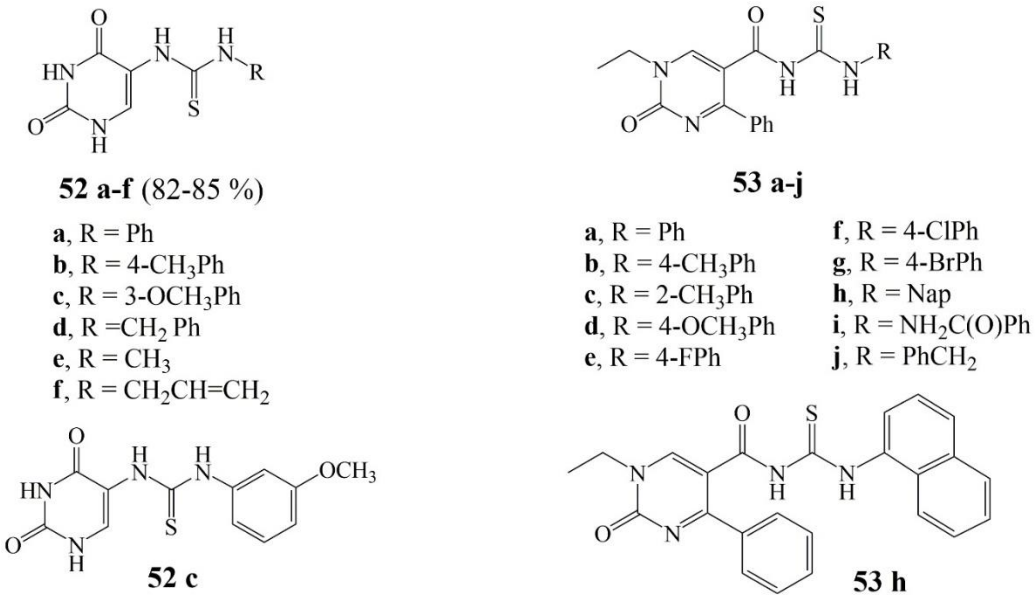

Figure 21. Line drawings of compounds **52a-f** and **53a-j**.

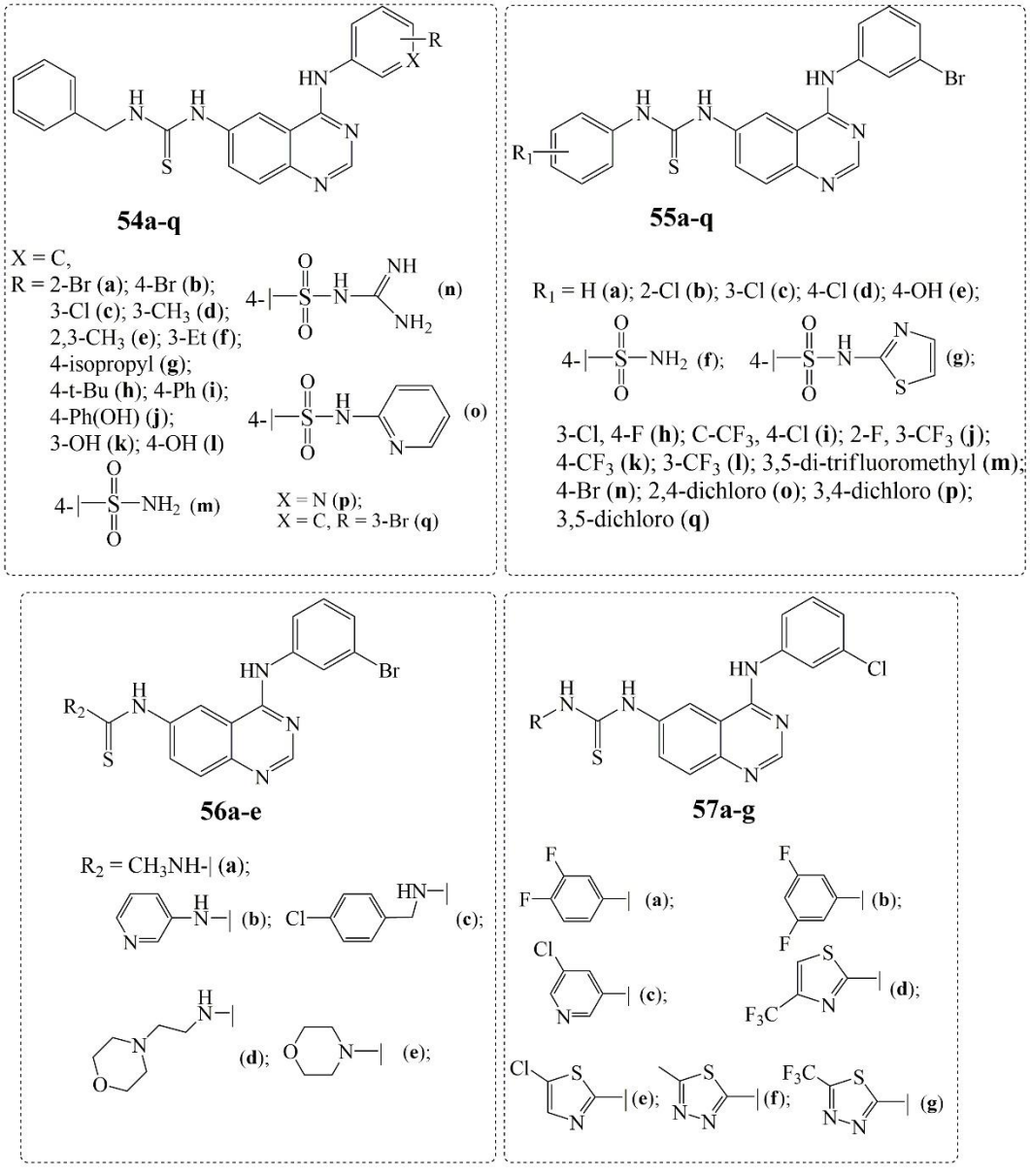

Figure 22. 1-(Quinazolin-6-yl)-3-phenyl-substituted thioureas.

Molecular docking studies showed that these derivatives bind to the Hsp90 ATP-binding site, with binding energies ranging from −7.5 to −9.2 kcal/mol [104]. Halogenated compounds (**53e**, **53g**), methoxy (**53d**), and alpha-naphthyl-containing compounds (**53h**) were particularly effective, likely due to their ability to induce Hsp90 conformational changes, promoting a more inactive state of the protein. These findings suggest new therapeutic strategies for treating invasive ductal breast carcinoma and bone metastasis.

Activation of the NF-κB transcription factor is a key resistance mechanism triggered by EGFR kinase inhibitors in non-small cell lung cancer and other tumors. To overcome this, Hamed *et al*. [105] synthesized 1-(quinazolin-6-yl)-3-phenyl-substituted thioureas **54-57** (Figure 22), which inhibit both EGFR kinase and NF-κB. The 4-amino-phenylquinazoline motif was crucial for strong kinase inhibition in the nM range, and quinazoline also served as a scaffold for inhibitors of various enzymes.

Lipophilic substituents on the aniline ring were preferred, while polar groups (e.g., hydroxy, sulfonamide, pyridine) reduced activity. Halogens, particularly chlorine (**54c**), were most favorable for NF-κB inhibition, while ethyl (**54f**), methyl (**54d**), and bromine (**55a**) showed reduced potency. The position of substituents affected EGFR inhibition, with meta-position providing the best activity, especially for hydrophobic groups like halogens. The methylene spacer between the thiourea linker and the aromatic ring was non-essential, as replacing it with phenyl (**55a**, **55d**) did not alter activity.

The thiourea group was crucial for NF-κB inhibition, while the aromatic ring also played an important role. Replacing the aromatic ring with methyl (**56a**) or other groups significantly reduced activity. For EGFR inhibition, hydrophilic and heterocyclic substituents (**55e**, **55f**, **56b**, **56d**) were most potent, while bulky lipophilic groups decreased activity. Compounds **55c** and **55h** showed the best balance between EGFR and NF-κB inhibition [105].

In compound **57**, the heterocyclic rings were modified with lipophilic groups to balance polarity and lipophilicity. 3-Chloropyridine (**57c**) slightly reduced both activities, while thiazole and thiadiazole derivatives (**57d-g**) decreased EGFR activity but preserved or enhanced NF-κB inhibition. Thiazole rings improved water solubility compared to halogen-substituted phenyl analogs. Compounds **57b**, **57d**, and **57e** were the most effective, with **57b** showing the best NF-κB inhibition (IC_{50} = 0.3 μM) and **57d** exhibiting good NF-κB activity (IC_{50} = 0.6 μM) and inhibiting MDA-MB-231 cell growth, despite lower EGFR activity. Both compounds were nontoxic and well tolerated in pharmacokinetic and xenograft studies.

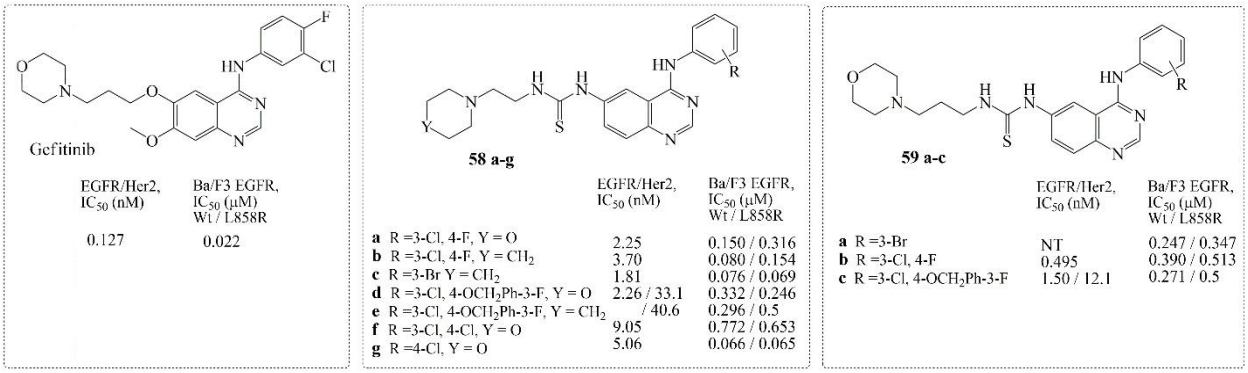

Figure 23. 4-Arylamino-6-thioureido-quinazoline derivatives and their effect on EGFR/Her2 enzymatic activity and antiproliferation against Ba/F3 expressing EGFRWT and L858R.

Mowafy *et al*. [106] synthesized a series of 4-arylamino-6-thioureido-quinazoline derivatives (**58**, **59**) as Gefitinib mimics, a first-generation EGFR inhibitor for non-small cell lung cancer. Most compounds inhibited EGFR kinase with IC_{50} values in the low nanomolar

range (0.495–9.05 nM). Their antiproliferative effects were tested on gefitinib-insensitive double mutant cell lines (Ba/F3 with Del19/T790M and L858R/T790M) (Figure 23).

In vitro results showed that urea derivatives had lower IC_{50} values than thiourea derivatives (**58b**), and the three-carbon linker (**59b**) was more potent than the two-carbon linker (**58a**). A docking study revealed that the binding modes of the compounds resembled Lapatinib, with the quinazoline scaffold forming a hydrogen bond with Met793 in the hinge region. Thiourea derivatives formed an additional hydrogen bond with Cys797, enhancing binding affinity compared to Gefitinib (Figure 24).

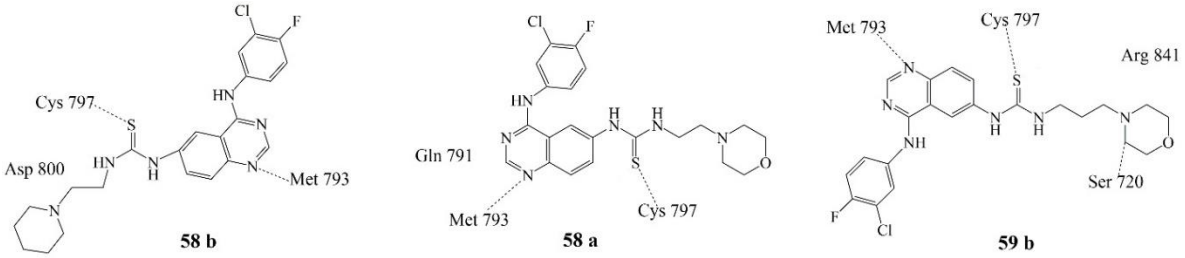

Figure 24. Binding mode of compounds **58b**, **58a,** and **59b**.

Thiourea compounds showed weaker binding due to sulfur's lower electronegativity compared to oxygen (C_{docker} energy = -24 kcal/mol). However, the three-carbon linker (**59b**) exhibited stronger inhibition ($IC_{50} \leq 0.5$ nM) due to extra interactions with Arg841 and Ser720, absent in the two-carbon linker compound (**58a**, IC_{50} = 2.3 nM) (Figure 24). The authors concluded that dual inhibitors targeting both EGFR and ErbB2 showed superior efficacy and better selectivity against mutant cell lines compared to EGFR-only inhibitors like Gefitinib. These dual inhibitors may also help counteract acquired resistance due to Her2 overexpression and avoid the cytotoxicity of covalent inhibitors.

Tandutinib, a quinazoline-based anticancer agent, is a dual PDGFR/FLT3 inhibitor currently under evaluation for acute myeloid leukemia treatment (Figure 25). Compound **A** is a potent and selective PDGFR inhibitor, with the thiourea moiety enhancing water solubility and pharmacokinetic properties [107]. Compound **B** and its analogs are novel FLT3 inhibitors, effectively inhibiting cell growth in both FLT3 ITD-mutated and wild-type human leukemia cell lines. These compounds feature a thiourea group and an N-heteroarene scaffold linked by a conformationally constrained piperazine ring.

Li *et al*. [107] synthesized a series of [1-3]triazolo[4,5-d]pyrimidine/thiourea hybrids (**60a-r**) through ring cleavage and scaffold replacement that should improve the flexibility of these molecules. These compounds were tested for antiproliferative activity against various human cancer cell lines (gastric MKN-45, MGC803, lung H1650, A549, and esophageal EC-109). The hybrids showed potent antiproliferative effects, especially compound **60r**, which was highly effective against H1650 and A549 lung cancer cell lines (IC_{50} = 1.9, 3.3 μM), with low toxicity against the normal gastric epithelial cell line GES-1 (IC_{50} = 27.4 μM). This compound shows promise for the development of new antitumor agents.

Thus, the aromatic system, along with the position and nature of substituents on the aniline ring, plays a key role in compound activity. Interactions with targets like EGFR and Hsp90 indicate that derivatives with various substituents can effectively bind to these targets, opening up new avenues for therapeutic development. Future research should focus on optimizing these hybrid thiourea derivatives for improved efficacy and selectivity. Detailed mechanistic studies are needed to clarify the pathways and molecular targets involved in their anticancer activity. Additionally, preclinical evaluations of pharmacokinetic properties and

safety profiles will be crucial for advancing these compounds to clinical trials. Exploring combination therapies and developing novel drug delivery systems could further enhance their therapeutic potential.

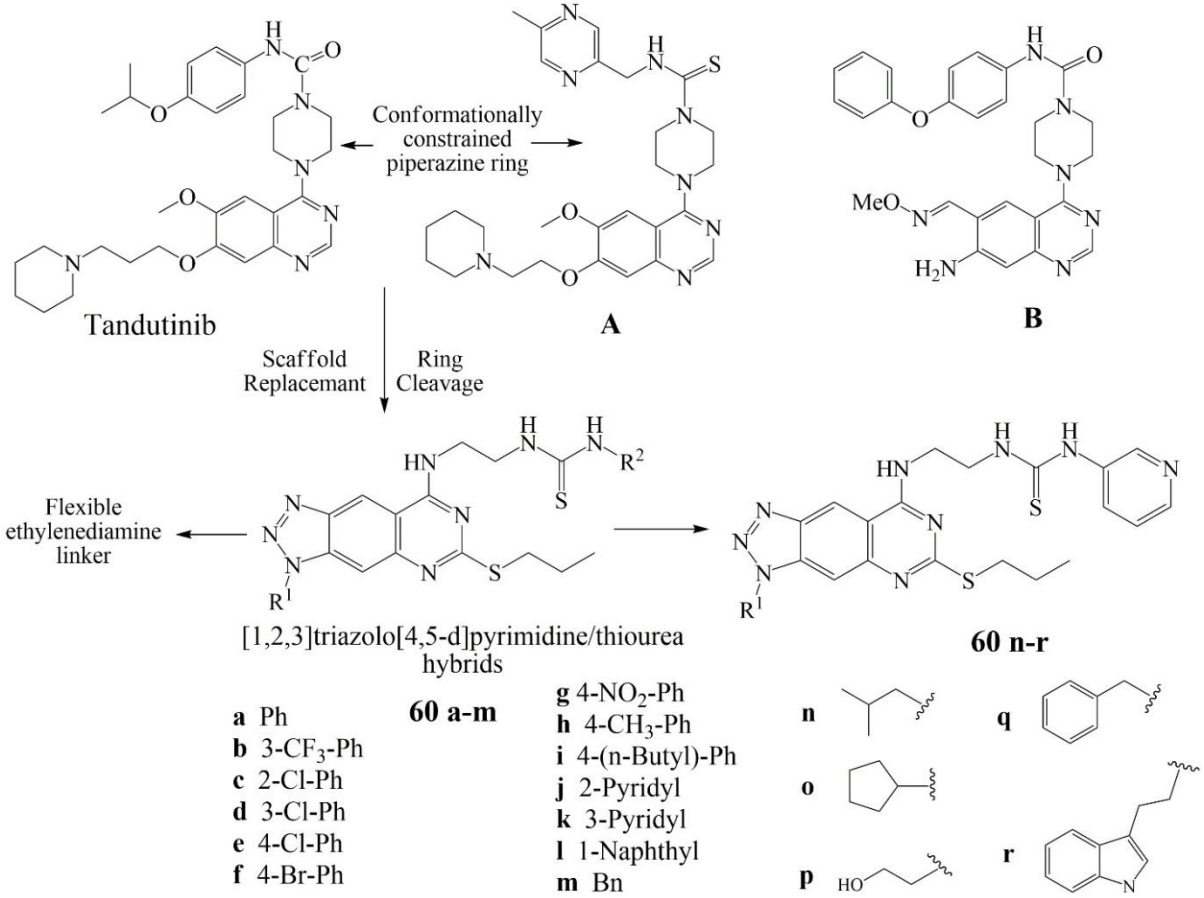

Figure 25. A series of [1,2,3]triazolo[4,5-*d*]pyrimidine-thiourea conjugates.

3.8. Thiourea derivatives as Sorafenib analogs have the ability of both antitumor and antiangiogenic activities.

Angiogenesis, along with uncontrolled cell division, plays a crucial role in cancer progression, as tumors cannot grow beyond 1–2 mm without a blood supply [108]. Targeting both antitumor and antiangiogenic pathways with a single agent is more effective than combination therapies, creating a need for new drugs that can block both processes.

Receptor tyrosine kinases (RTKs) are key cell proliferation, death, and differentiation regulators, making them important targets in cancer therapy. Approved small-molecule RTK inhibitors, such as Sorafenib, Regorafenib, Axitinib, Lenvatinib, and Sunitinib, are potent antitumor agents (Figures 11 and 26). Sorafenib (BAY 43-9006; Nexavar), a multi-targeted drug approved by the FDA, is effective against kidney and liver cancers by inhibiting kinases involved in tumor growth and angiogenesis [109].

Among Sorafenib derivatives, thiourea compounds **61a-h**, featuring a 3,5-(trifluoromethyl)phenyl group, showed strong antitumor and antiangiogenic effects (Figure 26a) [108]. They were tested on various human cancer cell lines (lung NCI-H460, colorectal Colo-205, colon HCT116, breast MDA-MB-231, and MCF-7, hepatocarcinoma HepG2 and PLC/PRF/5) and normal endothelial cells (HUVEC). Compound **61e**, with a phenylamino group, exhibited the best results. It inhibited cell growth across all lines (IC_{50} = 1.9–9.9 μM) and demonstrated superior antiangiogenic activity. Moreover, **61e** inhibited RTKs like

VEGFR2, VEGFR3, and PDGFRß and showed enhanced effects on tumor formation compared to Sorafenib. Its NH group forms a hydrogen bond with Glu885 in the kinase hinge region and interacts hydrophobically with residues such as Leu1035, Val899, and Phe918 (Figure 26b). These findings suggest that compound **61e** could be a promising candidate for cancer therapy.

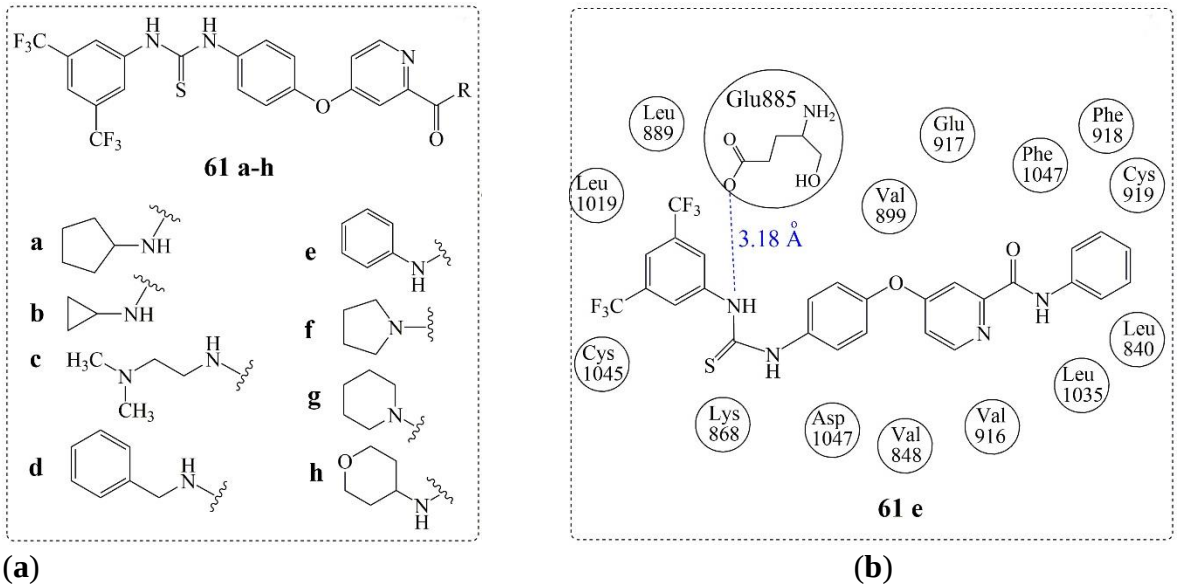

Figure 26. Compounds (**a**) **61a-h**; the schematic (**b**) **61e**-VEGFR2 interaction.

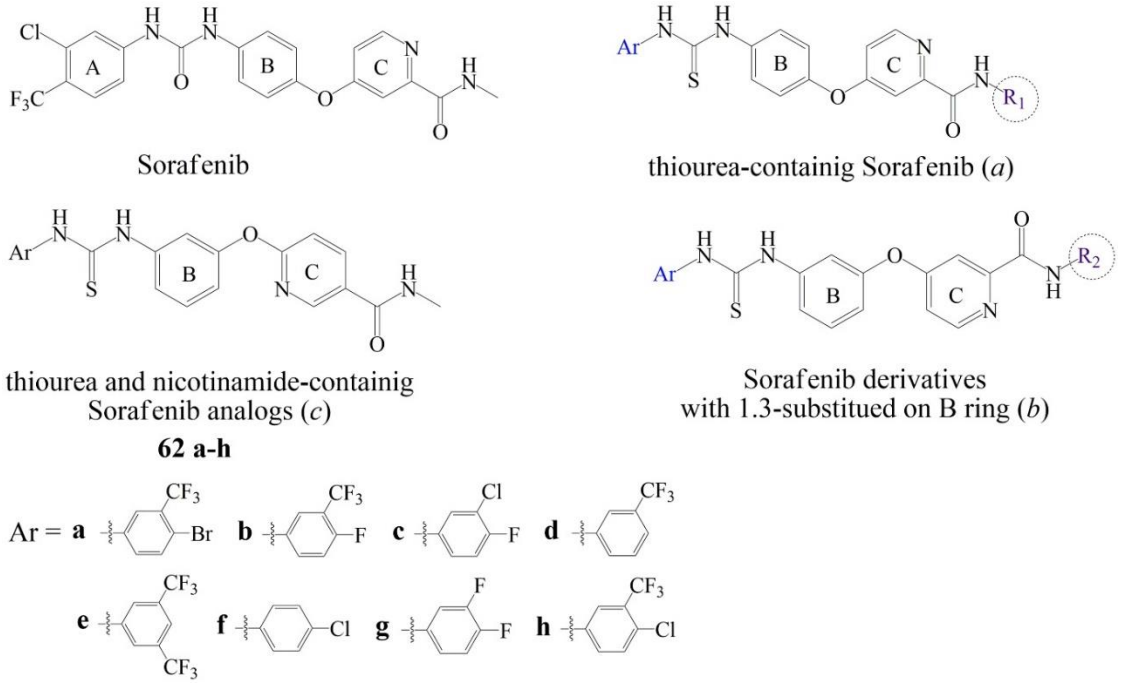

Figure 27. Modifications of Sorafenib.

Inspired by the bioisosteric approach, where thiopental replaces urea with thiourea in pentobarbital, Jianwen Yao's group synthesized a series of diarylthiourea-based Sorafenib derivatives (Figure 27a, b) [110–112]. These compounds exhibited stronger antiproliferative activity against HCT-116 and MDA-MB-231 cells compared to Sorafenib. Some also inhibited VEGFR phosphorylation and demonstrated antiangiogenic effects in rat aortic ring assays. Key structure-activity relationships (SARs) include: 1) Replacing urea with thiourea enhances antiproliferative activity, 2) Methyl groups on the terminal amide improve activity, and 3) 1,3-substitution on the B ring enhances antiangiogenic effects.

Building on these findings, Kong *et al*. [110, 112] synthesized a new series of nicotinamide-containing thiourea analogs of Sorafenib (Figure 27). These compounds retained the thiourea group, formamide terminal, and 1,3-substitution on the B ring while replacing the C ring's 2,4-substitution with 2,5-substitution, as nicotinamide improves drug-likeness.

Compared to Sorafenib, most compounds showed potent inhibitory activity against HCT116, MDA-MB-231, PC-3, and HepG2 cells. Compounds **62a**, **62e**, and **62h**, with electron-withdrawing groups on the terminal phenyl ring, exhibited the highest activity, with **62h** showing the best antiproliferative effects across all cell lines [110, 111]. *In vitro* HUVEC tuber formation and *in vivo* rat aorta ring assays confirmed the excellent antiangiogenic properties of compounds **62e** and **62h**, suggesting their potential as effective anticancer agents.

Tumor survival, growth, and metastasis depend on processes like cell proliferation, differentiation, apoptosis, and angiogenesis, all regulated by protein kinases and signaling pathways [112]. Inhibiting the Raf/MEK/ERK pathway, especially at the Raf-kinase level, can target tumors driven by this pathway, such as melanomas, thyroid, ovarian, colorectal, and lung cancers. Raf-1, A-Raf, and B-Raf isoforms, with the B-Raf^{V600E} mutation being common in many cancers, are key targets. VEGFR-2, involved in metastasis and angiogenesis, is also critical for tumor progression. Thus, targeting B-Raf^{V600E} and VEGFR-2 with multikinase inhibitors is a promising cancer treatment strategy.

Sun *et al*. [112] synthesized Sorafenib derivatives with thioether and nicotinamide moieties (compounds **63-65**, Figure 28) to enhance antiproliferative activity through the thiourea group.

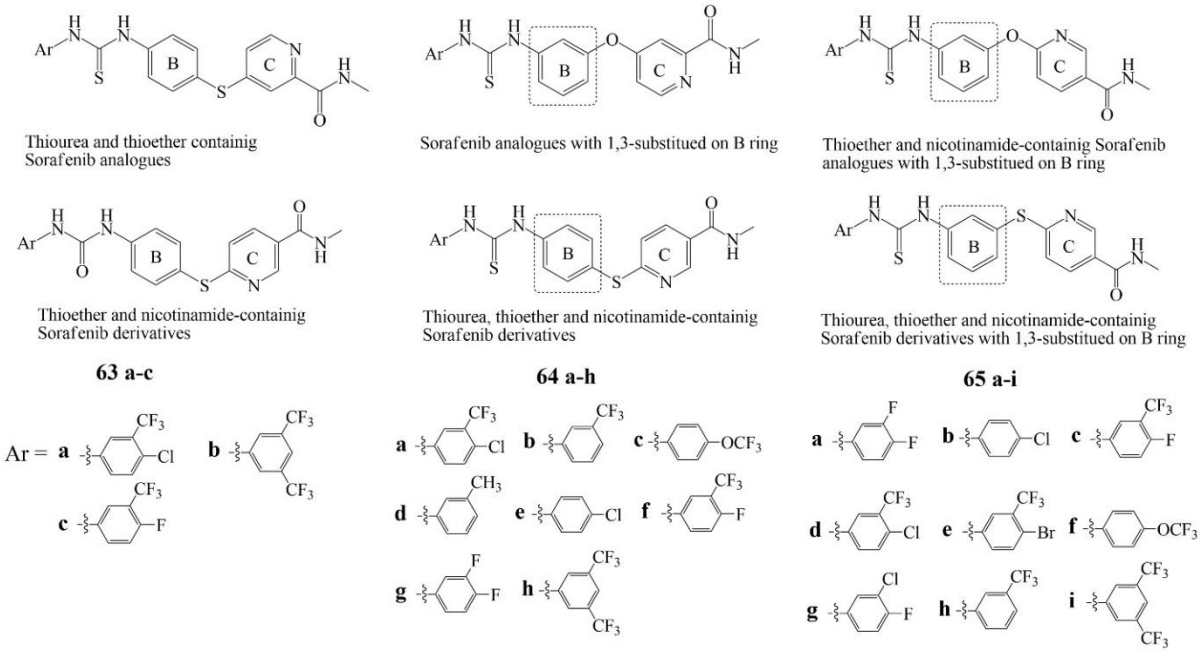

Figure 28. Thiourea, thioether, and nicotinamide-containing Sorafenib analogs.

Compared to Sorafenib, compounds **63-65** showed potent inhibition of B-Raf, B-Raf^{V600E}, and VEGFR-2 and strong antiproliferative effects against HCT-116 and B16BL6 cell lines. Compounds with electron-withdrawing substituents (4-Cl, 4-F, 3-CF3) at the 3,4-position on the phenyl ring exhibited the strongest activity. Notably, **63a**, **64a**, and **65d** had IC_{50} values of 21, 27, and 17 nM for B-Raf, 29, 28, and 16 nM for B-Raf^{V600E}, and 84, 46, and 63 nM for VEGFR-2. These compounds also displayed significant antiangiogenic effects in the HUVEC tube formation assay.

In conclusion, the structural modifications to Sorafenib, including thiourea and thioether groups, significantly improved its therapeutic activity. Compounds with electron-withdrawing groups showed potent inhibition of B-Raf and VEGFR-2, suggesting the potential for more effective cancer treatments. Future research should focus on optimizing these molecules to enhance efficacy and minimize side effects, advancing them toward clinical use.

3.9. Thiourea derivatives as zinc-binding agents.

Zinc-dependent histone deacetylases (HDACs) play a key role in carcinogenesis and are important targets in anticancer drug design. Acyl-substituted thioureas, which contain a zinc-binding group (ZBG), are promising candidates for HDAC inhibitors. These inhibitors typically include a ZBG, a linker chain mimicking acetylated lysine to fit into the cavity, and a hydrophobic cap to interact with the enzyme's outer surface. The FDA-approved inhibitor suberoylanilide hydroxamic acid 1 (SAHA, vorinostat, Figure 29) serves as a classic example [113].

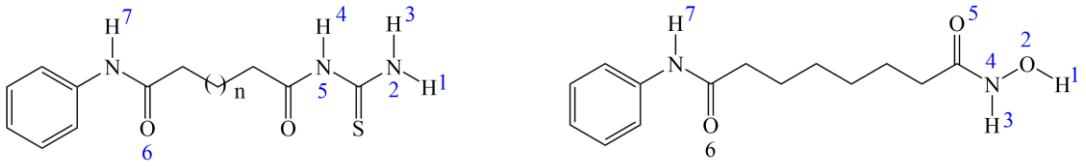

Figure 29. Line drawings of SAHA and acyl-substituted thioureas **66**, **67**.

Hydroxamates, while effective, suffer from poor pharmacokinetic properties and non-specific enzyme targeting. To address these issues, the authors of Al-Amily *et al.* [114] designed new compounds (**66** and **67**) that retain the hydrophobic cap and linker but feature a novel ZBG. N-Adipoyl monoanilide thiourea (**66**) and N-pimeloyl monoanilide thiourea (**67**) replace the typical N-hydroxyamide group with an acylthiourea moiety.

Table 1. Docking results of compounds **66** and **67** against HDAC2 and HDAC7.

Isoform/ PDP Code	Ligand	Pose Rank	*B*inding energy (Kcal/mol)	K_i	H-Bonds			Distance from Zn^{2+} (Å)
					Involved ligand atoms	Involved residue	Length (Å)	
HDAC2/ 4LXZ, chain A	SAHA	1st	-7.1	6.2×10^{-6}	1H	Tyr 297	2.029	2O: 5.117 5O: 2.198
	66	1st	-7.3	4.4×10^{-6}	1H 3H 3H 4H	His 135 Asp170(OD1) Asp170(OD2) Asp 258	2.3 2.326 2.163 2.705	2N: 2.028 S: 3.890 6O: 4.278
	67	1st	-7.1	6.2×10^{-6}	1H	Asp 170	2.483	2N: 2.012 S: 3.889 6O: 4.247
HDAC7/ 3ZNR, chain A	SAHA	6th	-6.9	8.7×10^{-6}	5O 5O	His 137 His 136	2.311 2.180	2O: 5.314 5O: 2.224
	66	3rd	-7.2	5.4×10^{-6}	1H 1H 1H 6O	Asp174(OD2) Asp174(OD1) Asp 268 His 176	2.343 2.204 2.308 2.152	2N: 1.445 S: 3.781 6O: 4.819
	67	2nd	-7.2	5.4×10^{-6}	6O 1H S	Gly309 His 176 His 136	1.986 2.353 3.176	2N: 2.555 S: 3.693 6O: 4.267

Docking studies showed that compounds **66** and **67** bind to HDAC2 and HDAC7 with affinities comparable to or better than SAHA. The sulfur atom of compounds **66** and **67** is

positioned closer to the zinc ion in HDAC2 and HDAC7 (distances of 3.693 Å and 3.781 Å, respectively), suggesting strong coordination with the zinc ion, unlike the oxygen atom in SAHA's hydroxyl group (5.314 Å), as shown in Table 1.

In vitro tests demonstrated that compounds **66** and **67** inhibited the growth of cancer cell lines (human colon adenocarcinoma HRT-18, mouse hepatic carcinoma HC-04, and epithelial cells from healthy human breast milk HBL-100) at micromolar concentrations. Both compounds exhibited higher inhibitory activity in cancer cells compared to normal cells, with IC_{50} values differing by no more than 3 μM across the cancer cell lines [114]. The similar binding energies in HDAC2 docking further suggest their selective inhibition of HDAC2. Moreover, their low toxicity against normal cells and high affinity for HDAC2 make them promising selective HDAC2 inhibitors, with the acyl thiourea group acting as a unique ZBG.

Thus, future research should further optimize these thiourea derivatives to enhance their specificity and pharmacokinetic profiles. At the same time, exploring their effects in preclinical models will be crucial for assessing their potential as therapeutic agents. Developing these novel HDAC inhibitors is a promising avenue for creating more selective and effective anticancer therapies.

4. Conclusions

Functionalized thiourea derivatives exhibit a broad range of biological activities due to their involvement in non-covalent interactions (hydrogen, π-π, hydrophobic) with key biological targets like proteins, enzymes, and receptors. Their high antitumor activity is attributed to their ability to target multiple drug-related proteins involved in carcinogenesis. Analysis of experimental data reveals that substituents in thiourea derivatives play a critical role in their anticancer activity.

Recent studies highlight the promising potential of *N*-aryl and *N*, *N′*-diaryl thioureas as anticancer agents. Notably, modifications such as introducing electron-withdrawing substituents have been shown in Section 3.1, enhancing their biological activity and selectivity. Compounds like 1,3-*bis*(4-(trifluoromethyl)phenyl)thiourea and 1-(3,4-dichlorophenyl)-3-[3-(trifluoromethyl)phenyl]thiourea show strong activity against various cancer cell lines, indicating their potential for drug development.

The introduction of heterocyclic substituents, like pyridine or thiadiazine, significantly improves the activity of thiourea derivatives and their binding ability to target proteins, increasing their antitumor potential (Section 3.2). These compounds effectively interact with critical targets such as HER2, VEGFR2, and B-RAF, influencing their pathways through hydrogen bonds and hydrophobic interactions. As a result, they often outperform traditional drugs such as Sorafenib, exhibiting higher selectivity for cancer cells and promising safety profiles.

Hybrid thiourea derivatives (Section 3.3), created by combining pharmacophore groups of known drugs with thiourea moieties, demonstrate potent activity against key cancer targets (such as VEGFR-2). For instance, 3-(4-methoxy-3-(2-methoxypyridin-4-yl)phenyl)-N-(4-methoxyphenyl)azetidine-1-carbothioamide shows exceptional potency, surpassing traditional chemotherapeutics like Doxorubicin. These hybrids highlight their effectiveness in inhibiting tumor growth and angiogenesis.

As a continuation of this, Section 3.4 demonstrates that benzo[d][1,3]dioxol-5-yl thiourea hybrids show potent antiproliferative activity against various cancer cell lines through their ability to target key molecular pathways. They exhibited impressive IC_{50} values. Further

optimization of these derivatives could enhance selectivity and reduce side effects, with the potential for combination therapies to improve efficacy.

Bis-thiourea derivatives (Section 3.5) exhibit enhanced biological activity, interacting with multiple targets, including protein kinases and DNA. Quantitative structure-activity relationship (QSAR) studies reveal that properties like mass, polarizability, and bond types are crucial for anticancer activity. Such compounds as meta-bis-thiourea derivatives show significant antiproliferative effects and could serve as potent agents in cancer therapy. As follows, the dual thiourea groups in these derivatives facilitate robust binding and potential synergistic effects, making them effective in combating tumor growth.

Sulfur heteroarotinoids containing thiourea derivatives represent a novel class of anticancer agents that combine the bioactivity of thiourea with the unique properties of heteroarotinoids (Section 3.6). Compounds like SHetA2 show significant apoptotic effects against various cancers and potential inhibition of key targets. SHetA2 induces apoptosis in cancer cells primarily through mitochondrial pathways, involving downregulating anti-apoptotic proteins Bcl-2 and Bcl-xL. This selective induction of apoptosis in cancer cells, as opposed to normal cells, is attributed to its ability to target mitochondria directly and influence mitochondrial integrity and reactive oxygen species generation. A new series of SHetA2 analogs was evaluated for biological activity. These analogs were modified to enhance their pharmacological properties and binding affinity to the target protein, mortalin. Notably, the analogs with substitutions on Ring B, particularly those with CF3 and OCF3 groups, showed enhanced potency compared to SHetA2. The structure-activity relationship studies revealed that substitutions at specific positions on Ring B and modifications of the linker played crucial roles in the potency of the compounds. Along with this, the flexibility of the linker in the SHetA2 scaffold and the spatial orientation of substituents influence the binding affinity and overall activity of the compounds. Thus, incorporating sulfur heteroarotinoids into thiourea structures enhances their ability to interact with biological macromolecules, potentially leading to more effective and selective anticancer agents.

Integrating pyrimidine and quinazoline pharmacophores (Section 3.7) into thiourea derivatives improves their biological activity and selectivity against cancer targets. Modifying these compounds with groups such as α-naphthol, methoxy groups, and halogens affects their activity against targets, including Hsp90 and EGFR. In particular, halogens in the meta position on the aniline ring effectively inhibit NF-κB. The absence of a methylene spacer between the thiourea linker and the aromatic ring does not reduce activity, and compounds without an aromatic ring show reduced activity. The presence of an aromatic system, as well as optimization of the position and nature of the substituents on the aniline ring, is crucial for the activity of these compounds. These investigations highlight the importance of optimizing substituents for maximum anticancer efficacy.

Research on thiourea derivatives as Sorafenib analogs (Section 3.8) demonstrates significant antitumor and antiangiogenic potential. Compounds with the phenylamino group exhibit strong activity against various cancer cell lines, while their modifications improve selectivity and potency. For example, introducing a nicotinamide substituent significantly enhances these compounds' inhibitory activity and antiangiogenic effects. For example, introducing a nicotinamide substituent significantly enhances these compounds' inhibitory activity and antiangiogenic effects. Compounds with electron-withdrawing groups have demonstrated notable inhibitory effects against B-Raf and VEGFR-2. In general, this Section

highlights the importance of structural changes in improving the therapeutic properties of compounds.

The development of thiourea derivatives as zinc-binding agents (Section 3.9) highlights their potential as selective histone deacetylases (HDACs) inhibitors. These compounds show promising antiproliferative activity and high selectivity, offering new avenues for cancer therapy. Docking studies demonstrate that these derivatives have binding affinities comparable to, or better than, the FDA-approved inhibitor suberoylanilide hydroxamic acid (SAHA). Additionally, in vitro evaluations revealed their significant antiproliferative activity against cancer cell lines and a good selectivity reflected in their binding affinity for HDAC2.

In conclusion, thiourea derivatives are an excellent foundation for developing new anticancer agents. The introduction of various functional groups and structural modifications has significantly improved their potency and selectivity, suggesting their potential for future clinical applications. Molecular docking and high-throughput screening will continue to play crucial roles in optimizing these compounds for cancer treatment.

Funding

This research received no external funding.

Acknowledgments

This research was supported by the National Academy of Sciences of Ukraine (according to fundamental research on the topic “Prediction of bioactivity and creation of modern approaches to the synthesis of heterocyclic compounds for the needs of the pharmaceutical industry” 0123U101194 of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine).

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Suzuki, T.; Shibuya, Y.; Sato, T.; Nishizawa, S.; Sato, I.; Yamaguchi, A. Thermodynamics of Complexation between Thiourea-based Receptor and Acetate in Water/Acetonitrile Mixture. *Analyt. Sci.* **2016**, *32*, 741–744, https://doi.org/10.2116/analsci.32.741.
2. Yuan, Y.-X.; Wang, L.; Han, Y.-F.; Li, F.-F.; Wang, H.-B. A novel azo-thiourea based visible light switchable anion receptor. *Tetrahedron Lett.* **2016**, *57*, 878–882, http://dx.doi.org/10.1016/j.tetlet.2016.01.037.
3. Zhou, X.-b.; Yip, Y.-W.; Chan, W.-H.; Lee, A.W.M. An easy assembled fluorescent sensor for dicarboxylates and acidic amino acids. *Beilstein J. Org. Chem.* **2011**, *7*, 75–81, https://doi.org/10.3762/bjoc.7.11.
4. Blanco, J.L.J.; Benito, J.M.; Mellet, C.O.; Fernández, J.M.G. Sugar Thioureas as Anion Receptors. Effect of Intramolecular Hydrogen Bonding in the Carboxylate Binding Properties of Symmetric Sugar Thioureas. *Org. Lett*. **1999**, *1*, 1217–1220, https://doi.org/10.1021/ol990889s.
5. Ehrhard, A.A.; Jäger, S.; Malm, C.; Basaran, S.; Hunger, J. CF_3-groups critically enhance the binding of thiourea catalysts to ketones – a NMR and FT-IR study. *J. Mol. Liq*. **2019**, *296*, 111829, https://doi.org/10.1016/j.molliq.2019.111829.
6. Zheng, W.; Fu, Y.; Liu, L.; Guo, Q. Hydrogen Bonding Interaction between Ureas or Thioureas and Carbonyl Compounds. *Acta Phys.-Chim. Sin.* **2007**, *23*, 1018–1024, https://doi.org/10.1016/S1872-1508(07)60057-6.

7. Lippert, K.M.; Hof, K.; Gerbig, D.; Ley, D.; Hausmann, H.; Guenther, S.; Schreiner, P.R. Hydrogen-Bonding Thiourea Organocatalysts: The Privileged 3,5-Bis(trifluoromethyl)phenyl Group. *Eur. J. Org. Chem*. **2012**, *2012*, 5919–5927, https://doi.org/10.1002/ejoc.201200739.
8. Spiliopoulou, N.; Nikitas, N.F.; Kokotos, C.G. Photochemical synthesis of acetals utilizing Schreiner's thiourea as the catalyst. *Green Chem*. **2020**, *22*, 3539–3545, https://doi.org/10.1039/D0GC01135E.
9. Zavala-Contreras, B.; Santacruz-Ortega, H.; Orozco-Valencia, A.U.; Inoue, M.; Lara, K.O.; Navarro, R-E. Optical Anion Receptors with Urea/Thiourea Subunits on a TentaGel Support. *ACS Omega* **2021**, *6*, 9381–9390, https://doi.org/10.1021/acsomega.0c05554.
10. Manna, U.; Portis, B.; Egboluche, T.K.; Nafis, M.; Hossain, Md.A. Anion Binding Studies of Urea and Thiourea Functionalized Molecular Clefts. *Front. Chem*. **2021**, *8*, 575701, https://doi.org/10.3389/fchem.2020.575701.
11. Mitrea, D.G.; Cîrcu, V. Synthesis and characterization of novel acylthiourea compounds used in ions recognition and sensing in organic media. *Spectrochim. Acta -A: Mol. Biomol. Spectrosc.* **2021**, *258*, 119860, https://doi.org/10.1016/j.saa.2021.119860.
12. Pattnaik, C.; Singhamahapatra, A.; Parida, R.; Sahoo, S.C.; Sahoo, L.N.; Chary, K.V.R.; Sahoo, S. Structural elucidation of *N*-amidothiourea derived from nicotinic acid hydrazide: A detailed study of its application as selective anion sensor. *Res. Chem.* **2023**, *5*, 100693, https://doi.org/10.1016/j.rechem.2022.100693.
13. Kumar, V.; Kaushik, M.P.; Srivastava, A.K.; Pratapa, A.; Thiruvenkatam, V.; Guru Row, T.N. Thiourea based novel chromogenic sensor for selective detection of fluoride and cyanide anions in organic and aqueous media. *Analyt. Chim. Acta* **2010**, *663*, 77–84, https://doi.org/10.1016/j.aca.2010.01.025.
14. Cao, C.; You, X.; Feng, L.; Luo, G.; Yue, G.; Ji, X. Synthesis of new chromogenic sensors containing thiourea and selective detection for F^-, $H_2PO_4^-$, and Ac^- anions. *Canad. J. Chem*. **2020**, *98*, 659–666, https://doi.org/10.1139/cjc-2020-0120.
15. Takahashi, Y.; Sato, T.; Ito, K. Recognition of Anions Using p-Nitrophenyl Thiourea Based on 1,1-Binaphthyl-2,2-diol. *Lett. Org. Chem*. **2008**, *5*, 676–683, https://doi.org/10.2174/157017808786857480.
16. Kumar, V.; Rana, H.; Kaushik, M.P. A naked-eye selective detection of cyanide ion: studies on the effect of chromophores and spacers on ditopic receptors. *Analyst* **2011**, *136*, 1873–1880, https://doi.org/10.1039/C0AN00959H.
17. Odago, M.O.; Colabello, D.M.; Lees, A.J. A simple thiourea based colorimetric sensor for cyanide anion. *Tetrahedron* **2010**, *66*, 7465–7471, https://doi.org/10.1016/j.tet.2010.07.006.
18. Khansari, M.E.; Johnson, C.R.; Basaran, I.; Nafis, A.; Wang, J.; Leszczynski, J.; Hossain, Md.A. Synthesis and anion binding studies of tris(3-aminopropyl)amine-based tripodal urea and thiourea receptors: proton transfer-induced selectivity for hydrogen sulfate over sulfate. *RSC Adv*. **2015**, *5*, 17606–17614, https://doi.org/10.1039/C5RA01315A.
19. Khansari, M.E.; Mirchi, A.; Pramanik, A.; Johnson, C.R.; Leszczynski, J.; Hossain, Md.A. Remarkable hexafunctional anion receptor with operational ureabased *inner cleft* and thioureabased *outer cleft*: Novel design with high-efficiency for sulfate binding. *Sci. Rep*. **2017**, *7*, 6032, https://doi.org/10.1038/s41598-017-05831-x.
20. Emami Khansari, M.; Hasan, M.H.; Johnson, C.R.; Williams, N.A.; Wong, B.M.; Powell, D.R.; Tandon, R.; Hossain, M.A. Anion Complexation Studies of 3-Nitrophenyl-Substituted Tripodal Thiourea Receptor: A Naked-Eye Detection of Sulfate via Fluoride Displacement Assay. *ACS Omega* **2017**, *2*, 9057–9066, https://doi.org/10.1021/acsomega.7b01485.
21. Basaran, I.; Khansari, M.E.; Pramanik, A.; Wong, B.M.; Hossain, M.A. Binding and selectivity of dihydrogen phosphate by H-bond donors and acceptors in a tripodal-based thiourea receptor. *Tetrahedron Lett*. **2015**, *56*, 115–118, https://doi.org/10.1016/j.tetlet.2014.11.025.
22. Beletskiy, E.V.; Kass, S.R. Selective binding and extraction of aqueous dihydrogen phosphate solutions *via* three-armed thiourea receptors. *Org. Biomol. Chem*. **2015**, *13*, 9844–9849, https://doi.org/10.1039/C5OB01450F.
23. Al-Saidi, H.M.; Khan, S. Recent Advances in Thiourea Based Colorimetric and Fluorescent Chemosensors for Detection of Anions and Neutral Analytes: A Review. *Crit. Rev. Analyt. Chem*. **2024**, *54*, 93–109, https://doi.org/10.1080/10408347.2022.2063017.
24. Kundu, S.; Egboluche, T.K.; Hossain, M.A. Urea- and Thiourea-Based Receptors for Anion Binding. *Acc. Chem. Res*. **2023**, *56*, 1320–1329, https://doi.org/10.1021/acs.accounts.2c00701.

25. Nural, Y.; Karasu, E.; Keleş, E.; Aydıner, B.; Seferoğlu, N.; Efeoğlu, Ç.; Şahin, E.; Seferoğlu, Z. Synthesis of novel acylthioureas bearing naphthoquinone moiety as dual sensor for high-performance naked-eye colorimetric and fluorescence detection of CN^- and F^- ions and its application in water and food samples. *Dyes Pigments* **2022**, *198*, 110006, https://doi.org/10.1016/j.dyepig.2021.110006.
26. Chung, Y.K.; Ha, S.; Woo, T.G.; Kim, Y.D.; Song, C.; Kim, S.K. Binding thiourea derivatives with dimethyl methylphosphonate for sensing nerve agents. *RSC Adv*. **2019**, *9*, 10693–10701, https://doi.org/10.1039/C9RA00314B.
27. Ellaby, R.J.; Chu, D.F.; Pépés, A.; Clark, E.R.; Hiscock, J. Predicting the hydrolytic breakdown rates of organophosphorus chemical warfare agent simulants using association constants derived from hydrogen bonded complex formation events. *Supramolecular Chem*. **2021**, *33*, 309–317, https://doi.org/10.1080/10610278.2021.1999 450.
28. Kumar, V. Urea/Thiourea Based Optical Sensors for Toxic Analytes: A Convenient Path for Detection of First Nerve Agent (Tabun). *Bull. Chem. Soc. Jpn*. **2021**, *94*, 309–326, https://doi.org/10.1246/bcsj.20200285.
29. Kumar, V.; Rana, H.; Raviraju, G.; Garg, P.; Baghel, A.; Gupta, A.K. Chromogenic and fluorogenic multianalyte detection with a tuned receptor: refining selectivity for toxic anions and nerve agents. *RSC Adv*. **2016**, *6*, 59648–59656, https://doi.org/10.1039/C6RA07080A.
30. Steppeler, F.; Iwan, D.; Wojaczyńska, E.; Wojaczyński, J. Chiral Thioureas—Preparation and Significance in Asymmetric Synthesis and Medicinal Chemistry. *Molecules* **2020**, *25*, 401, https://doi.org/10.3390/molecules25020401.
31. Vera, S.; García-Urricelqui, A.; Mielgo, A; Oiarbide, M. Progress in (Thio)urea- and Squaramide-Based Brønsted Base Catalysts with Multiple H-Bond Donors. *Eur. J. Org. Chem*. **2023**, *26*, e202201254, https://doi.org/10.1002/ejoc.202201254.
32. Phillips, A.M.F.; Prechtl, M.H.G.; Pombeiro, A.J.L. Non-Covalent Interactions in Enantioselective Organocatalysis: Theoretical and Mechanistic Studies of Reactions Mediated by Dual H-Bond Donors, Bifunctional Squaramides, Thioureas and Related Catalysts. *Catalysts* **2021**, *11*, 569, https://doi.org/10.3390/catal11050569.
33. Parvin, T.; Yadav, R.; Choudhury, L.H. Recent applications of thiourea-based organocatalysts in asymmetric multicomponent reactions (AMCRs). *Org. Biomol. Chem*. **2020**, *18*, 5513–5532, https://doi.org/10.1039/D0OB00595A.
34. Jain, I.; Malik, P. Advances in urea and thiourea catalyzed ring opening polymerization: A brief overview. *Eur. Polym. J*. **2020**, *133*, 109791, https://doi.org/10.1016/j.eurpolymj.2020.109791.
35. McDermott, P.E.; Fearraigh, M.P.Ó.; Horan, A.M.; McGarrigle, E.M. Thiourea-catalysed conjugate additions of amines to vinyl phosphonates and phosphinates. *Org. Biomol. Chem*. **2023**, *21*, 1027–1032, https://doi.org/10.1039/d2ob02116a.
36. Ehrhard, A.A.; Gunkel, L.; Jäger, S.; Sell, A.C.; Nagata, Y.; Hunger, J. Elucidating Conformation and Hydrogen-Bonding Motifs of Reactive Thiourea Intermediates. *ACS Catal*. **2022**, *12*, 12689–12700, https://doi.org/10.1021/acscatal.2c03382.
37. Li, M.; Zhang, S.; Zhang, X.; Wang, Y.; Chen, J.; Tao, Y.; Wang, X. Unimolecular Anion-Binding Catalysts for Selective Ring-Opening Polymerization of *O*-carboxyanhydrides. *Angew. Chem. Inter. Ed*. **2021**, *60*, 6003–6012, https://doi.org/10.1002/anie.202011352.
38. Mohite, A.R.; Phatake, R.S.; Dubey, P.; Agbaria, M.; Shames, A.I.; Lemcoff, N.G.; Reany, O. Thiourea-Mediated Halogenation of Alcohols. *J. Org. Chem*. **2020**, *85*, 12901–12911, https://doi.org/10.1021/acs.joc.0c01431.
39. Smajlagic, I.; Guest, M.; Durán, R.; Herrera, B.; Dudding, T. Mechanistic Insight toward Understanding the Role of Charge in Thiourea Organocatalysis. *J. Org. Chem*. **2020**, *85*, 585−593, https://doi.org/10.1021/acs.joc.9b02682.
40. Mayfield, A.B.; Metternich, J.B.; Trotta, A.H.; Jacobsen, E.N. Stereospecific Furanosylations Catalyzed by Bis-thiourea Hydrogen-Bond Donors. *J. Am. Chem. Soc*. **2020**, *142*, 4061−4069, https://dx.doi.org/10.1021/jacs.0c00335.
41. Rufino, V.C.; Pliego Jr., J.R. Bifunctional Primary Amino-thiourea Asymmetric Catalysis: The Imine-Iminium Ion Mechanism in the Michael Addition of Nitromethane to Enone. *Asian J. Org Chem*. **2021**, *10*, 1472–1485, https://doi.org/10.1002/ajoc.202100160.
42. Arshad, N.; Parveen, U.; Channar, P.A.; Saeed, A.; Saeed, W.S.; Perveen, F.; Javed, A.; Ismail, H.; Mir, M.I.; Ahmed, A.; Azad, B.; Khan, I. Investigation of Newly Synthesized Bis-Acyl-Thiourea Derivatives of

4-Nitrobenzene-1,2-Diamine for Their DNA Binding, Urease Inhibition, and Anti-Brain-Tumor Activities. *Molecules* **2023**, *28*, 2707, https://doi.org/10.3390/molecules28062707.
43. Ronchetti, R.; Moroni, G.; Carotti, A.; Gioiello, A.; Camaioni, E. Recent advances in urea- and thiourea-containing compounds: focus on innovative approaches in medicinal chemistry and organic synthesis. *RSC Med. Chem*. **2021**, *12*, 1046–1064, https://doi.org/10.1039/D1MD00058F.
44. Shakeel, A.; Altaf, A.A.; Qureshi, A.M.; Badshah, A. Thiourea Derivatives in Drug Design and Medicinal Chemistry: A Short Review. *J. Drug Des. Med. Chem*. **2016**, *2*, https://dx.doi.org/10.11648/j.jddmc.20160201.12.
45. Breton, M.; Leblond, J.; Tranchant, I.; Scherman, D.; Bessodes, M.; Herscovici, J.; Mignet, N. Lipothioureas as Lipids for Gene Transfection: A Review. *Pharmaceuticals* **2011**, *4*, 1381–1399, https://doi.org/10.3390/ph4101381.
46. Shivakumara, K.N.; Sridhar, B.T. Review on role of urea and thiourea derivatives of some heterocyclic scaffold in drug design and medicinal chemistry. *Int. J. Chem. Res. Dev*. **2021**, *3*, 20–25, https://doi.org/10.33545/26646552.2021.v3.i1a.23.
47. Phetsuksiri, B.; Jackson, M.; Scherman, H.; McNeil, M.; Besra, G.S.; Baulard, A.R.; Slayden, R.A.; DeBarber, A.E.; Barry, C.E.; Baird, M.S.; Crick, D.C.; Brennan, P.J. Unique Mechanism of Action of the Thiourea Drug Isoxyl on *Mycobacterium tuberculosis*. *J. Biol. Chem*. **2003**, *278*, 53123–53130, https://doi.org/10.1074/jbc.M311209200.
48. Alahari, A.; Trivelli, X.; Guérardel, Y.; Dover, L.G.; Besra, G.S.; Sacchettini, J.C.; Reynolds, R.C.; Coxon, G.D.; Kremer, L. Thiacetazone, an Antitubercular Drug that Inhibits Cyclopropanation of Cell Wall Mycolic Acids in Mycobacteria. *PLoS ONE* **2007**, *12*, e1343, https://doi.org/10.1371/journal.pone.0001343.
49. Browne, M.K.; Stoller, J.L. Intraperitoneal noxythiolin in faecal peritonitis. *Brit. J. Surg*. **1970**, *57*, 525–529, https://doi.org/10.1002/bjs.1800570711.
50. Wyllie, J.H.; Hesselbo, T.; Black, J.W. EFFECTS IN MAN OF HISTAMINE H_2-RECEPTOR BLOCKADE BY BURIMAMIDE. *Lancet* **1972**, *300*, 1117–1120, https://doi.org/10.1016/S0140-6736(72)92719-5.
51. Black, J.W.; Duncan, W.A.M.; Emmett, J.C.; Ganellin, C.R.; Hesselbo, T.; Parsons, M.E.; Wyllie, J.H. Metiamide – An orally active histamine H_2-receptor antagonist. *Agents Actions* **1973**, *3*, 133–137, https://doi.org/10.1007/BF01965723.
52. Parsons, M.E.; Ganellin, C.R. Histamine and its receptors. *Brit. J. Pharmacol*. **2006**, *147*, S127–S135, https://doi.org/10.1038/sj.bjp.0706440.
53. Elks, J.; Ganellin, C.R. L. In *Dictionary of Drugs: Chemical Data, Structures and Bibliographies*, Elks, J., Ganellin, C.R., Eds.; Springer US: Boston, MA, **1990**; pp. 723-747, https://doi.org/10.1007/978-1-4757-2085-3_12.
54. Lain, S.; Hollick, J.J.; Campbell, J.; Staples, O.D.; Higgins, M.; Aoubala, M.; McCarthy, A.; Appleyard, V.; Murray, K.E.; Baker, L.; Thompson, A.; Mathers, J.; Holland, S.J.; Stark, M.J.R.; Pass, G.; Woods, J.; Lane, D.P.; Westwood, N.J. Discovery, In Vivo Activity, and Mechanism of Action of a Small-Molecule p53 Activator. *Cancer Cell* **2008**, *13*, 454–463, https://doi.org/10.1016/j.ccr.2008.03.004.
55. Dai, W.; Zhou, J.; Jin, B.; Pan, J. Class III-specific HDAC inhibitor Tenovin-6 induces apoptosis, suppresses migration and eliminates cancer stem cells in uveal melanoma. *Sci. Rep*. **2016**, *6*, 22622, https://doi.org/10.1038/srep22622.
56. Liu, S.; Zhou, G.; Lo, S.N.H.L.; Louie, M.; Rajagopalan, V. SHetA2, a New Cancer-Preventive Drug Candidate. In Anti-cancer Drugs - Nature, Synthesis and Cell, Bankovic, J., Ed.; IntechOpen: Rijeka, **2016**; http://dx.doi.org/10.5772/65365.
57. Finch, R.A.; Liu, M.-C.; Cory, A.H.; Cory, J.G.; Sartorelli, A.C. Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone; 3-AP): an inhibitor of ribonucleotide reductase with antineoplastic activity. *Adv. Enzyme Regul*. **1999**, *39*, 3–12, https://doi.org/10.1016/s0065-2571(98)00017-x.
58. Yen, Y.; Margolin, K.; Doroshow, J.; Fishman, M.; Johnson, B.; Clairmont, C.; Sullivan, D.; Sznol, M. A phase I trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone in combination with gemcitabine for patients with advanced cancer. *Cancer Chemother. Pharmacol*. **2004**, *54*, 331–342, https://doi.org/10.1007/s00280-004-0821-2.
59. Hwang, D.F.; Chen, T.Y. Toxins in Food: Naturally Occurring. In *Encyclopedia of Food and Health*, Caballero, B., Finglas, P.M., Toldrá, F., Eds.; Academic Press: Oxford, **2016**; pp. 326-330, https://doi.org/10.1016/B978-0-12-384947-2.00698-X.

60. Mechoulam, R.; Sondheimer, F.; Melera, A.; Kincl, F.A. THE STRUCTURE OF ZAPOTIDINE. *J. Am. Chem. Soc*. **1961**, *83*, 2022–2023, https://doi.org/10.1021/ja01469a064.
61. D'Cruz, O.J.; Uckun, F.M. Novel tight binding PETT, HEPT and DABO-based non-nucleoside inhibitors of HIV-1 reverse transcriptase. *J. Enzyme Inhib. Med. Chem*. **2006**, *21*, 329–350, https://doi.org/10.1080/14756360600774413.
62. van Zeijl, M.; Fairhurst, J.; Jones Thomas, R.; Vernon Steven, K.; Morin, J.; LaRocque, J.; Feld, B.; O'Hara, B.; Bloom Jonathan, D.; Johann Stephen, V. Novel Class of Thiourea Compounds That Inhibit Herpes Simplex Virus Type 1 DNA Cleavage and Encapsidation: Resistance Maps to the UL6 Gene. *J. Virol.* **2000**, *74*, 9054–9061, https://doi.org/10.1128/jvi.74.19.9054-9061.2000.
63. Bielenica, A.; Stefańska, J.; Stępień, K.; Napiórkowska, A.; Augustynowicz-Kopeć, E.; Sanna, G.; Madeddu, S.; Boi, S.; Giliberti, G.; Wrzosek, M.; Struga, M. Synthesis, cytotoxicity and antimicrobial activity of thiourea derivatives incorporating 3-(trifluoromethyl)phenyl moiety. *Eur. J. Med. Chem*. **2015**, *101*, 111–125, https://doi.org/10.1016/j.ejmech.2015.06.027.
64. Doğan, Ş.D.; Gündüz, M.G.; Doğan, H.; Krishna, V.S.; Lherbet, C.; Sriram, D. Design and synthesis of thiourea-based derivatives as *Mycobacterium tuberculosis* growth and enoyl acyl carrier protein reductase (InhA) inhibitors. *Eur. J. Med. Chem*. **2020**, *199*, 112402, https://doi.org/10.1016/j.ejmech.2020.112402.
65. Wang, H.; Zhai, Z.-W.; Shi, Y.-X.; Tan, C.-X.; Weng, J.-Q.; Han, L.; Li, B.-J.; Liu, X.-H. Novel Trifluoromethylpyrazole Acyl Thiourea Derivatives: Synthesis, Antifungal Activity and Docking Study. *Lett. Drug. Des. Discov*. **2019**, *16*, 785–791, https://doi.org/10.2174/1570180815666180704103047.
66. Müller, J.; Limban, C.; Stadelmann, B.; Missir, A.V.; Chirita, I.C.; Chifiriuc, M.C.; Nitulescu, G.M.; Hemphill, A. Thioureides of 2-(phenoxymethyl)benzoic acid 4-R substituted: A novel class of anti-parasitic compounds. *Parasitol. Int*. **2009**, *58*, 128–135, https://doi.org/10.1016/j.parint.2008.12.003.
67. Lambert, W.T.; Goldsmith, M.E.; Sparks, T.C. Insecticidal activity of novel thioureas and isothioureas. *Pest Manag. Sci*. **2016**, *73*, 743–751, https://doi.org/10.1002/ps.4353.
68. Huong, D.Q.; Bay, M.V.; Nam, P.C. Antioxidant activity of thiourea derivatives: An experimental and theoretical study. *J. Mol. Liq*. **2021**, *340*, 117149, https://doi.org/10.1016/j.molliq.2021.117149.
69. Li, J.H.; Li, R.H.; Wang, Y.; Li, S.X.; Wu, Y.P.; Zhang, J.; Zhu, Y.G.; Xie, B.J. Synthesis, herbicidal activity, enzyme activity, and molecular docking of novel aniline thiourea. *Phosphorus Sulfur Silicon Relat. Elem*. **2021**, *196*, 664–671, https://doi.org/10.1080/10426507.2021.1901702.
70. Wahid, A.; Basra, S.M.A.; Farooq, M. Thiourea: A Molecule with Immense Biological Significance for Plants. *Int. J. Agric. Biol*. **2017**, *19*, 911–920.
71. Shankaraiah, N.; Kumar, N.P.; Amula, S.B.; Nekkanti, S.; Jeengar, M.K.; Naidu, V.G.M.; Reddy, T.S.; Kamal, A. One-pot synthesis of podophyllotoxin–thiourea congeners by employing NH_2SO_3H/NaI: Anticancer activity, DNA topoisomerase-II inhibition, and apoptosis inducing agents. *Bioorg. Med. Chem. Lett*. **2015**, *25*, 4239–4244, https://doi.org/10.1016/j.bmcl.2015.07.100.
72. Zang, W.; Hao, Y.; Wang, Z.; Zheng, W. Novel thiourea-based sirtuin inhibitory warheads. *Bioorg. Med. Chem. Lett*. **2015**, *25*, 3319–3324, https://doi.org/10.1016/j.bmcl.2015.05.058.
73. Farooqi, A.S.; Hong, J.Y.; Cao, J.; Lu, X.; Price, I.R.; Zhao, Q.; Kosciuk, T.; Yang, M.; Bai, J.J.; Lin, H. Novel Lysine-Based Thioureas as Mechanism-Based Inhibitors of Sirtuin 2 (SIRT2) with Anticancer Activity in a Colorectal Cancer Murine Model. *J. Med. Chem*. **2019**, *62*, 4131–4141, https://doi.org/10.1021/acs.jmedchem.9b00191.
74. Parmar, D.R.; Soni, J.Y.; Guduru, R.; Rayani, R.H.; Kusurkar, R.V.; Vala, A.G.; Talukdar, S.N.; Eissa, I.H.; Metwaly, A.M.; Khalil, A.; Zunjar, V.; Battula, S. Discovery of new anticancer thiourea-azetidine hybrids: design, synthesis, *in vitro* antiproliferative, SAR, *in silico* molecular docking against VEGFR-2, ADMET, toxicity, and DFT studies. *Bioorg. Chem*. **2021**, *115*, 105206, https://doi.org/10.1016/j.bioorg.2021.105206.
75. Abbas, S.Y.; Al-Harbi, R.A.K.; Sh El-Sharief, M.A.M. Synthesis and anticancer activity of thiourea derivatives bearing a benzodioxole moiety with EGFR inhibitory activity, apoptosis assay and molecular docking study. *Eur. J. Med. Chem.* **2020**, *198*, 112363, https://doi.org/10.1016/j.ejmech.2020.112363.
76. Sharma, S.K.; Wu, Y.; Steinbergs, N.; Crowley, M.L.; Hanson, A.S.; Casero Jr., R.A.; Woster, P.M. (Bis)urea and (Bis)thiourea Inhibitors of Lysine-Specific Demethylase 1 as Epigenetic Modulators. *J. Med. Chem*. **2010**, *53*, 5197–5212, https://doi.org/10.1021/jm100217a.
77. Kumar, V.; Chimni, S.S. Recent Developments on Thiourea Based Anticancer Chemotherapeutics. *Anti-Cancer Agents Med. Chem.* **2015**, *15*, 163–175, https://doi.org/10.2174/1871520614666140407123526.

78. Siegel, R.L.; Miller, K.D.; Wagle, N.S.; Jemal, A. Cancer statistics, 2023. *CA Cancer J. Clin*. **2023**, *73*, 17–48, https://doi.org/10.3322/caac.21763.
79. ReFaey, K.; Tripathi, S.; Grewal, S.S.; Bhargav, A.G.; Quinones, D.J.; Chaichana, K.L.; Antwi, S.O.; Cooper, L.T.; Meyer, F.B.; Dronca, R.S.; Diasio, R.B.; Quinones-Hinojosa, A. Cancer Mortality Rates Increasing vs Cardiovascular Disease Mortality Decreasing in the World: Future Implications. *Mayo Clin. Proc. Innov. Qual. Outcomes* **2021**, *5*, 645–653, https://doi.org/10.1016/j.mayocpiqo.2021.05.005.
80. Kerru, N.; Singh, P.; Koorbanally, N.; Raj, R.; Kumar, V. Recent advances (2015–2016) in anticancer hybrids. *Eur. J. Med. Chem*. **2017**, *2017*, 179–212, http://dx.doi.org/10.1016/j.ejmech.2017.07.033.
81. Fu, R.-G.; Sun, Y.; Sheng, W.-B.; Liao, D.-F. Designing multi-targeted agents: An emerging anticancer drug discovery paradigm. *Eur. J. Med. Chem*. **2017**, *136*, 195–211, https://doi.org/10.1016/j.ejmech.2017.05.016.
82. El-Atawy, M.A.; Alsubaie, M.S.; Alazmi, M.L.; Hamed, E.A.; Hanna, D.H.; Ahmed, H.A.; Omar, A.Z. Synthesis, Characterization, and Anticancer Activity of New N,N′-Diarylthiourea Derivative against Breast Cancer Cells. *Molecules* **2023**, *28*, 6420, https://doi.org/10.3390/molecules28176420.
83. Horvat, D.; Khansari, M.E.; Pramanik, A.; Beeram, M.R.; Kuehl, T.J.; Hossain, M.A.; Uddin, M.N. A Synthetic Thiourea-Based Tripodal Receptor that Impairs the Function of Human First Trimester Cytotrophoblast Cells. *Int. J. Environ. Res. Public Health* **2014**, *11*, 7456–7469, https://doi.org/10.3390/ijerph110707456.
84. Zhang, Y.; Meng, X.; Tang, H.; Cheng, M.; Yang, F.; Xu, W. Design, synthesis, and biological evaluation of novel substituted thiourea derivatives as potential anticancer agents for NSCLC by blocking K-Ras protein-effectors interactions. *J. Enzyme Inhib. Med. Chem*. **2020**, *35*, 344–353, https://doi.org/10.1080/14756366.2019.1702653.
85. Strzyga-Łach, P.; Chrzanowska, A.; Podsadni, K.; Bielenica, A. Investigation of the Mechanisms of Cytotoxic Activity of 1,3-Disubstituted Thiourea Derivatives. *Pharmaceuticals* **2021**, *14*, 1097, https://doi.org/10.3390/ph14111097.
86. Zou, H.; Sevigny, M.B.; Liu, S.; Madden, D.T.; Louie, M.C. Novel flexible heteroarotinoid, SL-1-39, inhibits HER2-positive breast cancer cell proliferation by promoting lysosomal degradation of HER2. *Cancer Lett*. **2019**, *443*, 157–166, https://doi.org/10.1016/j.canlet.2018.11.022.
87. Liu, S.; Louie, M.C.; Rajagopalan, V.; Zhou, G.; Ponce, E.; Nguyen, T.; Green, L. Synthesis and evaluation of the diarylthiourea analogs as novel anti-cancer agents. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1301–1305, https://doi.org/10.1016/j.bmcl.2015.01.042.
88. Ginn, E.; Baek, J.; Zou, H.; Fallatah, M.M.J.; Liu, S.; Sevigny, M.B.; Louie, M. Enantiomer of the novel flexible heteroarotinoid, SL-1-09, blocks cell cycle progression in breast cancer cells. *Eur. J. Pharmacol*. **2019**, *862*, 172634, https://doi.org/10.1016/j.ejphar.2019.172634.
89. Arshad, N.; Saeed, A.; Perveen, F.; Ujan, R.; Farooqi, S.I.; Ali Channar, P.; Shabir, G.; El-Seedi, H.R.; Javed, A.; Yamin, M.; Bolte, M.; Hökelek, T. Synthesis, X-ray, Hirshfeld surface analysis, exploration of DNA binding, urease enzyme inhibition and anticancer activities of novel adamantane-naphthyl thiourea conjugate. *Bioorg. Chem*. **2021**, *109*, 104707, https://doi.org/10.1016/j.bioorg.2021.104707.
90. Yeşilkaynak, T.; Nur Özkömeç, F.; Çeşme, M.; Demirdöğen, R.E.; Sezer, C.V.; Kutlu, H.M.; Emen, F.M. Novel thiourea derivative compounds: Thermal behavior, biological evaluation, Hirshfeld surfaces and frontier orbitals analyses, in silico ADMET profiling and molecular docking studies. *J. Mol. Struct*. **2023**, *1280*, 135086, https://doi.org/10.1016/j.molstruc.2023.135086.
91. Elseginy, S.A.; Hamdy, R.; Menon, V.; Almehdi, A.M.; El-Awady, R.; Soliman, S.S.M. Design, synthesis, and computational validation of novel compounds selectively targeting HER2-expressing breast cancer. *Bioorg. Med. Chem. Lett*. **2020**, *30*, 127658, https://doi.org/10.1016/j.bmcl.2020.127658.
92. Ragab, F.A.F.; Abdel-Aziz, S.A.; Kamel, M.; Ouf, A.M.A.; Allam, H.A. Design, synthesis and biological evaluation of some new 1,3,4-thiadiazine-thiourea derivatives as potential antitumor agents against non-small cell lung cancer cells. *Bioorg. Chem*. **2019**, *93*, 103323, https://doi.org/10.1016/j.bioorg.2019.103323.
93. Yang, B.; Yang, Y.-S.; Yang, N.; Li, G.; Zhu, H.-L. Design, biological evaluation and 3D QSAR studies of novel dioxin-containing pyrazoline derivatives with thiourea skeleton as selective HER-2 inhibitors. *Sci. Rep*. **2016**, *6*, 27571, https://doi.org/10.1038/srep27571.
94. Solomon, V.R.; Pundir, S.; Lee, H. Design and synthesis of 4-piperazinyl quinoline derived urea/thioureas for anti-breast cancer activity by a hybrid pharmacophore approach. *J. Enzyme Inhib. Med. Chem*. **2019**, *34*, 620–630, https://doi.org/10.1080/14756366.2019.1571055.

95. Al-Harbi, R.A.K.; Sh. El-Sharief, M.A.M.; Abbas, S.Y. Synthesis and anticancer activity of *bis*-benzo[*d*][1,3]dioxol-5-yl thiourea derivatives with molecular docking study. *Bioorg. Chem*. **2019**, *90*, 103088, https://doi.org/10.1016/j.bioorg.2019.103088.
96. Shing, J.C.; Won, C.J.; Robert, C.; A., S.M.; N., S.J.; Abdul, F.; and Bram, R.J. A novel synthetic 1,3-phenyl bis-thiourea compound targets microtubule polymerization to cause cancer cell death. *Cancer Biol. Ther*. **2014**, *15*, 895–905, https://doi.org/10.4161/cbt.28881.
97. Pingaew, R.; Prachayasittikul, V.; Worachartcheewan, A.; Thongnum, A.; Prachayasittikul, S.; Ruchirawat, S.; Prachayasittikul, V. Anticancer activity and QSAR study of sulfur-containing thiourea and sulfonamide derivatives. *Heliyon* **2022**, *8*, e10067, https://doi.org/10.1016/j.heliyon.2022.e10067.
98. Nowotarski, S.L.; Pachaiyappan, B.; Holshouser, S.L.; Kutz, C.J.; Li, Y.; Huang, Y.; Sharma, S.K.; Casero Jr, R.A.; Woster, P.M. Structure–activity study for (bis)ureidopropyl- and (bis)thioureidopropyldiamine LSD1 inhibitors with 3-5-3 and 3-6-3 carbon backbone architectures. *Bioorg. Med. Chem*. **2015**, *23*, 1601–1612, https://doi.org/10.1016/j.bmc.2015.01.049.
99. Arafa, W.A.A.; Ghoneim, A.A.; Mourad, A.K. *N*-Naphthoyl Thiourea Derivatives: An Efficient Ultrasonic-Assisted Synthesis, Reaction, and *In Vitro* Anticancer Evaluations. *ACS Omega* **2022**, *7*, 6210−6222, https://doi.org/10.1021/acsomega.1c06718.
100. Sharma, A.; Benbrook, D.M.; Woo, S. Pharmacokinetics and interspecies scaling of a novel, orally-bioavailable anti-cancer drug SHetA. *PLoS ONE* **2018**, *13*, e0194046, https://doi.org/10.1371/journal.pone.0194046.
101. Liu, T.; Hannafon, B.; Gill, L.; Kelly, W.; Benbrook, D. Flex-Hets differentially induce apoptosis in cancer over normal cells by directly targeting mitochondria. *Mol. Cancer Ther*. **2007**, *6*, 1814–1822, https://doi.org/10.1158/1535-7163.MCT-06-0279.
102. Nammalwar, B.; Bunce, R.A.; Berlin, K.D.; Benbrook, D.M.; Toal, C. Synthesis and biological evaluation of SHetA2 (NSC-721689) analogs against the ovarian cancer cell line A2780. *Eur. J. Med. Chem*. **2019**, *170*, 16–27, https://doi.org/10.1016/j.ejmech.2019.03.010.
103. Alshammari, M.B.; Aly, A.A.; Youssif, B.G.M.; Bräse, S.; Ahmad, A.; Brown, A.B.; Ibrahim, M.A.A.; Mohamed, A.H. Design and synthesis of new thiazolidinone/uracil derivatives as antiproliferative agents targeting EGFR and/or $BRAF^{V600E}$. *Front Chem*. **2022**, *10*, 1076383, https://doi.org/10.3389/fchem.2022.1076383.
104. Koca, İ.; Özgür, A.; Er, M.; Gümüş, M.; Coşkun, K.A.; Tutar, Y. Design and synthesis of pyrimidinyl acyl thioureas as novel Hsp90 inhibitors in invasive ductal breast cancer and its bone metastasis. *Eur. J. Med. Chem*. **2016**, *122*, 280–290, https://doi.org/10.1016/j.ejmech.2016.06.032.
105. Hamed, M.M.; Darwish, S.S.; Herrmann, J.; Abadi, A.H.; Enge, M. First Bispecific Inhibitors of the Epidermal Growth Factor Receptor Kinase and the NF-κB Activity As Novel Anticancer Agents. *J. Med. Chem*. **2017**, *60*, 2853−2868, https://doi.org/10.1021/acs.jmedchem.6b01774.
106. Mowafy, S.; Galanis, A.; Doctor, Z.M.; Paranal, R.M.; Lasheen, D.S.; Farag, N.A.; Jänne, P.A.; Abouzid, K.A.M. Toward discovery of mutant EGFR inhibitors; Design, synthesis and in vitro biological evaluation of potent 4-arylamino-6-ureido and thioureido-quinazoline derivatives. *Bioorg. Med. Chem*. **2016**, *24*, 3501–3512, https://doi.org/10.1016/j.bmc.2016.05.063.
107. Li, Z.-H.; Liu, X.-Q.; Zhao, T.-Q.; Geng, P.-F.; Guo, W.-G.; Yu, B.; Liu, H.-M. Design, synthesis and preliminary biological evaluation of new [1,2,3]triazolo[4,5-*d*]pyrimidine/thiourea hybrids as antiproliferative agents. *Eur. J. Med. Chem*. **2017**, *139*, 741–749 https://doi.org/10.1016/j.ejmech.2017.08.042.
108. Bai, W.; Ji, J.; Huang, Q.; Wei, W. Synthesis and evaluation of new thiourea derivatives as antitumor and antiangiogenic agents. *Tetrahedron Lett*. **2020**, *61*, 152366, https://doi.org/10.1016/j.tetlet.2020.152366.
109. Abdelgalil, A.A.; Alkahtani, H.M.; Al-Jenoobi, F.I. Chapter Four - Sorafenib. In Profiles of Drug Substances, Excipients and Related Methodology; Academic Press: **2019**; Volume 44, pp. 239-266, https://doi.org/10.1016/bs.podrm.2018.11.003.
110. Kong, X.; Yao, Z.; He, Z.; Xu, W.; Yao, J. Design, synthesis and biological evaluation of thiourea and nicotinamide-containing sorafenib analogs as antitumor agents. *Med. Chem. Commun*. **2015**, *6*, 867–870, https://doi.org/10.1039/C4MD00536H.
111. Yao, J.; He, Z.; Chen, J.; Sun, W.; Fang, H.; Xu, W. Design, synthesis and biological activities of sorafenib derivatives as antitumor agents. *Bioorg. Med. Chem. Lett*. **2012**, *22*, 6549–6553, https://doi.org/10.1016/j.bmcl.2012.09.031.

112. Sun, S.; He, Z.; Huang, M.; Wang, N.; He, Z.; Kong, X.; Yao, J. Design and discovery of thioether and nicotinamide containing sorafenib analogs as multikinase inhibitors targeting B-Raf, B-Raf^{V600E} and VEGFR-2. *Bioorg. Med. Chem*. **2018**, *26*, 2381–2391, https://doi.org/10.1016/j.bmc.2018.03.039.
113. Micheletti, G.; Boga, C.; Drius, G.; Bordoni, S.; Calonghi, N. Suberoylanilide Hydroxamic Acid Analogs with Heteroaryl Amide Group and Different Chain Length: Synthesis and Effect on Histone Deacetylase. *Molecules* **2024**, *29*, 238, https://doi.org/10.3390/molecules29010238.
114. Al-Amily, D.H.; Hassan Mohammed, M. Design, Synthesis, and Docking Study of Acyl Thiourea Derivatives as Possible Histone Deacetylase Inhibitors with a Novel Zinc Binding Group. *Sci. Pharm*. **2019**, *87*, 28, https://doi.org/10.3390/scipharm87040028.