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Article

Synthesis, Biological Evaluation, and Molecular Docking of Novel Azolylhydrazonothiazoles as Potential Anticancer Agents

Jehan Y. Al-Humaidi, Sobhi M. Gomha,* Sayed M. Riyadh, Mohamed S. Ibrahim, Magdi E. A. Zaki, Tariq Z. Abolibda,* Ohoud A. Jefri, and Amr S. Abouzied

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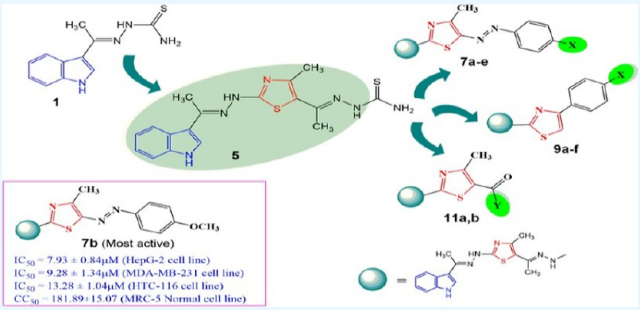
ABSTRACT: A novel set of thiazolylhydrazonothiazoles bearing an indole moiety were synthesized by subjection reactions of carbothioamide derivative and hydrazonoyl chlorides (or α-haloketones). The cytotoxicity of the synthesized compounds was evaluated against the colon carcinoma cell line (HCT-116), liver carcinoma cell line (HepG2), and breast carcinoma cell line (MDA-MB-231), and demonstrated encouraging activity. Furthermore, when representative products were assessed for toxicity against normal cells, minimal toxic effects were observed, indicating their potential safety for use in pharmacological studies. The mechanism of action of the tested products, as inhibitors of the epidermal growth factor receptor tyrosine kinase domain (EGFR TK) protein, was suggested through docking studies that assessed their binding scores and modes, in comparison to a reference standard (W19), thus endorsing their anticancer activity.



1. INTRODUCTION

The hallmark of cancer disease is an uncontrolled mechanism that regulates abnormal growth in normal cells.[1] The available anticancer drugs have inadequate merits such as toxicity, lack of selectivity, and long-dose resistance.[2] Therefore, the development of anticancer agents which have an advanced mechanism of action for suppression of cells is considered to be a valuable target in drug discovery. Triple-negative breast cancer could be caused by epidermal growth factor receptors (EGFR).[3] Compounds with a heterocyclic core play an important role in the design and development of an entirely novel category of structural features for medicinal purposes. Indole derivatives represent an important structural class in drug discovery due to its important biological activities.[4−8] The Indole skeleton is one of the most attractive structures with potent anticancer action, and it is widely found in both active chemicals and natural products.[9] As currently known, various indole derivatives, including mitraphylline, cediranib, indomethacin, indoximod, tryptophol, vincristine, and topsentine, have been shown to be effective anticancer medications[10−18] (Figure 1). A lot of indole-based derivatives additionally demonstrated tyrosine kinase (TK) inhibitory action against breast cancer cell lines, according to the literature study.[19,20] On the other hand, thiazoles tethered by heterocyclic compounds have a prominent role in medicinal chemistry due to their wide range of activities in the field of drug design and discovery. Their applications have been investigated for inhibition of EGFR as potential antitumor agents.[3] Pyrazolylthiazoles have revealed significant in vitro antiproliferative activity against MCF-7.[21] Also, *N*-pyridinyl-2-(6-phenylimidazo[2,1-*b*]thiazol-3-yl)acetamides have demonstrated inhibitory activity against VEGFR2 kinase.[22] Moreover, 5-benzylidene- 2,4-thiazolidine diones have been evaluated as VEGFR-2 kinase inhibitors and revealed anti-angiogenesis activity.[23] Recently, the utility of thiazolylhyrazono-thiazoles has been endorsed as potential anticancer drugs.[24−26] In addition, azolylthiazoles have been handled in several clinically available anticancer drugs, such as ixabepilone,[27] dabrafenib[28] and dasatinib[29] (Figure 1). Otherwise, the conjugated hydrazone system has been widely employed in pharmacological research as antitumor[30,31] agents (Figure 1). The integration of a single species of two or more pharmacophores is a useful structural modification method known as molecular hybridization. In recent years, hybrid drug design has been employed as a primary strategy for generating innovative anticancer medicines that can address many of the pharmacokinetic shortcomings of existing anticancer medications[32−36] (Figure 1).

Based on the examples described above, and in continuation of our attempts to synthesize heterocycles with anticancer

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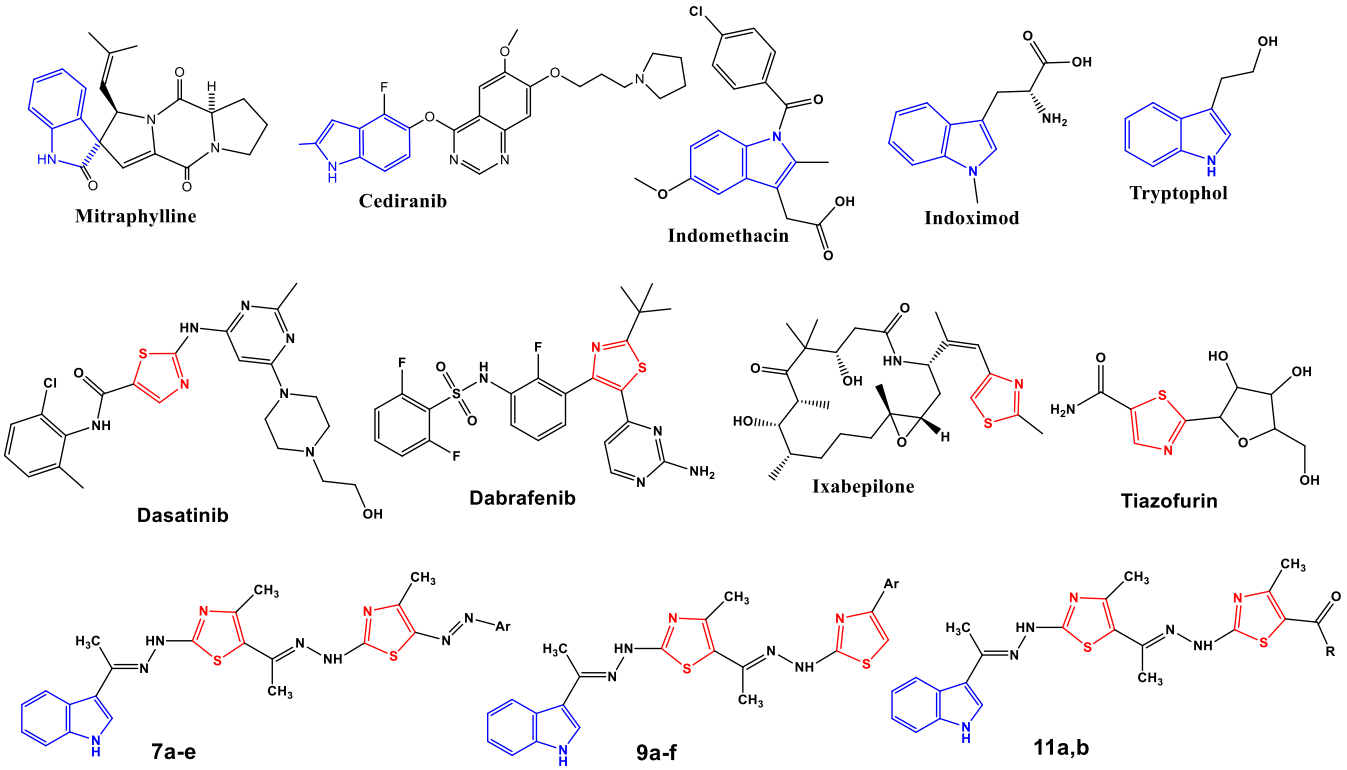
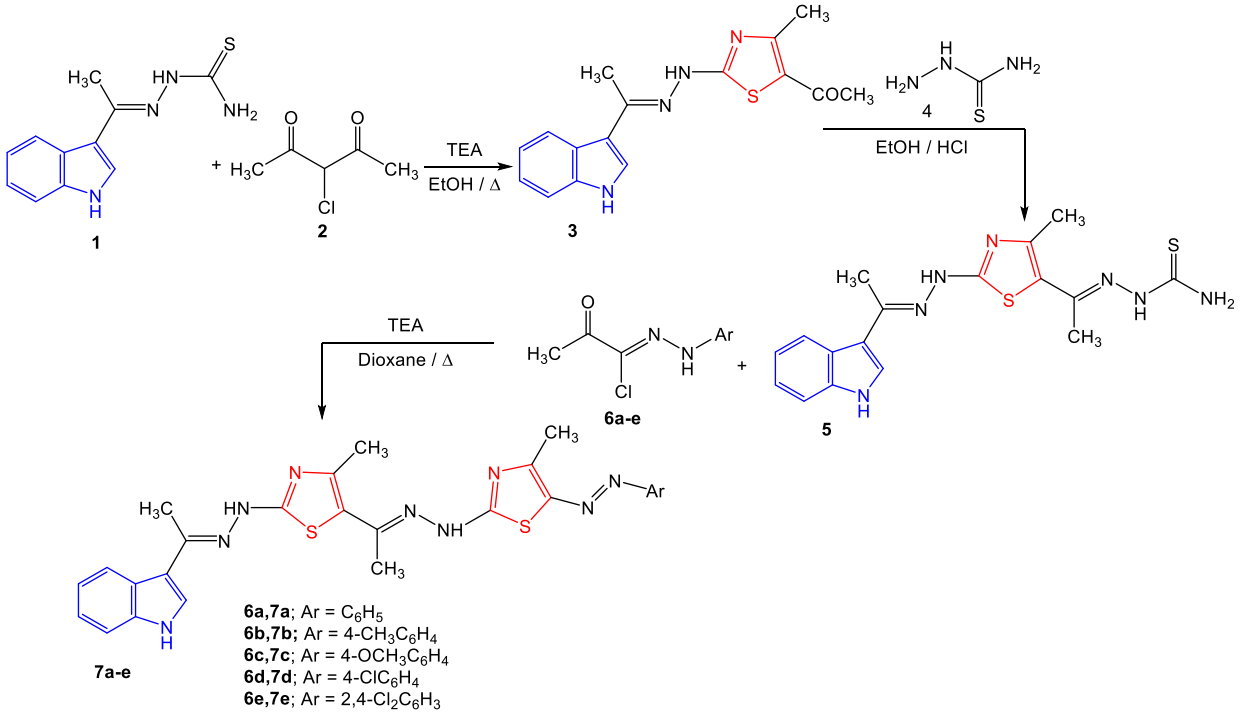


Figure 1. Indole and thiazole derivatives as anticancer drugs, as well as targeted compounds.

Scheme 1. Synthesis of Compound 5 and Compounds 7a−e



activity.[24−26,31,37−41] We aimed in this study to prepare a hybrid structural scaffold of thiazolylthiazoles, with hydrazone linker, and investigate their potential activities as therapeutic agents for cancer therapy. The molecular docking study of the isolated products into epidermal growth factor receptor tyrosine kinase domain (EGFR TK) binding site was also demonstrated to predict the binding affinity and determine the interactions of the proposed derivatives **7a−e**, **9a−f** and **11a,b** (Figure 1).

2. RESULTS AND DISCUSSION

In our study, we have settled on the preparation of 1-{2-[2-(1-(1*H*-indol-3-yl)ethylidene)hydrazinyl]-4-methylthiazol-5-yl}-ethan-1-one (**3**), as a key intermediate, from the reaction of 2-

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Scheme 2. Synthesis of Hydrazonothiazoles 9a−f and 11a,b

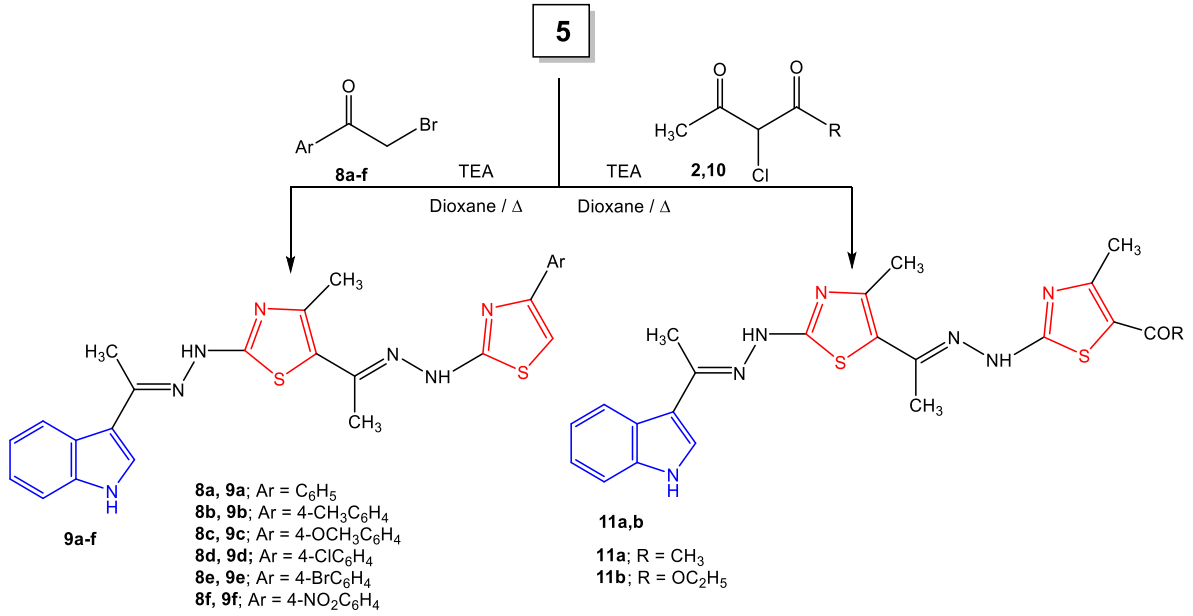

Table 1. Inhibitory Effects of the Tested Compounds against Various Carcinoma Cell Lines Were Evaluated through IC_{50} Measurements (The Mean ± Standard Error was Used to Express the Results)[a]

	IC_{50} values (μM)			CC_{50} values (μM)[b]
compound no	HepG-2	MDA-MB-231	HCT-116	MRC-5
doxorubicin	6.18 ± 0.29	8.37 ± 0.64	7.18 ± 0.44	89.17 ± 2.75
3	93.2 ± 3.17	74.02 ± 3.39	138.38 ± 5.14	[c]
5	62.30 ± 1.49	44.26 ± 2.95	83.03 ± 1.38	
7a	15.38 ± 1.46	13.39 ± 1.48	16.49 ± 1.08	315.94 ± 16.20
7b	9.36 ± 0.92	12.97 ± 1.00	12.34 ± 1.92	175.92 ± 18.24
7c	7.93 ± 0.84	9.28 ± 1.34	13.28 ± 1.04	181.89 ± 15.07
7d	27.35 ± 1.37	18.49 ± 3.39	47.40 ± 2.48	
7e	39.48 ± 3.37	48.04 ± 4.58	83.04 ± 3.58	
9a	48.01 ± 1.46	77.85 ± 4.03	36.29 ± 2.58	169.91 ± 11.37
9b	10.38 ± 1.82	14.47 ± 2.93	15.18 ± 4.48	177.28 ± 7.26
9c	13.39 ± 2.39	17.03 ± 3.05	17.29 ± 3.00	186.27 ± 10.65
9d	92.04 ± 2.38	83.28 ± 4.35	64.15 ± 3.33	
9e	81.68 ± 1.74	133.45 ± 7.91	61.39 ± 2.25	
9f	58.18 ± 2.28	81.02 ± 5.32	106.32 ± 6.02	
11a	37.25 ± 1.45	63.28 ± 4.49	41.37 ± 2.34	195.16 ± 17.26
11b	13.04 ± 1.06	16.30 ± 4.35	31.26 ± 2.28	214.08 ± 9.36

[a]IC_{50} (μM): 1−10 (good results); 11−40 (moderate); 41−100 (poor) and above 100 (inactive). [b]The data for CC_{50} values, which indicate the cytotoxic effects on normal human lung fibroblast (MRC-5) cell line, were recorded as mean ± standard error. [c]Not measured.

(1-(1*H*-indol-3-yl)ethylidene)hydrazine-1-carbothioamide (**1**)[42] and 3-chloro-2,4-pentanedione (**2**) in ethanolic solution and catalytic amounts of triethylamine under the thermal condition as depicted in Scheme 1.

Construction of our target 2-{1-[2-(2-(-1-(1*H*-indol-3-yl)-ethylidene)hydrazinyl)-4-methylthiazol-5-yl]ethylidene}-hydrazine-1-carbothioamide (**5**), was achieved via condensation of compound **3** with thiosemicarbazide (**4**) in ethanol and few drops of hydrochloric acid as a catalyst (Scheme 1). Elemental analyses and spectral data of IR, NMR, and MS were used to confirm the structure of compounds **3** and **5** (see Experimental Section).

Carbothioamide derivative **5** was subjected to cyclization with 2-oxo-*N*-arylpropanehydrazonoyl chlorides (**6a−e**)[26,37] via nucleophilic substitution and condensation reactions to give 4-methyl-5-(aryldiazenyl)-2-hydrazonothiazole derivatives **7a−e** (Scheme 1).

^{1}H NMR spectrum of compound **7a**, as a representative example, was consistent with the assigned structure. It revealed four singlet signals at δ = 2.26, 2.34, 2.41, and 2.48 ppm attributed to methyl groups on thiazoles and hydrazone moieties,[24,26] respectively. H-2 and (NH) exchangeable protons of the indole ring were resonated at δ = 8.26 and 11.85 ppm,[43] respectively. In the mass spectrum, the molecular ion peak of **7a** was recorded at m/z 527 which acquiesced with the molecular weight of the assigned structure.

The synthetic strategy of the previous cyclization reaction was extended towards α-haloketones. Thus, carbothioamide derivative **5** was allowed to react with 2-bromo-1-arylethan-1-ones (**8a−f**) or 3-chloro-2,4-pentanedione (**2**) or ethyl 2-chloro-3-

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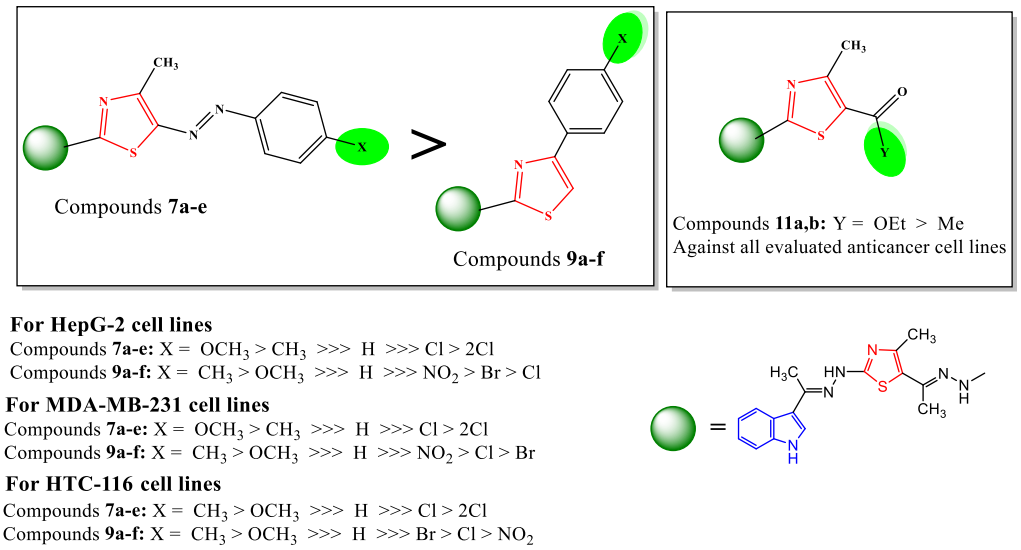

Figure 2. SAR of tested compounds against HepG-2, MDA-MB-231, and HCT-116.

oxobutanoate (**10**) in dioxane and employed triethylamine as basic catalyst under refluxing condition. This reaction allowed for the synthesis of hydrazonothiazoles (**9a−f**) or **11a** or **11b**, respectively, as illustrated in Scheme 2. The distinctive features in the structure of isolated products were elucidated by spectroscopic tools and elemental analyses. ^{1}H NMR spectra of products **9a−f** showed in each case a singlet signal at δ = 7.40−7.48 ppm corresponding to H-5 of thiazole ring.[44] In IR, the bands of (C═O) group of compounds **11a** and **11b** were revealed at 1700[45] and 1695[46] cm^{-1}, respectively.

2.1. Cytotoxic Activity. The in vitro growth inhibiting capability of the synthesized products was evaluated in comparison to the Doxorubicin anticancer reference drug in the liver carcinoma cell line (HepG-2), colon carcinoma cell line (HCT-116), and breast carcinoma cell line (MDA-MB-231). A dose−response curve was constructed from the obtained data, and the IC_{50} (the concentration of the test chemical required to kill 50% of the cell population) was calculated. The average IC_{50} of three independent studies was used to calculate cytotoxic activity. The findings presented in Table 1 and Figure 2 demonstrated that the majority of the tested compounds exhibited a considerable range of activity in comparison to the standard drug.

Based on the SAR (structure−activity relationship) analysis, it can be observed that certain structural features may contribute to cytotoxic activity:

- Generally, the in vitro inhibition effect of the 5-arylazothiazoles **7** is greater than 4-arylthiazoles **9** (**7b** > **9b**; **7c** > **9c**) towards all the examined anticancer cell lines.
- The in vitro inhibition effect of most of the synthesized compounds towards HepG-2 > MDA-MB-231 > HCT-116 cell lines (For example, the IC_{50} of compound **7c** towards HepG-2, MDA-MB-231, and HCT-116 cell lines = 7.93 ± 0.84, 9.28 ± 1.34, and 13.28 ± 1.04 μM, respectively).
- The order of the in vitro inhibition effect of the synthesized compounds against the liver carcinoma cell line (HepG-2) is: **7c** > **7b** > **9b** > **11b** > **9c** > **7a** (good results) ≫> **7d** > **11a** > **7e** (moderate results) ≫> **9a** > **9f** > **5** > **9e** > **9d** > **3** (poor results or inactive).
- The order of the in vitro inhibition effect of the synthesized compounds against the breast carcinoma cell line (MDA-MB-231) is: **7c** > **7b** > **7a** > **9b** > **11b** > **9c** > **7d** (good results) ≫> **5** > **7e** (moderate results) ≫> **11a** > **3** > **9a** > **9f** > **9d** > **9e** (poor results or inactive).
- The order of the in vitro inhibition effect of the synthesized compounds against the colon carcinoma cell line (HCT-116) is: **7b** > **7c** > **9b** > **7a** > **9c** (good results) ≫> **11b** > **9a** > **11a** > **7d** (moderate results) ≫> **9e** > **9d** > **5** > **7e** > **9f** > **3** (poor results or inactive).
- For thiazoles **7a−e**: the electron donating groups (e.g., Me and MeO) at the 4-position of phenylazothiazoles enhance the activity while the electron-withdrawing groups (e.g., Cl) decrease the activity (**7c**, **7b** ≫>**7a** ≫> **7d**, **7e**).
- For thiazoles **9a−f**: the electron donating groups (e.g., Me and MeO) at the 4-position of phenylazothiazoles enhance the activity while the electron-withdrawing groups (e.g., Cl, Br, NO_2) decrease the activity (**9b**, **9c** ≫> **9a** ≫> **9d, 9e, 9f**).
- For thiazoles **11a,b**: Carboxylate group (COOEt) at position 5 of the thiazole moiety has greater inhibition activity than the acetyl group ($COCH_3$) (**11b** > **11a**).

In order to establish a dose−response curve and calculate the fifty percent cytotoxic concentration (CC_{50}), the impact of both the investigated compounds and the Doxorubicin were evaluated on the normal human lung fibroblast (MRC-5) cell line. The resulting values are presented in Table 1. The selectivity index (SI) was calculated by dividing the CC_{50} by the IC_{50} values. The outcomes indicated that the majority of the compounds exhibited good selectivity index values (SI value >1). Although the examined compounds displayed limited toxicity against normal cells, indicating their safety, further investigations in vivo and pharmacology may be required.

2.2. Molecular Docking. In order to propose the mechanism of action of the screened compounds as protein Epidermal Growth Factor Receptor Tyrosine Kinase Domain (EGFR TK) inhibitors, docking studies were conducted using the Molec-ular Operating Environment 2019.012 suite.[47] This was done by comparing the binding scores and modes of the screened compounds to a compound named 4-[4-(1-benzo-

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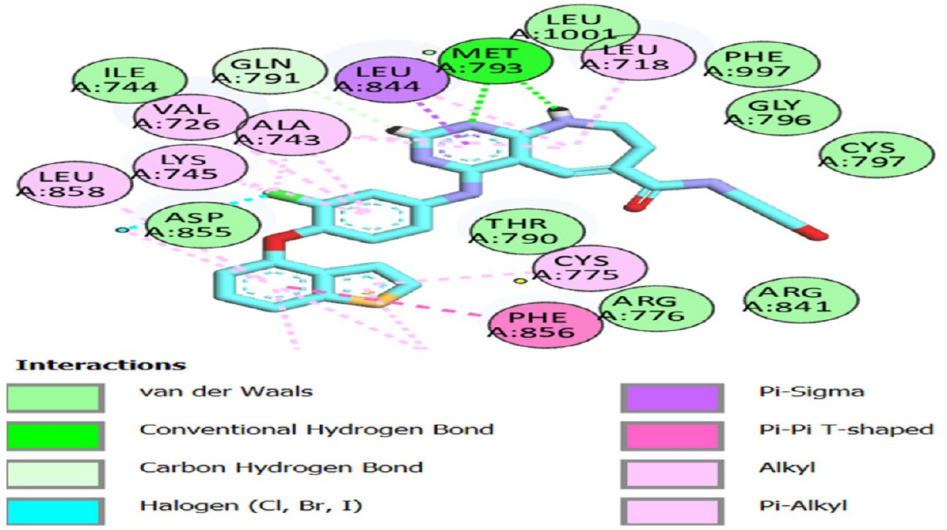

Figure 3. Redocked co-crystalized ligand (**W19**) interactions with **EGFR TK** residues in two dimensions.

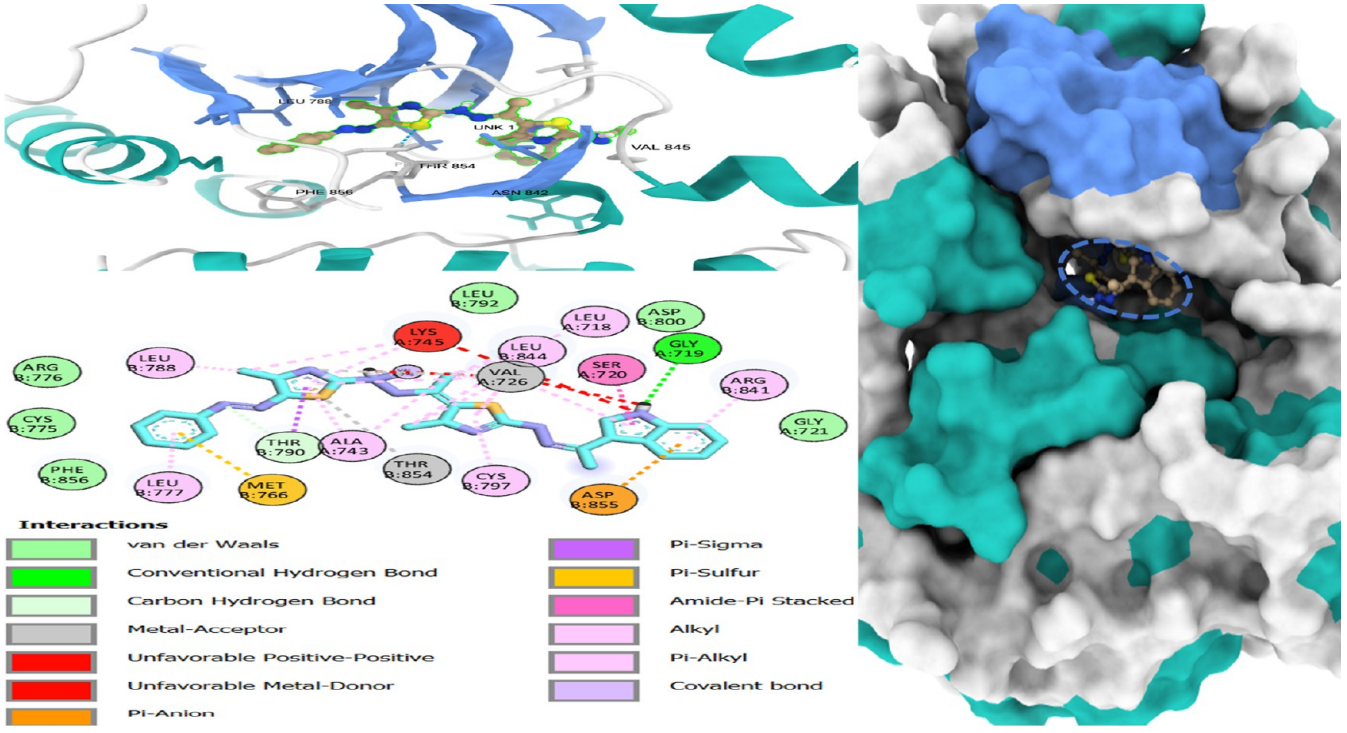

Figure 4. 3D, 2D, and mapping surface showing binding modes between **7a** and EGFR TK residues at the sites of activity.

thiophen-4-yloxy)-3-chlorophenyl]4-{[4-(1-benzothiophen-4-yloxy)-3-chlorophenyl]amino}-N-(2-hydroxyethyl)-8,9-dihydro-7H-pyrimido[4,5-b]azepine-6-carboxamide (W19) as a reference standard. The screened compounds were drawn using PerkinElmer ChemOffice Suite 2017. The three-dimensional (3D) structures of the small molecules were generated. The structures were assigned appropriate bond orders, hydrogen atoms, and ionization. The results were refined using the London DG force and force field energy. All minimizations were performed until a root mean square deviation (RMSD) gradient 0.01 kcal·mol^{-1}Å^{-1} using MMFF 94× (Merck molecular force field 94×), and the partial charges were determined automatically. The binding affinity of the ligand was evaluated using the scoring function and dock function (S, Kcal/mol) created by the MOE software.[48,49] The screened compounds and the co-crystallized inhibitor (W19) were prepared for the docking process toward (EGFR TK) by importing them into one database and storing them as an MDB file. From the Protein Data Bank (https://www.rcsb.org/structure/3W33), the (EGFR TK) X-ray was retrieved.[50] Furthermore, the docking of the compound was conducted by meticulously adhering to the methods that had been previously outlined.[49,50] Importantly, the protein that was obtained was corrected for errors, had 3D hydrogen-loading performed, and underwent energy minimization[50,51] before the docking process began. The file for the ready active site was loaded after which the overall docking procedure began. The scoring method was (London dG), the docking procedure site was (ligand atoms), and the placement strategy was (triangle matcher). The scoring method was GBVI/WSA dG, and the top 10 poses for each tested substance were chosen

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Table 2. Tested Compounds' Interactions and Binding Scores with the EGFR TK's Binding Pocket (3W33)

compounds no	binding scores (kcal/mol)	hydrogen bond interactions	distance (Å)	hydrophobic interactions	distance (Å)
7a	−10.9	GLY 719	2.22	THR 790	3.83
				LYS 745	3.71
				ALA 743	3.75
				LEU 788	3.55
				LEU 844	3.93
				ASP 855	3.84
				MET 766	3.66
				VAL 726	3.81
				ALA 743	3.79
7b	−10.2	LEU 718	1.99	LYS 745	3.44
		ASP 855	3.35	LEU 788	3.60
				PHE 997	3.97
				PHE 856	3.88
				ALA 1000	3.24
				ALA 743	3.50
				LYS 745	3.93
				LEU 788	3.47
7c	−10.2	ASP 855	2.15	PHE 856	3.22
		ARG 841	2.37	CYS 797	3.64
				ASP 800	3.31
				MET 766	3.11
				VAL 726	3.41
				LEU 788	3.26
				PHE 856	3.08
9a	−10.3	ASP 855	2.70	VAL 726	3.79
		ARG 841	3.16	ALA 743	3.47
				THR 854	3.28
				LEU 844	3.71
				LYS 745	3.71
9b	−10.9	ASP 855	2.70	LEU 788	3.24
		CYS 797	2.78	PHE 997	3.33
				VAL 726	3.76
				ALA 743	3.43
				PHE 856	3.20
				ASP 800	3.11
				LEU 844	3.75
				MET 766	3.87
				LEU 777	3.77
9c	−9.4	ASP 800	3.11	ARG 841	3.92
				LEU 718	3.64
				CYS 797	3.82
				ALA 743	3.22
11a	−10.0	ASP 855	2.39	VAL 726	3.93
		PHE 723	2.12	LEU 844	3.88
				ALA 743	3.29
				LYS 745	3.19
				PHE 856	3.14
				VAL 726	3.45
11b	−10.4	LYS 745	2.38	LEU 788	3.23
		ARG 841	2.12	GLY 721	3.18
		ALA 722	2.11	ALA 743	3.92
				LYS 745	3.26
				PHE 723	3.21
W19	−10.5	MET 793	2.51	PHE 856	3.88
		SER 720	2.29	LEU 788	3.82
				LYS 745	3.48
				LEU 844	3.93
				ALA 743	3.81
				VAL 726	3.68

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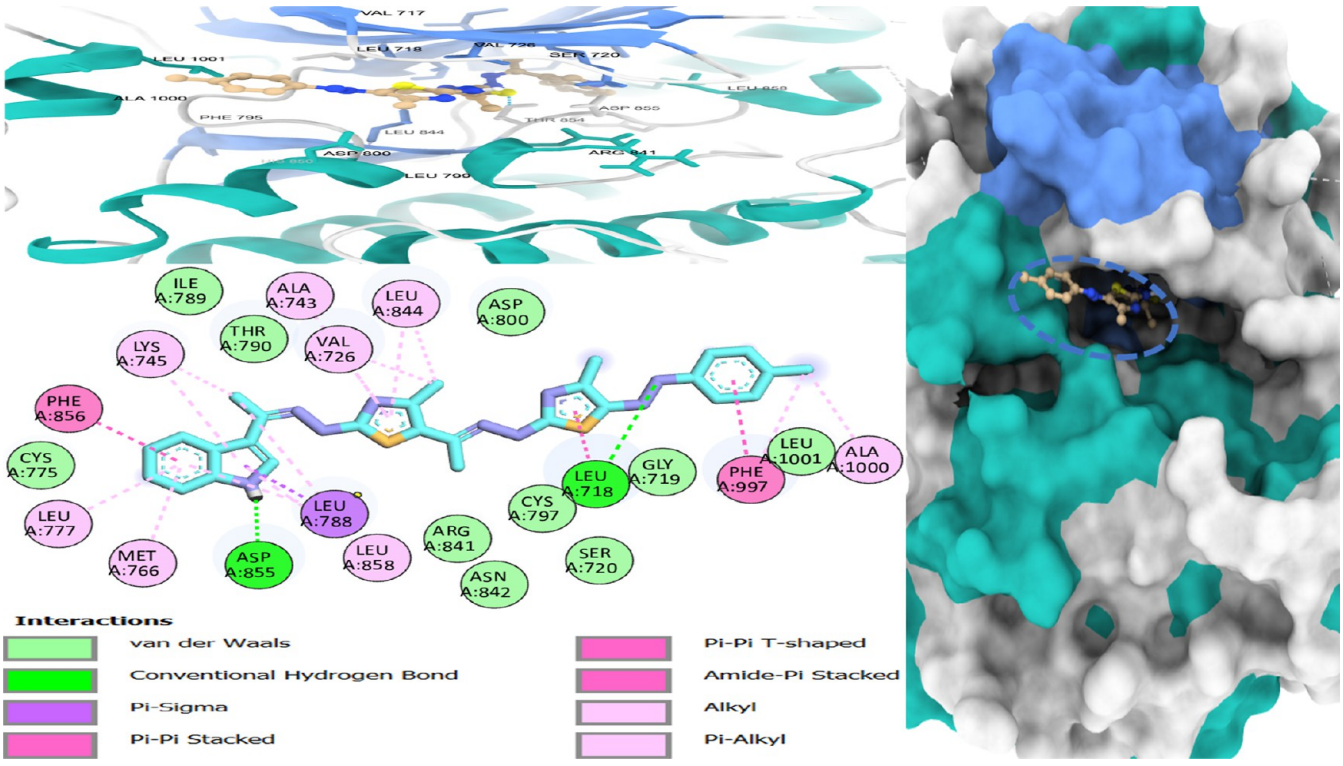

Figure 5. 3D, 2D, and mapping surface showing binding modes between **7b** and EGFR TK residues at the sites of activity.

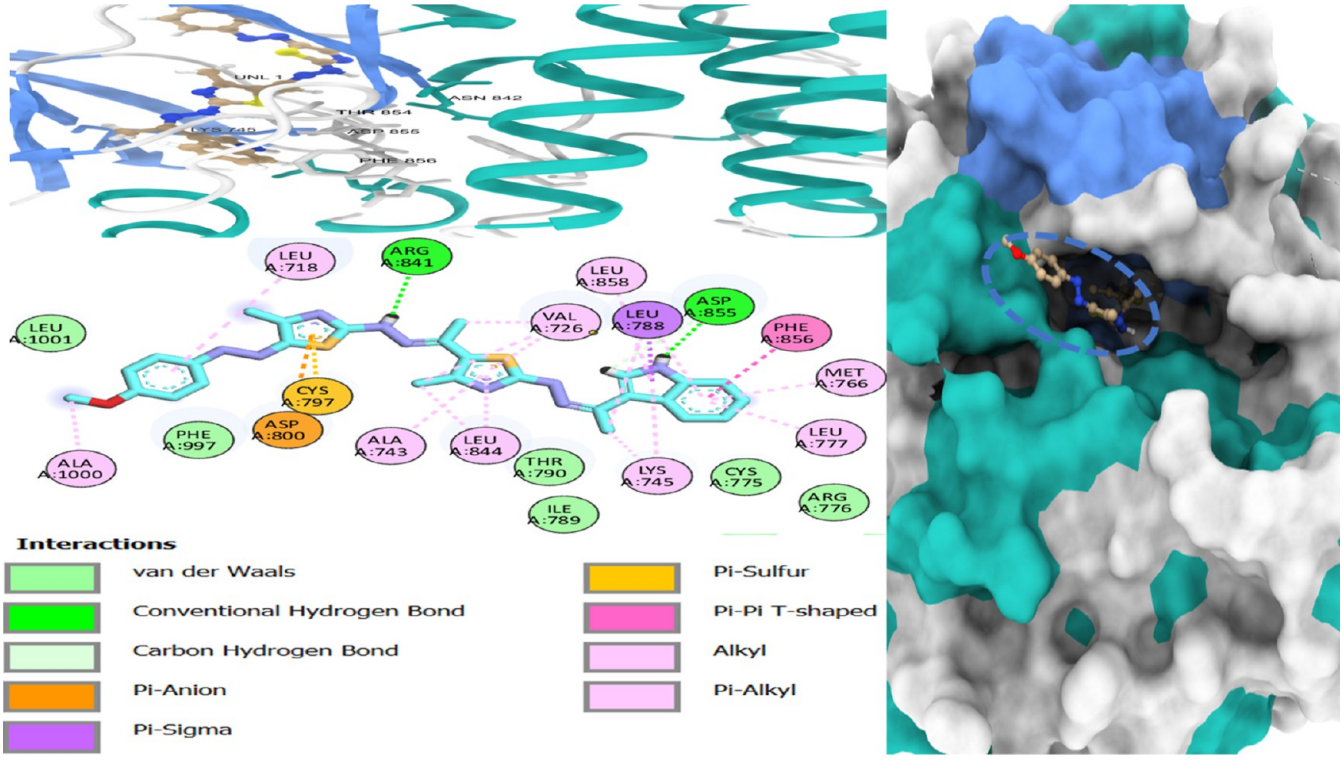

Figure 6. 3D, 2D, and mapping surface showing binding modes between **7c** and EGFR TK residues at the sites of activity.

from a pool of 30 poses using rigid receptor docking as the refining process. The MDB file for the examined ligands was then supplied to the application, and the calculations for the ongoing docking were carried out automatically. After everything was done, the resulting poses were analyzed, and the best ones with the largest scores, reasonable RMSD values, and better ligand−protein target interactions were picked and kept for subsequent studies. It is important to note that the co-crystallized ligand (W19) was redocked at its binding pocket on the prepared Target as part of a program validation phase for the applicable MOE program[52−54] (Figure 3). By getting a low RMSD value (1.21) between the screened compounds and the

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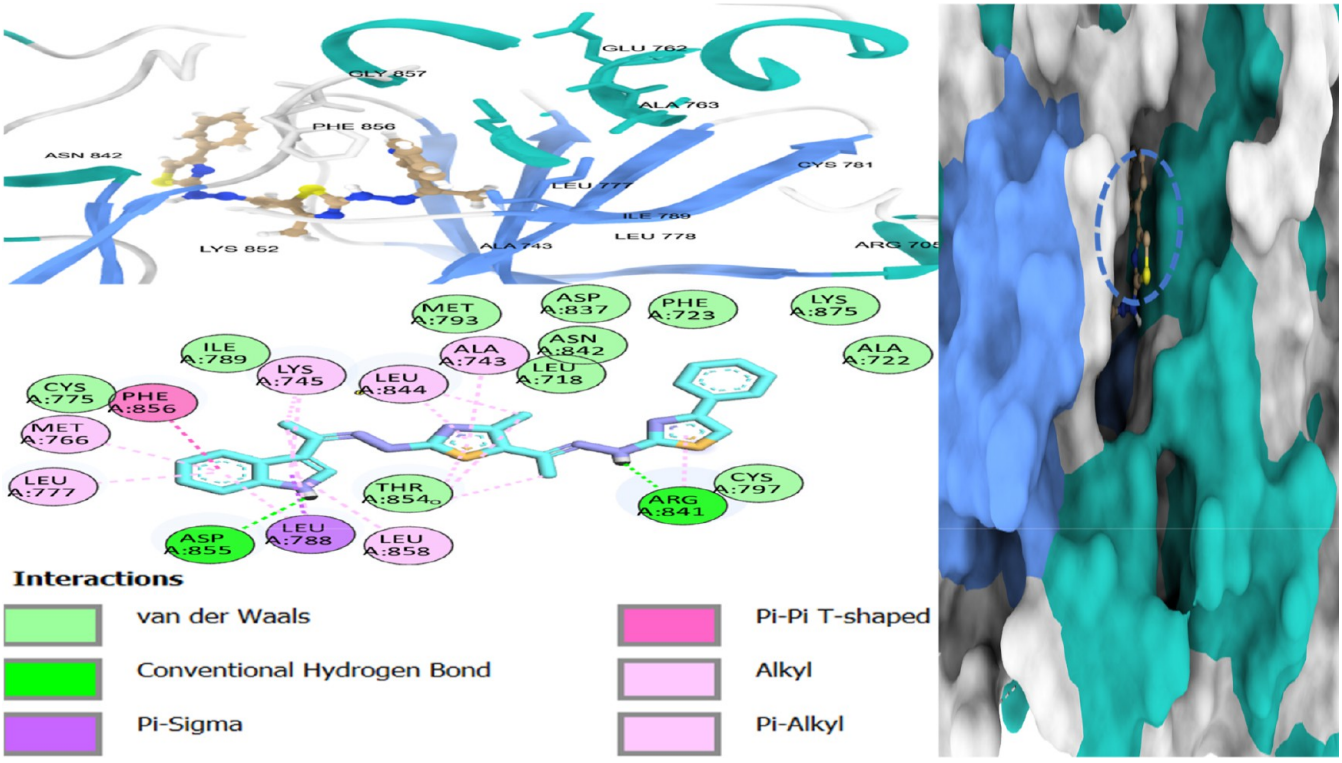

Figure 7. 3D, 2D, and mapping surface showing binding modes between **9a** and EGFR TK residues at the sites of activity.

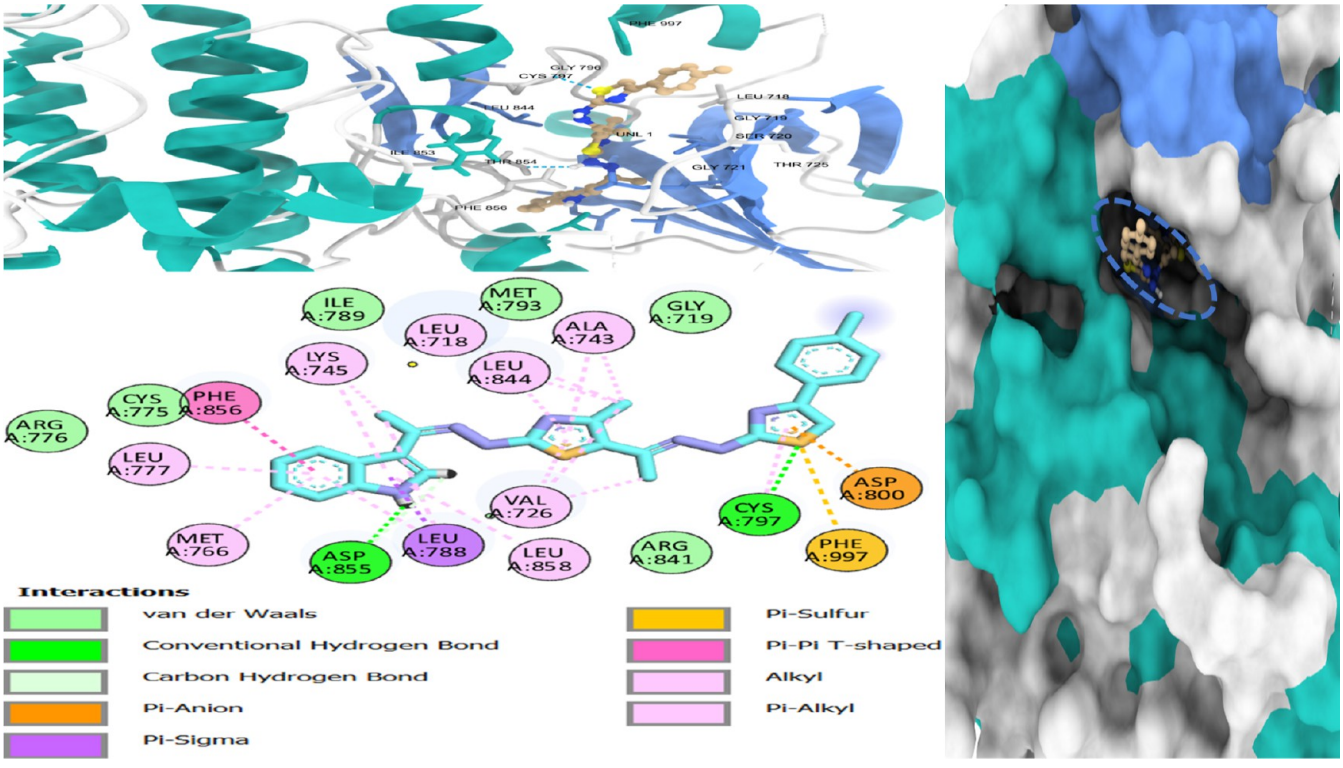

Figure 8. 3D, 2D, and mapping surface showing binding modes between **9b** and EGFR TK residues at the sites of activity.

redocked co-crystallized ligand (**W19**), a valid performance was demonstrated. Discovery Studio 4.0 software was used to further visualize the output from MOE software.

The binding interactions of the newly synthesized compounds were analyzed using molecular docking studies with the MOE 2019 package. Blocking the growth pathway by inhibiting this receptor is a promising strategy for developing anti-cancer agents. Molecular docking studies were employed to investigate the suggested binding interactions of the tested compounds with the EGFR TK. Comparing the compounds to the native co-crystallized ligand (W19), which serves as a reference control and exhibits binding energy (G of −10.5 kcal/mol) as shown in

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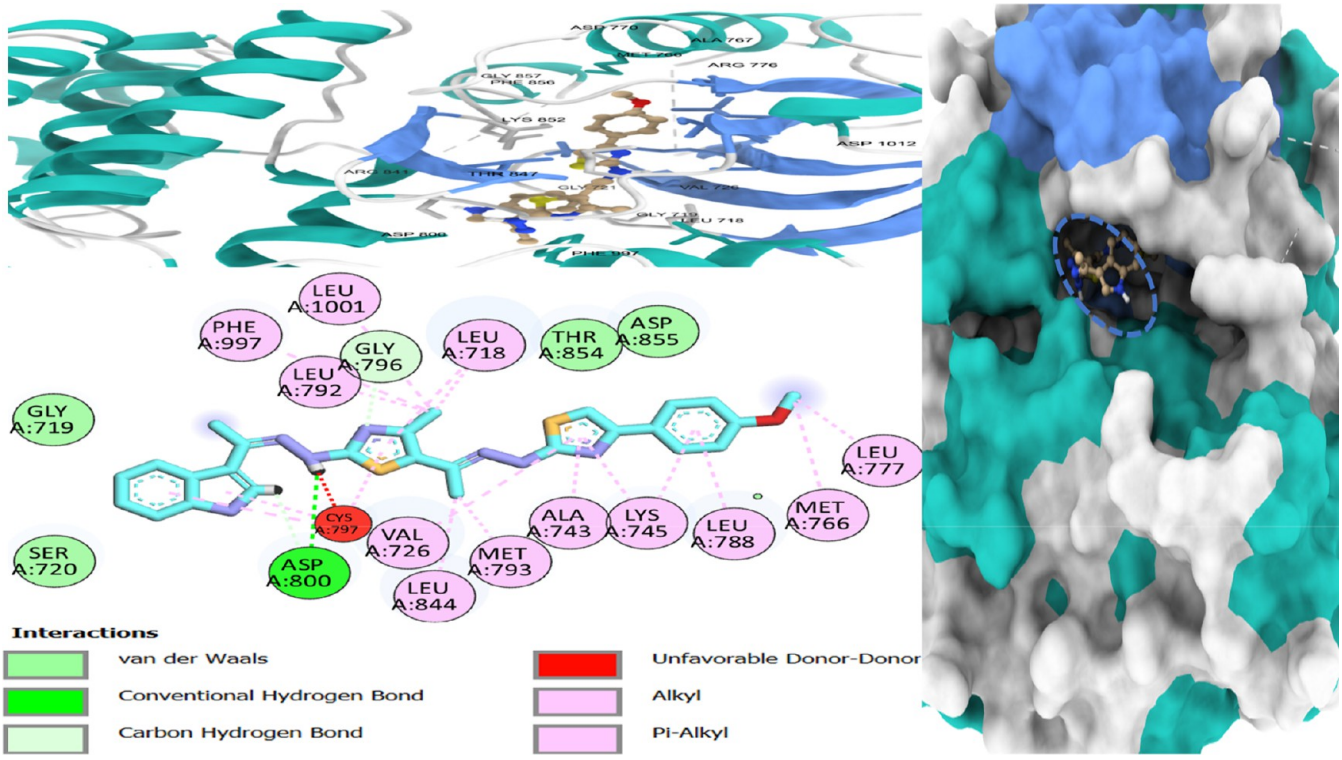

Figure 9. 3D, 2D, and mapping surface showing binding modes between **9c** and EGFR TK residues at the sites of activity.

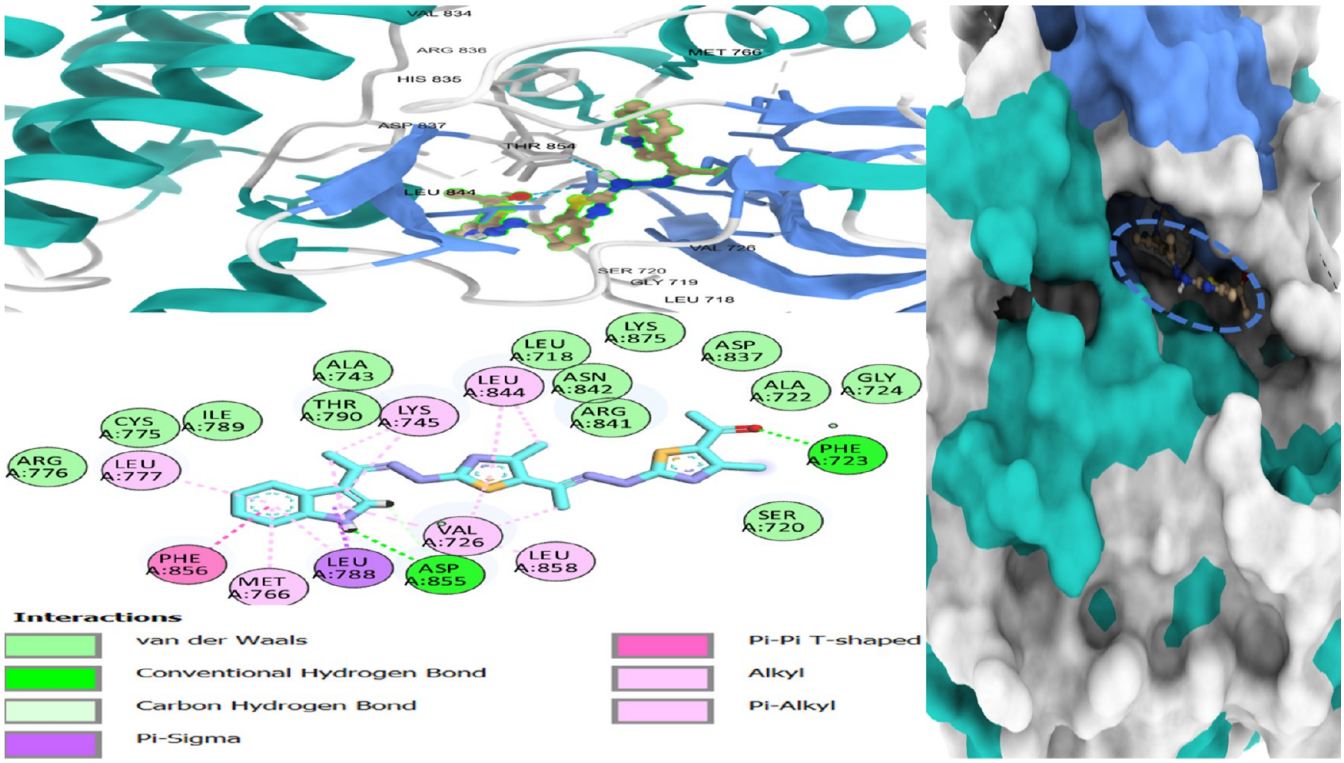

Figure 10. 3D, 2D, and mapping surface showing binding modes between **11a** and EGFR TK residues at the sites of activity.

Table 1 and binding interactions shown in Figure 4, the decrease in binding energy upon association of the compounds with the target protein suggests a greater binding efficiency. The binding scores, as well as the details of the binding interactions with their corresponding bond types for the most promising synthesized compounds (**7a−c**, **9a−c**, **11a**, and **11b**) besides, the co-crystallized ligand (**W19**), are depicted in Table 2.

It is worth noting that compounds **7a** and **9b** exhibited the best binding scores among synthesized compounds. Hence, compounds **7a** and **9b** revealed binding scores of −10.9 kcal/mol, which is superior to that of the co-crystallized inhibitor.

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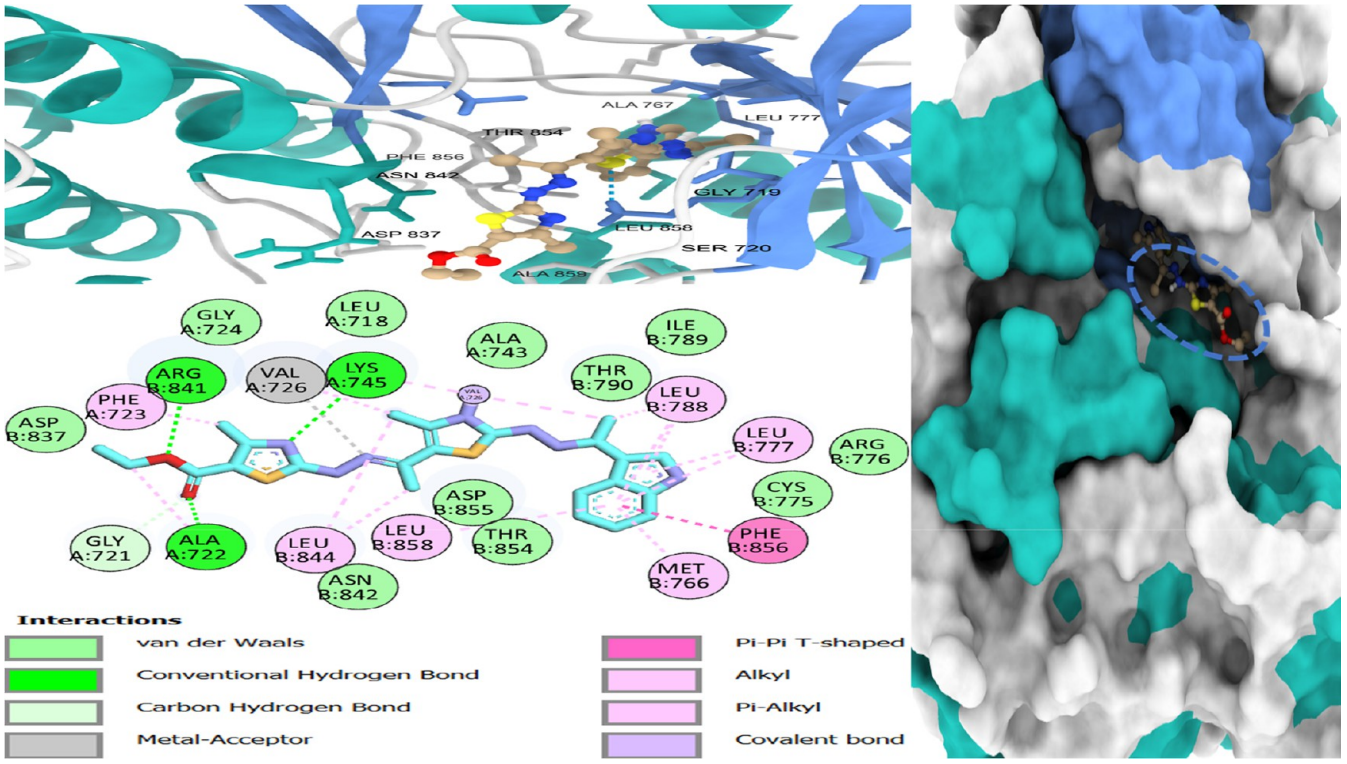

Figure 11. 3D, 2D, and mapping surface showing binding modes between **11b** and EGFR TK residues at the sites of activity.

Compound **7a** could compose H-bonds with the one key amino acid GLY 719 at a distance of 2.22 Å. In addition, compound **9b** could interact with receptor-forming H-bonds with amino acids ASP 855 and CYS 797 at a distance of 2.70 Å and 2.78 Å, respectively (Table 1). Table 1 displays the findings from the in-silico protein-screened compounds interaction, which revealed the active participation of certain amino acids in the protein target (PHE 997, PHE 856, LYS 745, MET 766, GLY 796, ASN 842, ASP 855, LEU 718, PHE 723, VAL 726, ALA 743, THR 790, LEU 844, THR 854) through a number of hydrogen and hydrophobic interactions. The screened synthesized showed binding energies from ΔG −9.4 to −10.9 kcal/mol with potential possibility of interactions with EGFR TK active sites as depicted in Table 1 and Figures 4−11.

3. EXPERIMENTAL SECTION

3.1. Instruments and Materials. The isolated products were evaluated for their melting points using an electrothermal Gallenkamp apparatus. To obtain the IR spectra, potassium bromide discs were used along with a Pye-Unicam SP300 instrument. The ^{1}H NMR and ^{13}C NMR spectra were recorded using a Jeol-500 spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR). The mass analysis was performed using a Thermo Scientific GC/MS model ISQ and/or an Agilent LC-MSD IQ Infinity II 1260 [(SpectraLab Scientific Inc., Markham, ON L3R 3V6, Canada)]. Using Merck silica gel GF254 plates (Merck, Darmstadt Germany), analytical thin-layer chromatography (TLC) was carried out. Finally, elemental analysis (C, H, and N) was executed with the Perkin-Elmer 2400 apparatus (Elementar Analysensysteme GmbH, Langenselbold, Germany).

*3.1.1. Synthesis of (E)-1-(2-(2-(1-(1H-Indol-3-yl)-ethylidene)hydrazineyl)-4-methylthiazol-5-yl)ethan-1-one (**3**).* 2-(1-(1*H*-Indol-3-yl)ethylidene)hydrazine-1-carbothioamide (**1**) (2.32 g, 0.01 mol) was licit to react with 3-chloro-2,4-pentanedione (**2**) (1.34 g, 0.01 mol) in ethanol (50 mL) and few drops of trimethylamine under reflux condition for 2 h. After completion of the reaction, the assembled precipitate was filtered off and rinsed by methanol. The isolated product was crystallized from ethanol to give pure compound **3**. Yellowish white solid; (yield 2.8 g, 90%) mp: 217−219 °C; IR, υ 2942 (C−H), 1698 (C═O), 1602 (C═N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.36 (s, 3H, CH_3), 2.41 (s, 3H, CH_3−C═N), 2.51 (s, 3H, $COCH_3$), 7.14−7.48 (m, 4H, Ar−H), 7.49 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 11.66 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 16.8 (CH_3), 23.7 (CH_3), 32.7 (CH_3), 122.8, 124.5, 127.6, 128.2, 130.4, 133.9, 140.4, 149.6, 151.6, 154.0, 162.0, 167.0, 174.1 ppm; MS, m/z (%) 312 (M$^+$, 18), 294 (27), 276 (100), 182 (86), 153 (67), 104 (26), 77 (38). Anal. Calcd for $C_{16}H_{16}N_4OS$ (312.10): C, 61.52; H, 5.16; N, 17.94; S, 10.26. Found: C, 61.74; H, 5.22; N, 17.71; S, 10.48%.

*3.1.2. Synthesis of (E)-2-(1-(2-(2-((E)-1-(1H-Indol-3-yl)-ethylidene)hydrazineyl)-4-methyl thiazol-5-yl)ethylidene)-hydrazine-1-carbothioamide (**5**).* Compound **3** (3.12 g, 0.01 mol) and thiosemicarbazide (0.9 g, 0.01 mol) were licit to dissolve in absolute ethanol (50 mL) under stirring conditions. A few drops of HCl were added and the reaction mixture was heated for 2 h. After cooling, sequential filtration and crystallization from ethanol have been achieved to afford pure product **5**. Yellow solid; (yield 3.08 g, 80%) mp: 251−253 °C; IR, υ 3410, 3248, 3158 (NH and NH_2), 2962 (C−H), 1601 (C═N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.36 (s, 3H, CH_3), 2.41 (s, 3H, CH_3−C═N), 2.43 (s, 3H, CH_3−C═N), 4.27 (br, 2H, NH_2), 7.14−7.49 (m, 4H, Ar−H), 8.26 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 10.85 (s, 1H, NH), 11.64 (s, 1H, NH) ppm; MS, m/z (%) 385 (M$^+$, 26), 366 (29), 280 (31), 252 (240), 158 (86), 142 (100), 138 (88), 89 (86), 75 (44). Anal.

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Calcd for $C_{17}H_{19}N_7S_2$ (385.11): C, 52.97; H, 4.97; N, 25.43; S, 16.63. Found: C, 52.79; H, 5.12; N, 25.61; S, 16.49%.

3.2. General Procedure for the Reactions of Thiosemicarbazones 5 with α-Haloketones. 2-{1-[2-(2-(-1-(1*H*-indol-3-yl)ethylidene)hydrazinyl)-4-methylthiazol-5-yl]-ethylidene}hydrazine-1-carbothioamide (**5**) (0.385 g, 1 mmol) was mixed with 2-oxo-*N*-arylpropanehydrazonoyl chlorides (**6a−e**) or 2-bromo-1-arylethan-1-ones (**8a−f**) or 3-chloro-2,4-pentanedione (**2**) or ethyl 2-chloro-3-oxobutanoate (**10**) [1 mmol of each] in dioxane (20 mL) and catalytic amount of triethylamine. The reaction mixture was heated under constant volume for 4 h. TLC (EtOAc/*n*-hexane 1:1) was used to monitor the reaction progress. Excess solvent was evaporated under reduced pressure and the formed precipitate was collected by filtration. Crystallization was achieved using an ethanol/dioxane mixture to isolate products **7a−e** or **9a−f** or **11a,b**, respectively.

*3.2.1. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-4-methyl-5-((E)-1-(2-(4-methyl-5-((E)-phenyldiazenyl)thiazol-2-yl)hydrazineylidene)ethyl)thiazole (**7a**).* Red solid; (yield 0.43 g, 80%) mp: 194−196 °C; IR, υ 3310, 3230, 3218 (3NH), 2914 (C−H), 1608 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.26 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.41 (s, 3H, CH_3−C═N), 2.48 (s, 3H, CH_3−C═N), 7.04−7.68 (m, 9H, Ar−H), 8.26 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 8.43 (s, 1H, NH), 11.85 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 9.1 (CH_3), 15.6 (CH_3), 16.3 (CH_3), 24.6 (CH_3), 112.0, 115.6, 116.3, 119.1, 120.8, 122.6, 123.9, 125.0, 128.2, 128.7, 128.9, 129.2, 130.3, 137.6, 138.6, 139.6, 141.3, 145.6, 158.7, 167.9 (Ar−C and C═N) ppm; MS, *m*/*z* (%) 527 (M^+, 18), 276 (28), 200 (14), 155 (37), 138 (100), 75 (33). Anal. Calcd for $C_{26}H_{25}N_9S_2$ (527.17): C, 59.18; H, 4.78; N, 23.89; S, 12.15. Found: C, 58.99; H, 4.62; N, 23.61; S, 12.29%.

*3.2.2. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-4-methyl-5-((E)-1-(2-(4-methyl-5-((E)-p-tolyldiazenyl)thiazol-2-yl)hydrazineylidene)ethyl)thiazole (**7b**).* Red solid; (yield 0.43 g, 80%) mp: 188−190 °C; IR, υ 3312, 3232, 3220 (3NH), 2916 (C−H), 1604 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.18 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.33 (s, 3H, CH_3−C═N), 2.37 (s, 3H, CH_3−C═N), 7.04−7.77 (m, 8H, Ar−H), 8.27 (s, 1H, pyrrole-H), 8.36 (s, 1H, NH), 8.43 (s, 1H, NH), 11.88 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 10.9 (CH_3), 13.8 (CH_3), 17.2 (CH_3), 21.7 (CH_3), 23.8 (CH_3), 113.8, 114.9, 120.6, 121.7, 122.8, 124.3, 126.6, 127.2, 128.7, 129.3, 130.1, 132.4, 133.4, 136.1, 137.6, 141.4, 160.6, 163.0, 169.0, 170.1 ppm (Ar−C and C═N); MS, *m*/*z* (%) 541 (M^+, 24), 412 (31), 317 (19), 263 (74), 229 (40), 183 (39), 138 (100), 96 (64), 77 (38). Anal. Calcd for $C_{27}H_{27}N_9S_2$ (541.18): C, 59.87; H, 5.02; N, 23.27; S, 11.84. Found: C, 59.93; H, 4.92; N, 23.16; S, 12.02%.

*3.2.3. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-5-((E)-1-(2-(5-((E)-(4-methoxyphenyl)diazenyl)-4-methylthiazol-2-yl)hydrazineylidene)ethyl)-4-methylthiazole (**7c**).* Red solid; (yield 0.46 g, 82%) mp: 174−176 °C; IR, υ 3312, 3230, 3221 (3NH), 2922 (C−H), 1600 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.29 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.36 (s, 3H, CH_3−C═N), 2.41 (s, 3H, CH_3−C═N), 3.71 (s, 3H, OCH_3), 7.11−7.37 (m, 8H, Ar−H), 7.79 (s, 1H, pyrrole-H), 8.35 (s, 1H, NH), 8.42 (s, 1H, NH), 11.88 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 11.9 (CH_3), 14.1 (CH_3), 17.3 (CH_3), 23.0 (CH_3), 51.8 (OCH_3), 114.3, 115.4, 120.6, 121.7, 122.6, 124.4, 126.7, 127.2, 128.7, 129.3, 130.4, 131.9, 134.1, 136.1, 137.6, 141.4, 161.6, 164.0, 168.0, 169.7 ppm (Ar−C and C═N); MS, *m*/*z* (%) 557 (M^+, 34), 412 (50), 341 (39), 246 (37), 188 (73), 117 (100), 83 (69), 77 (53). Anal. Calcd for $C_{27}H_{27}N_9OS_2$ (557.18): C, 58.15; H, 4.88; N, 22.60; S, 11.50. Found: C, 58.23; H, 4.94; N, 22.46; S, 11.62%.

*3.2.4. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-5-((E)-1-(2-(5-((E)-(4-chlorophenyl)diazenyl)-4-methylthiazol-2-yl)hydrazineylidene)ethyl)-4-methylthiazole (**7d**).* Yellow solid; (yield 0.47 g, 83%) mp: 203−205 °C; IR, υ 3312, 3236, 3219 (3NH), 2921 (C−H), 1599 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.28 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.36 (s, 3H, CH_3−C═N), 2.46 (s, 3H, CH_3−C═N), 7.11−7.34 (m, 8H, Ar−H), 7.79 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 8.42 (s, 1H, NH), 11.82 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 9.1 (CH_3), 14.7(CH_3), 16.2 (CH_3), 20.9 (CH_3), 109.8, 114.0, 114.1, 115.8, 118.2, 121.9, 122.3, 125.9, 126.1, 128.6, 129.8, 130.1, 132.1, 138.3, 142.7, 149.6, 149.7, 150.0, 156.5, 167.1 (Ar−C and C═N) ppm; MS, *m*/*z* (%) 563 (M^+ + 2, 5), 561 (M^+, 16), 320 (11), 252 (16), 197 (67), 138 (68), 126 (100), 116 (87), 77 (48). Anal. Calcd for $C_{26}H_{24}ClN_9S_2$ (561.13): C, 55.56; H, 4.30; N, 22.43; S, 11.41. Found: C, 55.49; H, 4.42; N, 22.61; S, 11.29%.

*3.2.5. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-5-((E)-1-(2-(5-((E)-(2,4-dichlorophenyl)diazenyl)-4-methylthiazol-2-yl)hydrazineylidene)ethyl)-4-methylthiazole (**7e**).* Yellow solid; (yield 0.48 g, 81%) mp: 236−238 °C; IR, υ 3315, 3232, 3224 (3NH), 2935 (C−H), 1605 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.28 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.36 (s, 3H, CH_3−C═N), 2.46 (s, 3H, CH_3−C═N), 7.11−7.57 (m, 7H, Ar−H), 7.85 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 8.40 (s, 1H, NH), 11.82 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 10.9 (CH_3), 13.4 (CH_3), 17.9 (CH_3), 20.9 (CH_3), 114.7, 115.8, 120.5, 121.8, 122.9, 124.6, 125.8, 126.7, 127.3, 128.9, 129.4, 130.3, 132.6, 133.4, 135.9, 136.6, 140.4, 142.3, 161.7, 163.9, 168.8, 170.1 ppm (Ar−C and C═N); MS, *m*/*z* (%) 595 (M^+, 21), 447 (26), 312 (83), 252 (62), 191 (28), 117 (100), 96 (29), 77 (79). Anal. Calcd for $C_{26}H_{23}Cl_2N_9S_2$ (595.09): C, 52.35; H, 3.89; N, 21.13; S, 10.75. Found: C, 52.49; H, 3.72; N, 21.21; S, 10.69%.

*3.2.6. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-4-methyl-5-((E)-1-(2-(4-phenylthiazol-2-yl)hydrazineylidene)-ethyl)thiazole (**9a**).* Yellow solid; (yield 0.43 g, 90%) mp: 173−175 °C; IR, υ 3318, 3236, 3228 (3NH), 2951 (C−H), 1598 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.29 (s, 3H, CH_3), 2.41 (s, 3H, CH_3−C═N), 2.49 (s, 3H, CH_3−C═N), 7.04−7.34 (m, 9H, Ar−H), 7.40 (s, 1H, thiazole-H5), 8.13 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 8.43 (s, 1H, NH), 11.85 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 13.2 (CH_3), 16.0 (CH_3), 20.8 (CH_3), 110.1, 113.0, 114.2, 115.8, 116.5, 117.0, 118.6, 120.1, 121.9, 125.3, 129.5, 129.9, 130.3, 131.9, 134.0, 141.9, 143.0, 150.7, 159.6, 163.3 (Ar−C and C═N) ppm; MS, *m*/*z* (%) 485 (M^+, 19), 313 (48), 270 (52), 243 (60), 190 (69), 138 (100), 117 (88), 91 (46), 75 (39). Anal. Calcd for $C_{25}H_{23}N_7S_2$ (485.15): C, 61.83; H, 4.77; N, 20.19; S, 13.20. Found: C, 61.99; H, 4.63; N, 20.31; S, 13.29%.

*3.2.7. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-4-methyl-5-((E)-1-(2-(4-(p-tolyl)thiazol-2-yl)hydrazineylidene)-ethyl)thiazole (**9b**).* Brown solid; (yield 0.45 g, 90%) mp: 181−183 °C; IR, υ 3318, 3233, 3221 (3NH), 2950 (C−H), 1599 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.29 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.39 (s, 3H, CH_3−C═N), 2.48 (s, 3H, CH_3−C═N), 7.18−7.34 (m, 8H, Ar−H), 7.46 (s, 1H, thiazole-H5), 8.12 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 8.42 (s, 1H, NH), 11.87 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125

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MHz) δ: 12.3 (CH_3), 18.4 (CH_3), 21.4 (CH_3), 22.6 (CH_3), 115.7, 116.3, 120.5, 121.7, 123.4, 125.8, 126.6, 127.5, 129.3, 130.2, 131.9, 134.4, 137.8, 140.5, 142.2, 145.1, 151.8, 156.8, 162.2, 168.9 ppm (Ar−C and C═N); MS, m/z (%) 499 (M^+, 14), 301 (100), 218 (76), 115 (74), 81 (51). Anal. Calcd for $C_{26}H_{25}N_7S_2$ (499.16): C, 62.50; H, 5.04; N, 19.62; S, 12.83. Found: C, 62.38; H, 4.93; N, 19.41; S, 13.01%.

*3.2.8. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-5-((E)-1-(2-(4-(4-methoxyphenyl)thiazol-2-yl)-hydrazineylidene)ethyl)-4-methylthiazole (**9c**).* Yellow solid; (yield 0.45 g, 88%) mp: 209−211 °C; IR, υ 3318, 3233, 3220 (3NH), 2951 (C−H), 1600 (C═N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.30 (s, 3H, CH_3), 2.36 (s, 3H, CH_3−C═N), 2.47 (s, 3H, CH_3−C═N), 3.82 (s, 3H, OCH_3), 7.18−7.35 (m, 8H, Ar−H), 7.47 (s, 1H, thiazole-H5), 8.12 (s, 1H, pyrrole-H), 8.32 (s, 1H, NH), 8.41 (s, 1H, NH), 11.77 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 13.0 (CH_3), 16.7 (CH_3), 19.0 (CH_3), 56.5 (OCH_3), 113.1, 116.5, 117.9, 119.4, 120.1, 125.2, 125.3, 129.6, 129.9, 131.6, 133.1, 133.4, 143.0, 143.4, 150.8, 153.8, 159.4, 159.6, 161.4, 164.5 (Ar−C and C═N) ppm; MS, m/z (%) 515 (M^+, 23), 399 (92), 269 (100), 185 (42), 138 (81), 112 (40), 96 (33), 77 (46). Anal. Calcd for $C_{26}H_{25}N_7OS_2$ (515.16): C, 60.56; H, 4.89; N, 19.01; S, 12.43. Found: C, 60.42; H, 4.97; N, 19.11; S, 12.21%.

*3.2.9. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-5-((E)-1-(2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazineylidene)-ethyl)-4-methylthiazole (**9d**).* Yellow solid; (yield 0.45 g, 86%) mp: 190−192 °C; IR, υ 3321, 3235, 3228 (3NH), 2961 (C−H), 1602 (C═N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.30 (s, 3H, CH_3), 2.41 (s, 3H, CH_3−C═N), 2.46 (s, 3H, CH_3−C═N), 7.18−7.58 (m, 8H, Ar−H), 7.48 (s, 1H, thiazole-H5), 8.13 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 8.42 (s, 1H, NH), 11.88 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 12.8 (CH_3), 17.9 (CH_3), 22.4 (CH_3), 115.7, 116.3, 120.8, 121.7, 123.5, 125.8, 126.8, 127.5, 129.5, 130.5, 132.3, 134.5, 136.4, 140.8, 142.6, 145.2, 152.1, 156.8, 162.2, 168.9 ppm (Ar−C and C═N); MS, m/z (%) 521 (M^+ + 2, 4), 519 (M^+, 13), 320 (98), 279 (83), 238 (52), 159 (74), 117 (100), 88 (78), 77 (42). Anal. Calcd for $C_{25}H_{22}ClN_7S_2$ (519.11): C, 57.74; H, 4.26; N, 18.85; S, 12.33. Found: C, 57.98; H, 4.33; N, 18.71; S, 12.29%.

*3.2.10. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-5-((E)-1-(2-(4-(4-bromophenyl)thiazol-2-yl)-hydrazineylidene)ethyl)-4-methylthiazole (**9e**).* Yellow solid; (yield 0.46 g, 82%) mp: 212−214 °C; IR, υ 3321, 3230, 3218 (3NH), 2958 (C−H), 1604 (C═N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.30 (s, 3H, CH_3), 2.40 (s, 3H, CH_3−C═N), 2.46 (s, 3H, CH_3−C═N), 7.18−7.50 (m, 8H, Ar−H), 7.48 (s, 1H, thiazole-H5), 8.14 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 8.44 (s, 1H, NH), 11.79 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 12.8 (CH_3), 17.6 (CH_3), 21.9 (CH_3), 114.9, 115.8, 120.8, 121.8, 123.6, 125.9, 126.9, 127.5, 129.6, 130.5, 132.2, 134.8, 136.4, 141.1, 143.2, 145.2, 152.7, 156.8, 163.9, 168.8 ppm (Ar−C and C═N); MS, m/z (%) 563 (M^+, 24), 385 (70), 251 (37), 197 (58), 138 (100), 115 (40), 77 (53). Anal. Calcd for $C_{25}H_{22}BrN_7S_2$ (563.06): C, 53.19; H, 3.93; N, 17.37; S, 11.36. Found: C, 53.06; H, 3.83; N, 17.51; S, 11.28%.

*3.2.11. 2-(2-((E)-1-(1H-Indol-3-yl)ethylidene)hydrazineyl)-4-methyl-5-((E)-1-(2-(4-(4-nitrophenyl)thiazol-2-yl)-hydrazineylidene)ethyl)thiazole (**9f**).* Yellow solid; (yield 0.42 g, 80%) mp: 219−221 °C; IR, υ 3320, 3240, 3230 (3NH), 2961 (C−H), 1608 (C═N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.28 (s, 3H, CH_3), 2.36 (s, 3H, CH_3−C═N), 2.48 (s, 3H, CH_3−C═N), 7.12−7.51 (m, 8H, Ar−H), 7.44 (s, 1H, thiazole-H5), 8.13 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 8.42 (s, 1H, NH), 11.81 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 12.8 (CH_3), 18.4 (CH_3), 22.7 (CH_3), 114.8, 115.4, 120.6, 121.7, 123.8, 125.9, 126.9, 127.7, 129.6, 130.8, 132.6, 134.8, 137.9, 140.7, 142.2, 145.4, 152.3, 156.8, 162.2, 168.9 ppm (Ar−C and C═N); MS, m/z (%) 530 (M^+, 6), 390 (26), 273 (45), 191 (71), 117 (100), 77 (50). Anal. Calcd for $C_{25}H_{22}N_8O_2S_2$ (530.13): C, 56.59; H, 4.18; N, 21.12; S, 12.08. Found: C, 56.78; H, 4.23; N, 20.99; S, 12.19%.

*3.2.12. 1-(2-(2-((E)-1-(2-(2-((E)-1-(1H-Indol-3-yl)-ethylidene)hydrazineyl)-4-methylthiazol-5-yl)ethylidene)-hydrazineyl)-4-methylthiazol-5-yl)ethan-1-one (**11a**).* Yellow solid; (yield 0.37 g, 80%) mp: 170−172 °C; IR, υ 3310, 3230, 3218 (3NH), 2914 (C−H), 1700 (C═O), 1604 (C═N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.39 (s, 3H, $COCH_3$), 2.44 (s, 3H, CH_3−C═N), 2.47 (s, 3H, CH_3−C═N), 7.14−7.42 (m, 4H, Ar−H), 8.29 (s, 1H, pyrrole-H), 8.31 (s, 1H, NH), 8.34 (s, 1H, NH), 11.81 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 13.1 (CH_3), 16.7 (CH_3), 17.8 (CH_3), 19.1 (CH_3), 25.3 (CH_3), 116.5, 117.8, 119.9, 124.6, 125.3, 125.8, 129.6, 131.6, 133.1, 135.4, 143.4, 143.9, 150.8, 153.8, 159.6, 164.5 (Ar−C and C═N), 193.0 (C═O) ppm; MS, m/z (%) 465 (M^+, 27), 230 (39), 270 (44), 187 (60), 138 (100), 115 (52), 83 (41), 77 (69). Anal. Calcd for $C_{22}H_{23}N_7OS_2$ (465.14): C, 56.75; H, 4.98; N, 21.06; S, 13.77. Found: C, 56.91; H, 4.82; N, 21.11; S, 13.82%.

*3.2.13. Ethyl 2-(2-((E)-1-(2-(2-((E)-1-(1H-indol-3-yl)-ethylidene)hydrazineyl)-4-methylthiazol-5- yl)ethylidene)-hydrazineyl)-4-methylthiazole-5-carboxylate (**11b**).* Yellow solid; (yield 0.39 g, 80%) mp: 214−216 °C; IR, υ 3312, 3235, 3221 (3NH), 2918 (C−H), 1695 (C═O), 1604 (C═N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 1.16 (t, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.39 (s, 3H, $COCH_3$), 2.44 (s, 3H, CH_3−C═N), 2.46 (s, 3H, CH_3−C═N), 4.20 (q, 2H, CH_2), 7.15−7.43 (m, 4H, Ar−H), 8.29 (s, 1H, pyrrole-H), 8.34 (s, 1H, NH), 8.36 (s, 1H, NH), 11.82 (s, 1H, NH) ppm; ^{13}C NMR (DMSO, 125 MHz) δ: 13.2 (CH_3), 14.9 (CH_3), 16.2 (CH_3), 17.1 (CH_3), 18.2 (CH_3), 114.6, 115.7, 120.3, 121.8, 122.6, 128.7, 131.9, 133.5, 134.1, 137.6, 141.2, 151.7, 152.2, 156.6, 156.9, 168.0, 169.2 (Ar−C and C═N), 172.3 (C═O) ppm; MS, m/z (%) 495 (M^+, 17), 243 (48), 297 (28), 270 (31), 216 (24), 197 (37), 138 (100), 115 (38), 96 (28), 77 (26). Anal. Calcd for $C_{23}H_{25}N_7O_2S_2$ (495.15): C, 55.74; H, 5.08; N, 19.78; S, 12.94. Found: C, 55.82; H, 4.92; N, 19.58; S, 12.81%.

3.3. Biological Evaluation. *3.3.1. Cytotoxicity Assay.* For the cytotoxicity and antitumor tests, cell lines were seeded in 96-well tissue culture plates at a cell density of 5×10^4 cells/well in media. Following a 24 h incubation period, compounds were added to the 96-well plates at eight different concentrations with six repetitions. Control wells containing only medium or 0.5% DMSO were included. After 24 h of incubation, cell viability was assessed using the MTT test.[55,56]

3.3.2. Safety and SI. To establish a dose−response curve and determine the 50% cytotoxic concentration (CC_{50}) of newly synthesized compounds and the reference drug doxorubicin, a normal human lung fibroblast (MRC-5) cell line procured from the American Type Culture Collection in Rockville, MD was utilized. GraphPad Prism software was utilized to perform the aforementioned calculations. The SI was calculated by dividing the CC_{50} by the IC_{50} values. Previous research has suggested that a compound is safe if its SI value is greater than 10.[57]

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4. CONCLUSIONS

In conclusion, three novel sets of thiazolylhydrazonothiazoles were synthesized from reactions of carbothioamide derivative **5** with various hydrazonoyl chlorides (or α-haloketones). The structure of the synthesized compounds was characterized and confirmed to be promising candidates for adjunctive therapeutic agents for cancer therapy. The cytotoxicity of the synthesized compounds was evaluated against the HCT-116, HepG2, and MDA-MB-231 cell lines and demonstrated that compounds **7a−c**, **9a−c**, **11a**, and **11b** have interesting activity. Based on the SAR analysis, it can be observed that the in vitro inhibition effect of the 5-arylazothiazoles **7** is greater than 4-arylthiazoles **9** (**7b** > **9b**; **7c** > **9c**) towards all the examined anticancer cell lines. For thiazoles **7a−e** and **9a−f**: the electron donating groups (e.g., Me and MeO) at 4-position of phenylazothiazoles enhances the activity while the electron withdrawing groups (e.g., Cl, Br, NO_2) decrease the activity (**7c, 7b** ≫>**7a** ≫> **7d, 7e**) and (**9b, 9c** ≫> **9a** ≫> **9d, 9e, 9f**). Most of the compounds tested in the set showed good selectivity index values. Also, the majority of them showed modest harmful effects when their toxicity against normal cells (MRC-5) was assessed, indicating that they might be used safely, however more in vivo and pharmacological research may be necessary. Furthermore, the docking studies for the most promising synthesized compounds (**7a-c, 9a-c, 11a**, and **11b**) utilizing the MOE 2019 suite toward the EGFR TK protein were studied and recorded high binding scores in comparison to a reference standard (W19), thus endorsing their anticancer activity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05038.

Mass, ^{1}H, and ^{13}C NMR spectra of most of the synthesized compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Sobhi M. Gomha − *Department of Chemistry, Faculty of Science, Islamic University of Madinah, Madinah 42351, Saudi Arabia;* orcid.org/0000-0002-7739-2837; Email: smgomha@iu.edu.sa

Tariq Z. Abolibda − *Department of Chemistry, Faculty of Science, Islamic University of Madinah, Madinah 42351, Saudi Arabia*; Email: t.z.a@iu.edu.sa

Authors

Jehan Y. Al-Humaidi − *Department of Chemistry, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh 11671, Saudi Arabia*

Sayed M. Riyadh − *Department of Chemistry, Faculty of Science, Cairo University, Cairo 12613, Egypt*

Mohamed S. Ibrahim − *Department of Chemistry, Faculty of Science, Islamic University of Madinah, Madinah 42351, Saudi Arabia*

Magdi E. A. Zaki − *Department of Chemistry, Faculty of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh 11623, Saudi Arabia*

Ohoud A. Jefri − *Department of Biological Science, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia*

Amr S. Abouzied − *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hail, Hail 81442, Saudi Arabia; Department of Pharmaceutical Chemistry, National Organization for Drug Control and Research (NODCAR), Giza 12311, Egypt;* orcid.org/0000-0002-9202-3909

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c05038

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