Dual Targeting of VEGFR2 and C-Met Kinases via the Design and Synthesis of Substituted 3-(Triazolo-thiadiazin-3-yl)indolin-2-one Derivatives as Angiogenesis Inhibitors

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1. INTRODUCTION

Receptor tyrosine kinases (RTKs) are a large family of kinase enzymes targeted by different chemotherapeutic medications. The main role of RTKs is to phosphorylate the tyrosine amino acid in several proteins aided by ATP (γ-phosphoryl group donor). This phosphorylation activates the signaling pathway at the cellular level, which is a crucial process for differentiation, proliferation, migration, and antiapoptotic pathway. However, dysregulation of RTK signaling at the cellular level leads to an assortment of human diseases, most notably cancers. The vascular endothelial growth factor receptor 2 (VEGFR2) and c-mesenchymal epithelial transition factor (c-Met) are RTKs which are linked to the hepatocyte growth factor and endothelial cells, respectively. These RTKs activate the phosphorylation cascade of different tyrosines through the dimerization process aided by ATP to terminally induce cell growth. The role of c-Met and VEGFR2 is complementary in the process of induction of angiogenesis of human cancer cells. This significant role in tumorigenesis has received great attention in different research groups for dual targeting VEGFR2 and c-Met kinases by novel antiangiogenic agents, as represented in Figure 1.

On the other hand, the isatin moiety was intensively reported as a key scaffold in several chemotherapeutic agents acting as VEGFR2 and/or c-Met inhibitors. Sunitinib (I; $K_i = 9.0 \text{ nM}$), an isatin derivative targeting multiple tyrosine kinase, was clinically approved in 2006 as an angiogenic anticancer agent for the therapy of renal cell carcinoma. Orantinib (II, SU6668) is another isatin analogue in phase I for the treatment of solid tumors exhibiting a significant effect on multiple tumors in xenograft models. It reveals its effect through inhibition of multiple kinases including VEGFR2 ($K_i = 9.0 \text{ nM}$). Likewise, SU14813 (III) is an orally active oxindole derivative, which inhibits multiple kinases including VEGFR2 ($IC_{50} = 0.04 \text{ μM}$). The preclinical trials proposed its use in combination with docetaxel to significantly affect different...
tumor xenograft models.\textsuperscript{17} Other isatin derivatives such as compounds (IV) and (V) (Figure 1) were designed to target VEGFR2 kinase ($IC_{50} = 0.31$ and $0.18 \mu M$, respectively).\textsuperscript{18,19} These analogues are indolinone-ureid hybrids that were designed to fit within the active site of the VEGFR2 enzyme targeting its key amino acids.

The c-Met kinase is the second target of interest to share VEGFR2 in the angiogenesis process within the cancer cells. Similar to VEGFR2, many isatin derivatives targeted c-Met kinase as inhibitors, for instance, an oxindole derivative (SU11274, VI Figure 2) that downregulates the viability of NSCLC cells via the inhibition of c-Met kinase with $IC_{50} = 12$ nM.\textsuperscript{20,21} 3-Hydrazinoindolin-2-one derivatives (VIIa,b, and VIII; Figure 2) are equipotent inhibitors for c-Met kinase as well, with $IC_{50} = 7$ nM.\textsuperscript{22} Compound VIII was cocry stallized with c-Met kinase and submitted to the protein data bank (PDB code: 3ZZE).\textsuperscript{23}

Many of the above-mentioned scaffolds with a triazole ring fused to aryl-substituted six-membered heterocycles and/or isatin functionalities were intensively reported to have potent c-Met kinase inhibitory activities.\textsuperscript{24–32} Moreover, different nitrogenous fused and hybrid heterocyclic compounds (pyrrolotriazine, benzimidazole, imidazopyridine, thienopyrimidine, and dioxoquinazoline) were reported to be promising dual-acting VEGFR2 and c-Met inhibitors.\textsuperscript{33–38} Besides, the compounds containing triazolothiadiazine fragments were
reported to possess a broad spectrum of activities such as antitumor, antibacterial, antiviral, antifungal, and PDE4 inhibitory activities.\(^{39-42}\)

### 1.1. Rational Design.

The rational design of our hybrid isatin and triazolothiadiazine compounds depends mainly on the aforementioned significant dual inhibitory activities of isatin and oxindole against VEGFR2 and c-Met-mediated tumorigenesis (Figure 3).

Such numerous reported studies for targeting VEGFR2 and c-Met-mediated tumorigenesis have drawn tremendous interest and prompt us to synthesize a series of hybrid 3-hydrazino-indolin-2-one derivatives (6a–γ; Scheme 1). The synthesized compounds include two series of 3-hydrazino-indolin-2-one derivatives in which the terminal N is directly attached to the isatin and triazolothiadiazine moieties. These compounds were investigated for their cytotoxic activity by the National Cancer Institute (NCI, USA) at 10 \( \mu \)M in a full NCI 58 cell panel. Then, the most active compound was screened for its inhibitory activities against VEGFR2 and c-Met kinases, and a molecular docking study was conducted to justify its mode of kinase inhibition. Additionally, the promising compounds were subjected to an in vivo hen egg chorioallantoic membrane (HET-CAM) study to check their angiogenic effects.

### 2. RESULTS AND DISCUSSION

#### 2.1. Chemistry.

The synthesis of target compounds 6a–γ was carried out, as shown in Scheme 1. The requisite N-unsubstituted isatin \( 2 \) was prepared by alkylation from their N-unsubstituted analogues as reported\(^{43-45}\) with bromoalkanes under anhydrous condition via stirring in dimethylformamide (DMF) or acetonitrile in the presence of \( \text{K}_2\text{CO}_3 \) in room temperature overnight or under reflux. The synthesis of the requisite series of compounds 2-(6-aryl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazin-1-ium chloride (5a–d) was conducted by the reflux of purpald (4; 4-amino-5-hydrazino-1,2,4-triazole-3-thiol) and phenacyl bromides (3) in the presence of hydrochloric acid (1 N).\(^{46}\) The target compounds 6a–γ were prepared by refluxing the intermediate 5 with isatin \( 2 \) in ethanol where the desired target product precipitates during the reaction, which is isolated by suction filtration, followed by recrystallization from aqueous DMF. As there is no literature precedence for the synthesis of intermediates 5a–d, a synthetic methodology was developed for these compounds taking into account the close nucleophilicity of sulfhydryl, amino, and hydrazino groups of purpald.\(^{46}\)

Many attempts were made to react 2'-bromoacetophenone (3; \( R^1 = H \)) with purpald (4) under reflux in ethanol in the absence or presence of a base (\( \text{NaOH} \), \( \text{Na}_2\text{CO}_3 \)) or in the presence of acetic acid. However, these attempts resulted in a mixture of products that were difficult to purify. This reaction was successful only by reflux in 1N hydrochloric acid for 50 min, as shown in Scheme 1.

In a related procedure,\(^{47}\) the benzaldehyde selectively reacted with the amino group of purpald (4) under reflux in aqueous ethanol in the presence of 1 N HCl to afford a very good yield. Upon application of the same method using phenacyl bromides instead of aldehydes, the desired intermediate compounds were isolated in good yields.

#### 2.2. Biological Screening.

##### 2.2.1. In Vitro Primary Single-Dose (10 \( \mu \)M Concentration) Screening against NCI 58 Human Cell Panel.

Eight representative compounds 6a, 6b, 6e, 6l, 6n, 6r, 6v, and 6y were investigated for their anticancer activities by single-dose in vitro screening at 10 \( \mu \)M concentration against NCI 58 human cell lines by the Developmental Therapeutics Program (DTP) in NCI (https://dtp.cancer.gov/).\(^{48}\) These cell lines were collected from nine different subpanels (hematopoietic system, lung cancer, colon, brain, skin melanoma, prostate, breast, kidney, and ovary). The antiproliferative data for each compound were reported as a mean percent cell growth of the treated cancer cells in comparison to the untreated negative control cells, and the
## Table 1. Growth Inhibition Percentage (GI %) of in Vitro Subpanel Tumor Cell Lines at Single-Dose (10 μM) Concentration for Compounds 6a, 6b, 6e, 6l, 6n, 6r, 6v, and 6y

| subpanel/cell line | 6a | 6b | 6e | 6l | 6n | 6r | 6v | 6y |
|--------------------|----|----|----|----|----|----|----|----|
| leukemia           |    |    |    |    |    |    |    |    |
| K-562              | 23 | 85 | 14 | 15 | 19 | 14 | 13 | 53 |
| MOLT-4             | 13 | 51 | 17 | 12 | 18 | 13 | 10 | 30 |
| RPMI-8226          | 21 | 20 | 21 | 16 | 15 | 12 | 15 | 16 |
| SR                 | 23 | 64 | 27 | 23 | 36 | 21 | 44 | 28 |
| NSCLS              |    |    |    |    |    |    |    |    |
| A549/ATCC          | 31 | 45 | 18 | 23 | 29 | 17 | 25 | 31 |
| EKV                | 13 |    |    |    | 15 |    |    |    |
| HOP-62             | 54 | 66 | 20 | 15 | 17 | 11 | 27 | 37 |
| HOP-92             | 36 | 22 | 15 | 15 | 18 | 11 | 24 | 16 |
| NCI-H23            | 31 | 35 | 19 | 16 |    | 16 | 22 |    |
| NCI-H322M          |    |    |    |    |    |    |    |    |
| NCI-H460           | 17 | 65 | 20 | 20 | 19 | 16 | 23 | 51 |
| NCI-H522           | 57 | 60 | 39 | 34 | 25 | 41 | 41 | 35 |
| colon cancer       |    |    |    |    |    |    |    |    |
| COLO 205           | 17 |    |    |    |    |    |    |    |
| HCC-2998           | 19 | 12 | 13 |    |    |    |    |    |
| HCT-116            | 14 | 70 | 17 |    |    |    |    |    |
| HCT-15             | 10 |    |    |    |    |    |    |    |
| HT29               | 28 | 57 | 27 | 27 | 24 | 44 | 16 | 16 |
| KM12               | 40 | 24 | 14 | 16 |    | 18 | 20 | 22 |
| SW-620             | 21 |    |    |    |    |    |    |    |
| CNS cancer         |    |    |    |    |    |    |    |    |
| SF-268             | 31 | 12 | 16 | 12 | 31 | 25 | 26 | 30 |
| SF-295             | 12 |    |    |    | 12 | 10 | 16 | 30 |
| SF-539             | 18 | 15 | 13 | 12 | 18 |    |    |    |
| SNB-19             | 17 | 37 | 10 | 18 | 16 | 16 | 26 | 26 |
| SNB-75             | 35 | 17 | 12 | 16 | 18 |    |    |    |
| U251               | 50 | 37 |    |    |    |    |    |    |
| melanoma           |    |    |    |    |    |    |    |    |
| LOX IMVI           | 38 | 27 |    |    |    |    |    |    |
| MALME-3M           | 25 |    |    |    |    |    |    |    |
| M14                | 29 | 14 | 12 | 12 | 33 | 29 | 33 | 29 |
| MDA-MB-435         | 22 | 19 | 12 | 12 | 52 | 14 | 16 | 16 |
| SK-MEL-2           | 13 | 27 | 11 | 14 | 16 | 16 | 20 | 16 |
| SK-MEL-28          | 15 |    |    |    | 15 | 12 | 12 | 12 |
| SK-MEL-5           | 20 |    |    |    | 16 | 17 |    |    |
| UACC-257           | 11 | 35 | 20 | 24 | 20 | 23 | 44 | 16 |
| UACC-62            | 23 |    |    |    | 28 | 21 |    |    |
| ovarian cancer      |    |    |    |    |    |    |    |    |
| IGROV1             | 17 |    |    |    |    |    |    |    |
| OVCAR-3            | 12 | 17 |    |    |    |    |    |    |
| OVCAR-4            | 41 | 21 | 26 | 22 | 22 |    |    |    |
| OVCAR-5            | 18 |    |    |    |    |    |    |    |
| OVCAR-8            | 41 | 51 |    |    | 13 | 20 |    |    |
| NCI/ADR-RES        | 11 |    |    |    |    |    |    |    |
| SK-OV-3            | 11 |    |    |    |    |    |    |    |
| renal cancer       |    |    |    |    |    |    |    |    |
| 786-0              | 19 | 20 | 12 |    |    |    |    |    |
| A498               | 23 | 18 | 10 | 24 |    |    |    |    |
| ACHN               | 35 |    |    |    | 17 | 23 |    |    |
| Caki-1             | 21 | 34 | 13 | 11 | 33 | 25 |    |    |
| RXF 393            | 18 | 45 | 30 |    |    |    |    |    |
| SN12C              | 21 |    |    |    |    |    | 12 | 25 |
| TK-10              | 29 |    |    |    |    |    |    | 17 |
| UO-31              |    | 27 | 23 |    |    |    |    |    |
| breast cancer      |    |    |    |    |    |    |    |    |
| PC                 |    |    |    |    |    |    |    |    |
| PC-3               | 20 | 44 | 10 |    |    |    |    |    |
| DU-145             | 16 | 50 | 23 |    |    |    |    |    |
| MCF7               | 29 | 28 | 21 |    |    |    |    |    |
| MDA-MB-231         | 12 | 23 | 11 |    |    |    |    |    |
| HS 578T            | 28 | 14 |    |    | 27 |    |    |    |
| BT-549             | 28 | 41 | 29 |    |    | 24 | 24 |    |
| T-47D              | 36 | 47 | 29 | 15 | 12 | 18 | 16 | 29 |
| MDA-MB-468         | 33 |    |    |    |    |    |    |    |
| mean growth, %     |    |    |    |    |    |    |    |    |
| sensitive cell lines no. | 16 | 31 | 10 |    |    |    |    |    |
|                   | 34 | 52 | 30 | 11 | 12 | 14 | 21 | 44 |
Percent growth inhibition (GI %) was calculated by the deduction of the percent cell growth from 100. As depicted in Table 1, compounds 6a, 6b, 6e, 6v, and 6y of the tested 3,5-substituted-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl-hydrazineylidine)indolin-2-one derivatives exhibited low to moderate nonselective inhibition of different cancer cell lines.

Compound 6b revealed the most efficient antiproliferative activity (GI %), with a broad-spectrum activity against numerous cell lines that belong to diverse cancer subpanels. In particular, compound 6b displayed a potent growth inhibitory impact against leukemia (CCRF-CEM, K-562, MOLT-4, RPMI-8226, and SR), nonsmall cell lung cancer (NCI-H522, NCI-H460, and HOP-62), colon cancer (HT29 and HCT-116), ovarian cancer (OVCA-8), and prostate cancer (DU-145) cell lines with inhibition percents of 51, 85, 51, 61, 64, 66, 65, 60, 70, 57, 51, and 50%, respectively. In addition, compound 6b exerted cytotoxic activity with GI equal to or greater than 35% against nonsmall cell lung cancer (A549/ATCC and NCI-H23), colon cancer (KM12), CNS cancer (SNB-19 and U251), melanoma (LOX IMVI and UACC-257), renal cancer (ACHN and RXF 393), and breast cancer (BT-549 and T-47D) cell lines with inhibition percents of 45, 35, 40, 37, 37, 35, 35, 45, 41, and 47%, respectively.

Moreover, compound 6y was found to be the second most potent analogue (mean GI = 21) with broad-spectrum activity against different cell lines. Compound 6y showed GI equal to or greater than 35% over leukemia (CCRF-CEM and K-562), nonsmall cell lung cancer (HOP-62, NCI-H460, and NCI-H522), colon cancer (HCT-116 and HCT-15), melanoma (LOX IMVI), and breast cancer (MCF7) cell lines with inhibition percents of 46, 53, 37, 51, 35, 46, 43, 37, and 35%, respectively.

Further investigation of the obtained results in Table 1 unveiled that leukemia (K-562 and SR), nonsmall cell lung cancer (A549/ATCC and NCI-H522), colon cancer (HT29), and melanoma (UACC-257) cell lines were susceptible to the cytotoxic effect of all tested compounds including 6a, 6b, 6e, 6i, 6n, 6r, 6v, and 6y. The susceptible cell lines to the effect of compounds 6a, 6v, and 6y with GI > 35 are listed in Table 1. The cell lines HOP-62, HOP-92, NCI-H522, SNB-75, U251, OVCAR-4, OVCAR-8, and T-47D are susceptible to compound 6a by GI of 35–57%. However, SR, NCI-H460, NCI-H522, HCT-15, and MDA-MB-435 cells are sensitive to compound 6y by a GI of 37–52%. Compound 6y exhibited a GI of 35–53% against CCRF-CEM, K-562, HOP-62, NCI-H460, HCT-116, HCT-15, LOX IMVI, and MCF7 cell lines.

2.2.2. Angiogenesis Study. Twenty-one test compounds were subjected to an in vivo HET-CAM study to check their angiogenic effects. In this study, dexamethasone (DEXA) which was reported to demonstrate potent antiangiogenic activity was used as a positive control. This study was conducted in two phases to check the mortality and toxicity of the test compounds at 40 and 100 μM concentrations against HEM-CAM. In the first phase, 40 μM of each of the test compounds dissolved in 100% dimethyl sulfoxide (DMSO) equivalent was applied onto HET-CAM on the 9th day, and the embryos were examined on the 11th day. All compounds produced 100% mortality of the chick embryos. In the second phase, 40 μL of each compound dissolved in 40% DMSO in sterile saline equivalent 40 μM was applied onto HEM-CAM on the 9th day, and then, the embryos and CAM were examined on the 11th day. Yet, at lower concentration (40 μM), eight compounds, namely, 6c, 6e, 6g, 6j, 6u, 6v, 6w, and 6y, were toxic against the HEM-CAM and the chick embryos. The other 13 compounds did not exhibit any detectable mortality against the chick embryos. Figure 4 shows the number of newly formed blood capillaries in HET-CAM during exposure to 40 μM of the 13 nontoxic compounds in comparison to the formed ones by DEXA (0.02 μg) as a potent antiangiogenic agent and using 40% DMSO as a negative control. DEXA, the positive control, produced a 54% reduction in the number of newly formed blood capillaries (P < 0.001). Seven compounds 6b, 6d, 6f, 6n, 6o, 6l, and 6x showed a significant decrease in the number of blood vessels as well. Compound 6b is the most effective derivative producing 56% reduction of angiogenesis comparable to the effect of DEXA.

Compound 6n was the least significantly effective (27%, P < 0.05). In contrast, two compounds 6a and 6h produced a significant increase in the number of newly formed blood capillaries (33 and 35%, respectively), whereas the other four compounds 6i, 6x, 6l, and 6m revealed a nonstatistically significant change.

Further validation was carried out by quantitative assessment of angiogenesis on HET-CAM using image analysis. Compound 6b (as the most effective antiangiogenic agent) and compound 6h (which increases angiogenesis) were tested. Their effects on the angiogenesis of CAM were examined on the 11th day using computerized image analysis to count newly formed small blood vessels in the circle of 100 mm² area representing a standard area for all the angiographs. Figure 5 shows the development of new blood vessels in HEM-CAM, after the application of DMSO (vehicle), compound 6b, and compound 6h, respectively. The results are expressed as mean ± standard error of three eggs using image analysis and
compared with one-way ANOVA and Dunnett’s multiple comparison test.

2.3. VEGFR-2, PDGFR, and C-Met Kinase Inhibitory Activities. As aforementioned, compound 6b superiorly emerged as the most efficient antiangiogenic and antiproliferative agent; accordingly, 6b was selected to evaluate its inhibitory activity toward VEGFR-2, PDGFR, and C-Met kinases. Table 2 displays the inhibition data, as IC50 values, for compound 6b and the clinically used sunitinib as a standard reference. As shown in Table 2, 6b exhibited good VEGFR-2, PDGFR, and C-Met submicromolar inhibitory activities with IC50 values equal to 435, 371, and 654 nM, respectively.

Table 2. IC50 Values for the Inhibitory Activities of Compound 6b toward VEGFR-2, PDGFR, and C-Met Kinases

| compound | VEGFR-2 (nM) | PDGFR (nM) | C-Met (nM) |
|----------|--------------|------------|------------|
| 6b       | 435          | 371        | 654        |
| sunitinib| 346          | 304        | 468        |

2.4. Molecular Docking Studies. The significant inhibitory activity of compound 6b was deeply investigated through molecular docking simulations inside the active site of the VEGFR-2 crystal structure (PDB: 4agd).56 This crystal structure contains sunitinib as a cocrystallized ligand, which was useful to obtain the key amino acids responsible for the activity. This crystal structure showed that the oxindole moiety played an important role in the biological activity by forming two hydrogen bonds with Cys919 and Glu917 (CDOCKER energy = −27.56 kcal/mol). Docking simulations showed that the oxindole moiety of compound 6b played the same role as the H-bond with Cys919 was 2.03 Å, whereas the H-bond with Glu917 was 2.10 Å. The triazine ring and phenyl ring aided compound 6b to be embedded in the pocket by forming a π-hydrophobic interaction and π-cation interaction with Leu840 and Lys838, respectively (Figure 6).

Compound 6b revealed additional inhibitory activity against c-Met kinase which can be illustrated by molecular docking studies using the crystal structure of c-Met kinase downloaded from protein databank (3ZZE) cocrystallized with an inhibitor with oxindole moiety.56 Compound 6b accepts a hydrogen bond with Met1160 (2.16 Å) by an oxindole ring, which is similar to the cocrystallized ligand (CDOCKER energy = −31.23 kcal/mol). This hydrogen bond was supported by another one between the triazine ring with Tyr1159 (2.69 Å). The oxindole moiety was embedded inside the hydrophobic region consisting of Ala1108, Val 1092, Met1211, and Tyr1230 (Figure 7).

3. CONCLUSIONS

Twenty-five 3-hydrazinoindolin-2-one derivatives directly attached to triazolo-thiadiazin-3-yl moiety (6a–y) were designed, synthesized, and investigated for their cytotoxicity. Eight compounds 6a, 6b, 6e, 6l, 6n, 6r, 6v, and 6y were assessed for their anticancer activity against a panel of NCI 58
tumor cell lines including nine different subpanels (hematopoietic system, lung cancer, colon, brain, skin melanoma, ovary, kidney, prostate, and breast). Five substituted 3-((triazolo-thiadiazin-3-yl)indolin-2-one derivatives revealed moderate to low broad-spectrum activity against most of NCI cancer cell lines. Among them, compound 6b was the most effective analogue revealing an average growth inhibition of 40%, specifically against leukemia, NSCLC, colon, ovarian, and prostate cancers within the range of 51−85%. Further angiogenesis assay was conducted using HET-CAM to check the mortality and toxicity of the test compounds at 100 and 40 μM concentrations. Thirteen out of 21 test compounds were nontoxic for the chick embryos, and 7 of these compounds revealed a remarkable reduction of the blood vessels developed including compound 6b as the most effective angiogenic agents. Therefore, compound 6b was subjected for VEGFR-2, PDGFR, and C-Met kinase inhibitory assays to exhibit submicromolar inhibitory activities of IC50 435, 371, and 654 nM, respectively. Docking of compound 6b inside the active site of both C-Met kinase and VEGFR2 showed the crucial role of the oxindole moiety as it revealed a hydrogen bond with Met1160 (2.16 Å) in c-Met kinase active site and hydrogen bond with D1222 in the VEGFR2 active site.

4. EXPERIMENTAL SECTION

4.1. Chemistry. 4.1.1. General. The Bruker spectrometer was used to record the proton magnetic resonance 1H NMR, 13C NMR, and 13C DEPT135 at 300 MHz and 75 MHz. DMSO-d6 was used as a solvent to generate all NMR spectra. The strong peak of DMSO at δ 2.50 was used for spectral calibration. The coupling constants are expressed in Hz. Both protons of NH and OH protons were measured as D2O exchangeable. The chemical shifts were expressed in values (ppm). The coupling constant (J) is given in hertz (Hz). The abbreviations used are as follows: s, singlet; d, doublet; and m, multiplet. High-resolution mass spectrometry (HRMS) was performed at Campus Chemical Instrument Center (CCIC) Mass Spectrometry and Proteomics Facility at The Ohio State University, USA. Melting points (mp) were measured with a Stuart melting point apparatus and were uncorrected. Thin-layer chromatography using precoated glass plates silica gel plates of 60F254-Merck was used for monitoring the progress of reactions, and our compound products were visualized by a UV lamp.

4.1.2. General Procedures for the Preparation of (6-Aryl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazin-1-ium Chloride (5a). Purpald (4; 2.92 g, 20 mmol, 1.1 equiv) was added to a stirred solution of 30 mL of 1 N HCl. The mixture was heated whereby a clear solution is obtained, and then a solution of 2’-bromoacetophenone (3; 18 mmol, 1.0 equiv) in 30 mL of ethanol was added dropwise over 10 min. Heating of the reaction mixture was continued for another 50 min. The reaction mixture was then left to cool unstirred for 2–3 h, whereby a precipitate of 5 is formed. The formed precipitate was suction filtered, washed with 10 mL of ice-cold water, and dried. The crude product 5 was used without further purification.

4.1.2.1. 2-(6-Phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazin-1-ium Chloride (5a). Yield 3.87 g (76%) of yellowish-white powder; mp 205–206 °C; 1H NMR (300 MHz, DMSO-d6): δ 10.19 (2H, br s, D2O exchangeable, 3-NH), 8.11 (2H, br s, Ar-2’-6’-H), 7.61 (3H, br s, Ar-3’-4’-5’-H), 4.49 (2H, s, 7-CH2). 13C NMR (125 MHz, DMSO): δ 154.99 (1C, 6C), 151.38 (1C, 8a-C), 137.56 (1C, 3C), 132.25 (1C, Ar-1’-C), 129.97 (2C, Ar-2’-6’-CH), 129.58 (3C, Ar-3’-4’-5’-CH), 23.60 (1C, 7-CH3). DEPT C135 (125 MHz, DMSO): δ 129.97 (2C, Ar-2’-6’-CH), 129.58 (3C, Ar-3’-4’-5’-CH), 23.26 (1C, 7-CH3). HRMS (ES+): m/z calcd for C16H13N3S [M + H]+, 247.0766; found, 247.0763.

4.1.2.2. 2-(6-(p-Tolyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazin-1-ium Chloride (5b). Yield 4.2 g (79%) of yellowish-white powder; mp 205–206 °C; 1H NMR (300 MHz, DMSO-d6): δ 9.21 (2H, br s, D2O exchangeable, 3-NH-NH2), 9.21 (1H, br s, D2O exchangeable, 3-NH), 7.98 (2H, dd, J = 7.3 Hz, Ar-2’-6’-H), 7.39 (2H, br s, Ar-3’-5’-H), 4.43–4.50 (2H, br s, 7-CH2), 2.40 (3H, s, Ar-3’-5’-H). 13C NMR (125 MHz, DMSO): δ 154.99 (1C, 6C), 151.38 (1C, 8a-C), 137.56 (1C, 3C), 132.25 (1C, Ar-1’-C), 129.97 (2C, Ar-2’-6’-CH), 129.58 (3C, Ar-3’-4’-5’-CH), 23.60 (1C, 7-CH3). DEPT C135 (125 MHz, DMSO): δ 129.97 (2C, Ar-2’-6’-CH), 129.58 (3C, Ar-3’-4’-5’-CH), 23.26 (1C, 7-CH3). HRMS (ES+): m/z calcd for C16H13N3S [M + H]+, 261.0912; found, 261.0918.

4.1.2.3. 2-(6-(4-Methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazin-1-ium Chloride (5c). Yield 4.4 g (78%) of yellowish-white powder; mp 209–210 °C; 1H NMR (300 MHz, DMSO-d6): δ 9.17 (1H, br s, D2O exchangeable, 3-NH), 7.95–8.10 (2H, dd, J = 37.0, 8.8 Hz, Ar-2’-6’-H), 7.61 (3H, d, J = 8.8 Hz, Ar-3’-5’-H), 4.45 (2H, s, J = 5.5 Hz, 7-CH2); 13C NMR (125 MHz, DMSO): δ 154.99 (1C, 6C), 151.38 (1C, 8a-C), 137.56 (1C, 3C), 132.25 (1C, Ar-1’-C), 129.97 (2C, Ar-2’-6’-CH), 129.58 (3C, Ar-3’-4’-5’-CH), 23.60 (1C, 7-CH3). HRMS (ES+): m/z calcd for C16H13N3S [M + H]+, 261.0912; found, 261.0918.

Figure 7. Docking mode of compound 6b into the binding site of the c-Met kinase (PDB: 3zze). It was docked within an rmsd of 2.0 Å from the cocrystallized ligand (6XP; cyan lines), revealing one hydrogen bond with D1222 in addition to electrostatic and multiple hydrophobic binding interactions.
7-CH2), 3.86 (3H, s, 5′-OCH3). 13C NMR (125 MHz, DMSO): δ 163.01 (1C, 6′-C), 161.44 (1C, Ar-4′-C), 155.85 (1C, 8a-C), 151.15 (1C, 3′-C), 130.15 (2C, Ar-1′-C), 125.35 (1C, Ar-1′-C), 114.90 (2C, Ar-3′, 5′-C), 56.09 (1C, 5′-OCH3), 23.17 (1C, 7-CH2). DEPT C135 (125 MHz, DMSO): δ 130.15 (2C, Ar-2′,6′-CH), 114.90 (2C, Ar-3′, 5′-CH), 56.09 (1C, 5′-OCH3), 23.17 (1C, 7-CH2). HRMS (ES+): m/z calcd for C19H16N7O2S[ M+H]+, 406.1086; found, 406.1082.

4.1.2.4. 2-(6-(4-Chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-hydrazine hydrochloride (5d). Yield 4.6 g (81%) of yellowish-white powder; mp 184–185 °C; 1H NMR (300 MHz, DMSO-d6): δ 9.18 (1H, br s, D2O exchangeable, 3-NH), 9.05 (1H, br s, D2O exchangeable, 3-NH-NH2), 8.13 (2H, d, J = 8.5 Hz, Ar-2′,6′-H), 7.68 (2H, d, J = 8.5 Hz, Ar-3′,5′-H), 4.47 (2H, s, 7-CH2). 13C NMR (125 MHz, DMSO): δ 154.99 (1C, 6′-C), 151.38 (1C, 8a-C), 139.70 (1C, 3′-C), 137.56 (2C, Ar-4′-C), 132.15 (1C, Ar-1′-C), 129.97 (2C, Ar-3′,5′-CH), 129.58 (2C, Ar-2′,6′-CH), 23.26 (1C, 7-CH2). DEPT C135 (125 MHz, DMSO): δ 129.97 (2C, Ar-3′,5′-CH), 129.58 (2C, Ar-2′,6′-CH), 23.26 (1C, 7-CH2). HRMS (ES+): m/z calcd for C19H16ClN3O2S[ M+H]+, 390.1376; found, 390.1367.

4.1.2.5. General Procedure for the Synthesis of Compound 6a–y. A mixture of the appropriate isatin (2; 1.0 mmol, 1.0 equiv) and appropriate compound (5; 1.3 mmol, 1.3 equiv) in ethanol (5 mL) was heated under reflux for 1 h. The reaction mixture was cooled to room temperature, and the crude product was filtered off, washed, and dried. The crude product was recrystallized from the DMF/H2O mixture (5:1).

4.1.3.1. 3-2-(6-Phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineyldiene)indolin-2-one (6a). Yield 304 mg (81%) of yellowish-white powder; mp 284–285 °C; 1H NMR (300 MHz, DMSO-d6): δ 13.03 (1H, br s, D2O exchangeable, 3-NH), 11.26 (1H, br s, D2O exchangeable, 3-NH), 8.05 (2H, d, J = 6.3 Hz, Ar-2′,6′-H), 7.71–7.59 (3H, m, Ar-3′,5′,6′-H), 7.56 (1H, d, J = 7.7 Hz, Ar-4′-H), 7.36 (1H, t, J = 7.7 Hz, Ar-6′-H), 7.11 (1H, t, J = 7.7 Hz, Ar-5′-H), 6.98 (1H, d, J = 7.7 Hz, Ar-7′-H), 4.48 (2H, s, 7-CH2). 13C NMR (125 MHz, DMSO): δ 163.88 (1C, 2′-C=O), 159.36 (1C, 6′-C), 155.37 (1C, 8a-C), 149.48 (1C, 3′-C), 141.74 (1C, 7′-C), 134.32 (1C, Ar-1′-C), 133.61 (1C, 3′-C), 135.27 (1C, 6′-CH), 131.00 (1C, Ar-4′-CH), 126.90 (2C, Ar-3′,5′-CH), 127.79 (2C, Ar-2′,6′-CH), 126.67 (1C, 4′-CH), 122.99 (1C, 5′-CH), 120.41 (1C, 3′-CH), 111.56 (1C, 7′-CH), 23.78 (2C, 7-CH2). DEPT C135 (125 MHz, DMSO): δ 132.57 (1C, 6′-CH), 131.01 (1C, Ar-4′-CH), 126.90 (2C, Ar-3′,5′-CH), 127.79 (2C, Ar-2′,6′-CH), 126.67 (1C, 4′-CH), 122.99 (1C, 5′-CH), 120.50 ? 111.56 (1C, 7′-CH), 23.78 (1C, 7-CH2). HRMS (ES+): m/z calcd for C19H16N3O2S[ M+H]+, 376.0981; found, 376.0976.

4.1.3.2. 5-Methoxy-3-(2-(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineyldiene)indolin-2-one (6b). Yield 357 mg (88%) of yellowish-white powder; mp 223 °C; 1H NMR (300 MHz, DMSO-d6): δ 13.34 (1H, br s, D2O exchangeable, 3-NH), 11.06 (1H, br s, D2O exchangeable, 1′-NH), 8.05 (2H, d, J = 6.3 Hz, Ar-2′,6′-H), 7.70–7.48 (3H, m, Ar-3′,5′,6′-H), 7.10 (1H, s, Ar-4′-H), 6.95–6.86 (1H, m, Ar-7′-H), 6.83–6.66 (1H, m, Ar-6′-H), 4.47 (2H, s, 7-CH2), 3.78 (3H, s, 6′-OCH3). 13C NMR (125 MHz, DMSO): δ 164.00 (1C, 2′-C=O), 155.79 (1C, 6′-C), 155.37 (1C, 8a-C), 154.72 (1C, 3′-C), 149.46 (1C, 7′-C), 135.42 (1C, Ar-1′-C), 134.60 (1C, 3′-C), 132.56 (1C, Ar-4′-CH), 129.59 (2C, Ar-3′,5′-CH), 127.79 (2C, Ar-2′,6′-CH), 121.14 (1C, 3′-a-C), 117.39 (1C, 7′-CH), 112.41 (1C, 4′-CH), 105.28 (1C, 6′-CH), 56.02 (1C, 5′-OCH3), 23.77 (1C, 7-CH2). DEPT C135 (125 MHz, DMSO): δ 132.57 (1C, Ar-4′-CH), 129.59 (2C, Ar-3′,5′-CH), 127.79 (2C, Ar-2′,6′-CH), 117.39 (1C, 7′-CH), 112.41 (1C, 4′-CH), 105.28 (1C, 6′-CH), 56.02 (1C, 5′-OCH3), 23.76 (1C, 7-CH2). HRMS (ES+): m/z calcd for C19H16N3O2S[ M+H]+, 406.1086; found, 406.1082.

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132.56 (1C, Ar-4′-CH), 129.59 (2C, Ar-3′,5′-CH), 127.79 (2C, Ar-2′,6′-CH), 121.14 (1C, 3′-a-C), 117.39 (1C, 7′-CH), 112.41 (1C, 4′-CH), 105.28 (1C, 6′-CH), 56.02 (1C, 5′-OCH3), 23.77 (1C, 7′-CH3). DEPT C135 (125 MHz, DMSO): δ 132.57 (1C, Ar-4′-CH), 129.59 (2C, Ar-3′,5′-CH), 127.79 (2C, Ar-2′,6′-CH), 117.39 (1C, 7′-CH), 112.41 (1C, 4′-CH), 105.28 (1C, 6′-CH), 56.02 (1C, 5′-OCH3), 23.76 (1C, 7′-CH3). HRMS (ES+): m/z calc'd for C18H16N7O2S [M + H]+, 410.0591; found, 410.0593.

4.1.3.6. 3-(2-(6-(4-Chlorophenyl)-7H-1,2,4]triazolo[3,4-b][1,3]thiadiazin-3-yl)hydrazine-eylidene)-5-methyloindolin-2-one (6f). Yield 396 mg (90%) of yellowish-white powder; mp 292 °C; 1H NMR (300 MHz, DMSO-d6): δ 13.13 (1H, br s, D2O exchangeable, 3-NH), 11.04 (1H, br s, D2O exchangeable, 1′-NH), 8.23 (2H, br s, Ar-2′,6′-H), 8.02 (2H, br s, Ar-3′,5′-H), 7.07 (1H, s, Ar-4′-H), 6.98–6.20 (2H, m, 6′-H and 7′-H), 4.44 (2H, s, 7′-CH3), 3.75 (3H, s, 5′-OCH3). 13C NMR (125 MHz, DMSO): δ 163.97 (1C, 2′-C=O), 161.41 (1C, 5′-C), 155.77 (1C, 6′-C), 154.31 (1C, 8a-C), 149.42 (1C, 3′-C), 137.41 (1C, 7′-a-C), 135.42 (1C, Ar-4′-C), 133.02 (1C, 3′-C), 132.46 (1C, Ar-1′-C), 129.68 (2C, Ar-3′,5′-CH), 129.50 (2C, Ar-2′,6′-CH), 124.46 (1C, 3′-a-C), 117.39 (1C, 7′-CH), 112.38 (1C, 6′-CH), 105.29 (1C, 4′-CH), 56.01 (1C, 5′-OCH3), 23.68 (1C, 7′-CH3). DEPT C135 (125 MHz, DMSO): δ 112.70 (2C, Ar-3′,5′-CH), 129.51 (2C, Ar-2′,6′-CH), 117.39 (1C, 7′-CH), 112.39 (1C, 6′-CH), 105.29 (1C, 4′-CH), 56.01 (1C, 5′-OCH3), 23.68 (1C, 7′-CH3). HRMS (ES+): m/z calc'd for C22H19Cl2N7O2S [M + H]+, 440.0696; found, 440.0691.

4.1.3.7. 3-(2-(6-(4-Methylphenyl)-7H-1,2,4]triazolo[3,4-b][1,3]thiadiazin-3-yl)hydrazine-eylidene)indolin-2-one (6g). Yield 361 mg (88%) of yellowish-white powder; mp 293 °C; 1H NMR (300 MHz, DMSO-d6): δ 13.28 (1H, br s, D2O exchangeable, 3-NH), 11.23 (1H, br s, D2O exchangeable, 1′-NH), 8.02 (2H, d, J = 8.7 Hz, Ar-2′-H), 7.56 (1H, d, J = 7.5 Hz, Ar-4′-H), 7.35 (1H, t, J = 7.7 Hz, Ar-6′-H), 7.20–7.08 (3H, m, Ar-3′,5′,6′-H), 6.98 (1H, d, J = 7.7 Hz, Ar-5′-H), 4.43 (2H, s, 7′-CH3), 3.86 (3H, s, 4′-OCH3). 13C NMR (125 MHz, DMSO): δ 163.87 (1C, 2′-C=O), 162.86 (1C, 6′-C), 154.93 (1C, 8a-C), 149.36 (1C, 3′-C), 141.69 (1C, 7′-a-C), 138.33 (1C, 4′-C), 134.15 (1C, 3′-C), 130.93 (1C, 6′-CH), 129.67 (2C, Ar-2′,6′-CH), 124.99 (2C, Ar-1′,5′-CH), 125.64 (1C, 3′-a-C), 129.97 (1C, 4′-CH), 120.45 (1C, 5′-CH), 110.24 (2C, Ar-3′,5′-CH), 111.54 (1C, 7′-CH), 56.05 (1C, 4′-OCH3), 23.56 (1C, 6′-CH3). DEPT C135 (125 MHz, DMSO): δ 130.93 (1C, 6′-CH), 126.97 (2C, Ar-2′,6′-CH), 122.97 (1C, 4′-CH), 120.45 (1C, 5′-CH), 110.24 (2C, Ar-3′,5′-CH), 111.54 (1C, 7′-CH), 56.06 (1C, 4′-OCH3), 23.55 (1C, 6′-CH3). HRMS (ES+): m/z calc'd for C22H20N7O2S [M + H]+, 443.1399; found, 443.1396.

4.1.3.8. 3-(2-(6-(4-Methylthiophenyl)-7H-1,2,4]triazolo[3,4-b][1,3]thiadiazin-3-yl)hydrazine-eylidene)-5-methylindolin-2-one (6h). Yield 364 mg (87%) of yellowish-white powder; mp 285–284 °C; 1H NMR (300 MHz, DMSO-d6): δ 13.26 (1H, br s, D2O exchangeable, 3-NH), 11.12 (1H, br s, D2O exchangeable, 1′-NH), 8.01 (2H, d, J = 8.4 Hz, Ar-2′-H), 7.37 (1H, s, Ar-4′-H), 7.24–7.05 (3H, m, Ar-3′,5′,6′-H), 6.86 (1H, d, J = 7.9 Hz, Ar-7′-H), 4.42 (2H, s, 7′-CH3), 3.86 (3H, s, 4′-OCH3), 2.31 (3H, s, 5′-CH3). 13C NMR (125 MHz, DMSO): δ 163.94 (1C, 2′-C=O), 162.84 (1C, 6′-C), 154.87 (1C, 8a-C), 149.38 (1C, 3′-C), 139.45 (1C, 7′-a-C), 138.27 (1C, Ar-4′-C), 134.26 (1C, 3′-a-C), 131.37 (1C, 6′-CH), 129.66 (2C, Ar-2′,6′-CH), 129.37 (1C, Ar-1′-C), 125.64 (1C, 3′-a-C), 120.83 (1C, 4′-CH), 115.02 (2C, Ar-3′,5′-CH), 111.29 (1C, 7′-CH), 56.04 (1C, 4′-OCH3), 23.54 (1C, 7′-CH3), 21.08 (1C, 5′-CH3). DEPT C135 (125 MHz, DMSO): δ 131.37 (1C, 6′-CH), 129.66 (2C, Ar-2′,6′-CH), 120.82 (1C, 4′-CH), 115.02 (2C, Ar-3′,5′-CH), 111.29 (1C, 7′-CH), 56.04 (1C, 4′-OCH3), 23.53 (1C, 7′-CH3), 21.08 (1C, 5′-CH3). HRMS (ES+): m/z calc'd for C24H17N5O5S [M + H]+, 420.1243; found, 420.1238.
OCH₃), 23.55 (1C, 7-CH₃). DEPT C¹³⁵ (125 MHz, DMSO): δ 129.68 (2C, Ar'-2',-6'-CH), 115.03 (2C, Ar-3',-5'-CH), 112.55 (1C, 7′-CH), 110.32 (1C, 4′-CH), 107.63 (1C, 6′-CH), 56.05 (1C, 4′-OCH₃), 23.54 (1C, 7-CH₃). HRMS (ES+): m/z calc'd for C₁₉H₂₃FN₂O₅S·[M + H]^+ 424.0992; found, 424.0989.

4.1.3.12. 5-Chloro-3-(2-(6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3]thiadiazin-3-yl)hydrazineyliden)-indolin-2-one (6f). Yield 440 mg (85%) of yellowish-white powder; mp 284–285 °C (decomp.). ¹H NMR (300 MHz, DMSO-d₆): δ 13.27 (1H, br s, D₂O exchangeable, 3-NH), 11.35 (1H, br s, D₂O exchangeable, 1′-NH), 8.72 (1H, s, Ar-4′-H), 8.06 (2H, d, J = 8.0 Hz, Ar-2′-,6′-H), 7.25 (1H, d, J = 8.0 Hz, Ar-7′-H), 7.13–7.08 (2H, m, Ar-3′-,5′-H), 6.84 (1H, d, J = 8.0 Hz, Ar-6′-H), 4.45 (2H, s, 7-CH₂). HRMS (ES+): m/z calc'd for C₂₀H₁₈BrN₇O₅S·[M + H]^+ 487.9696; found, 487.9697.

4.1.3.13. 5-Cloro-3-(2-(6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3]thiadiazin-3-yl)hydrazineyliden)-indolin-2-one (6m). Yield 416 mg (86%) of yellowish-white powder; mp 297–298 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 13.25 (1H, br s, D₂O exchangeable, 3-NH), 11.34 (1H, br s, D₂O exchangeable, 1′-NH), 8.87 (1H, s, Ar-4′-H), 8.12 (2H, d, J = 8.5 Hz, Ar-2′-,6′-H), 7.38 (1H, d, J = 8.2 Hz, Ar-7′-H), 7.16–7.05 (2H, m, Ar-3′-,5′-H), 6.80 (1H, d, J = 8.2 Hz, Ar-6′-H), 4.45 (2H, s, 7-CH₂). DEPT C¹³⁵ (125 MHz, DMSO): δ 129.51 (2C, Ar-2′-,6′-CH), 126.49 (1C, 6′-CH), 123.59 (1C, 4′-OCH₃), 22.61 (1C, 7-CH₂). HRMS (ES+): m/z calc'd for C₁₉H₂₃ClN₂O₅S·[M + H]^+ 424.0992; found, 424.0989.

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5-Methyl-3-(2-(6-(p-tolyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-1-methylindolin-2-one (65)

Yield 327 mg (81%) of yellow powder; mp 253-255 °C; 1H NMR (300 MHz, DMSO-d6): δ 13.27 (1H, br s, D2O exchangeable, 3-NH), 11.14 (1H, br s, D2O exchangeable, 1'-NH), 9.74 (2H, d, J = 8.1 Hz, Ar-3',5'-H), 7.41 (2H, J = 8.1 Hz, Ar-2',6'-H), 7.18 (1H, s, Ar-4'-H), 7.16 (1H, d, J = 8.0 Hz, Ar-3',6'-H), 6.86 (1H,d, J = 7.9 Hz, Ar-7'-H), 4.44 (2H, s, 7-CH2), 2.40 (3H, s, 4-CH3), 2.41 (3H, s, 5-CH3). 13C NMR (125 MHz, DMSO): δ 161.33 (1C, 2C=O=O), 163.96 (1C, 6-C), 155.28 (1C, 8a-C), 149.45 (1C, 3-C), 142.88 (1C, Ar-4'- C), 139.51 (1C, 3-C), 138.36 (1C, 7a-C), 134.43 (1C, 5'- C), 132.02 (1C, Ar-1'-C), 131.45 (1C, 6'-C), 130.77 (1C, 3'- C), 130.18 (2C, Ar-3,5'-CH), 127.76 (2C, Ar-2,6'-CH), 120.87 (1C, 4'-CH), 111.33 (1C, 7'-CH3), 23.65 (1C, 7'-CH), 21.54 (1C, 4'-CH3), 20.19 (1C, 5'-CH3). DEPT C13S (125 MHz, DMSO): δ 140.72 (1C, 2C=O=O), 141.28 (1C, 6-C), 146.37 (1C, 3-C), 142.00 (1C, 7a-C), 139.10 (1C, 5'-C), 130.21 (2C, Ar-3,5'-CH), 127.63 (2C, Ar-2,6'-CH), 120.76 (1C, 4'-CH), 111.73 (1C, 7'-CH3), 23.65 (1C, 7'-CH), 21.54 (1C, 4'-CH3), 20.19 (1C, 5'-CH3). HRMS (ES+): m/z calc for C28H19N3O2S [M + H]+, 468.0242; found, 468.0239.

3-Chloro-3-(2-(6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-1-propylindolin-2-one (66).

Yield 448 mg (93%) of yellowish-white powder; mp 266-268 °C; 1H NMR (300 MHz, DMSO-d6): δ 13.06 (1H, br s, D2O exchangeable, 3-NH), 8.75 (1H, s, Ar-4'-H), 8.09 (2H, d, J = 8.5 Hz, Ar-2',6'-H), 7.31 (1H, d, J = 8.1 Hz, Ar-7'-H), 7.18-6.98 (3H, m, Ar-3',5'-H and Ar-6'-H), 4.45 (2H, s, 7-CH2), 3.87 (3H, s, 4-OCH3), 3.26 (3H, s, 1'-NCH3). 13C NMR (125 MHz, DMSO): δ 155.65 (1C, 2C=O=O), 145.89 (1C, 6-C), 140.19 (1C, 3-C), 139.29 (1C, 7a-C), 138.77 (1C, 5'-C), 137.35 (1C, 5'-C), 130.25 (2C, Ar-3,5'-CH), 129.23 (1C, 6'-CH), 128.92 (1C, 4'-CH), 128.51 (1C, 4'-CH), 127.01 (1C, 6'-CH), 125.88 (1C, 4'-CH), 122.12 (1C, 3'-CH), 121.13 (1C, 1'-CH), 20.03 (1C, 5'-CH3). HRMS (ES+): m/z calc for C26H22ClN4O3S [M + H]+, 498.0392; found, 498.0352.
13.12 (1H, br s, D2O exchangeable, 3-NH), 8.01 (2H, d, 8.0 Hz, Ar-2, J = 8.5 Hz, Ar-2,′-H), 7.61 (1H, d, J = 7.4 Hz, Ar-4,′-H), 7.42–7.21 (6H, m, Ar-3,′,5,5′, 6, 2,′-H), 7.20–7.09 (3H, m, Ar-3,′,5,5′-H), 7.04 (1H, d, J = 7.5 Hz, Ar-7,′-H), 5.05 (2H, s, 1,′-N-CH2Ph), 4.44 (2H, s, 7-CH3), 3.85 (3H, s, 3,′-OCH3).

13C NMR (125 MHz, DMSO): δ 162.87 (1C, 2,−C=O), 162.02 (1C, 6,−C), 155.16 (1C, 8a-C), 154.74 (1C, 3−C), 154.91 (1C, 7,−C), 138.59 (1C, Ar-4′,C), 133.16 (1C, 3,−C), 130.76 (1C, 6′,C), 126.99 (2C, Ar-2,′−6,′-CH), 129.21 (2C, Ar-2,′−6,′-CH), 128.04 (1C, 4,−CH), 127.70 (2C, Ar-3,′−5,′-CH). 136.21 (1C, Ar-1,′-C), 124.38 (2C, Ar-1′-C and 3,′-C), 123.63 (1C, Ar-4,′-CH), 120.31 (1C, 5,−C), 115.08 (1C, Ar-3,′,5′-CH), 110.82 (1C, 7,−C), 56.05 (1C, 4,′-OCH3), 42.91 (2H, s, 1,′-N-CH2Ph), 23.66 (1C, 7-CH3). DEPT C135 (125 MHz, DMSO): δ 130.77 (1C, 6,−C), 129.69 (2C, Ar-2,′-6,′-CH), 129.21 (2C, Ar-2,′−6,′-CH), 128.04 (1C, 4,−CH), 127.70 (2C, Ar-3,′−5,′-CH), 126.63 (1C, Ar-4,′-CH), 120.32 (1C, 5,−C), 115.08 (1C, Ar-3,′,5′-CH), 110.82 (1C, 7,−C), 56.05 (1C, 4,′-OCH3), 42.91 (2H, s, 1,′-N-CH2Ph), 23.66 (1C, 7-CH3).

HRMS (ES+): m/z calcld for C26H22N7O2S [M + H]+, 496.1563; found, 496.1563.

4.1.3.25. 1-Benzyl-5-chloro-3-(2-(6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazinyliden)indolin-2-one (6y). Yield 477 mg (90%) of yellowish-white powder; mp 285–286 °C; 1H NMR (300 MHz, DMSO-d6): δ 13.10 (1H, br s, D2O exchangeable, 3-NH), 8.77 (1H, s, Ar-4,′-H), 8.10 (2H, d, J = 8.5 Hz, Ar-2,′−6,′-H), 7.39–7.30 (4H, m, Ar-6,′, 3,′,5,′,5′-H), 7.26 (2H, d, J = 8.0 Hz, Ar-2,′−6,′-H), 7.10 (2H, d, J = 8.5 Hz, Ar-3,′,5′-H), 6.93 (1H, d, J = 8.4 Hz, Ar-7,′-H), 4.98 (2H, s, 1,′-N-CH2Ph), 4.46 (2H, s, 7-CH3), 3.86 (3H, s, 4,′-OCH3). 13C NMR (125 MHz, DMSO): δ 164.93 (1C, 2,−C=O), 162.77 (1C, 6,−C), 154.92 (1C, 8a-C), 154.85 (1C, 3−C), 140.37 (1C, 7,−C), 139.43 (1C, Ar-4′,C), 138.16 (1C, 3,′-C), 137.23 (1C, Ar-1′,−C), 129.57 (2C, Ar-2,′−6,′-CH), 129.13 (2C, Ar-2,′−6,′-CH), 128.55 (1C, 4,−CH), 127.81 (1C, Ar-4,′-CH), 127.60 (2C, Ar-3,′,5′-CH, 126.35 (2C, 6,−CH and 5,−CH), 125.99 (1C, Ar-1′,−C), 119.55 (1C, Ar-3,−C), 119.41 (2C, Ar-3,′,5′−CH), 110.30 (1C, 7,′−CH), 56.05 (1C, 4,′-OCH3), 42.89 (2H, s, 1,′-N-CH2Ph), 22.62 (1C, 7-CH3). DEPT C135 (125 MHz, DMSO): δ 129.57 (2C, Ar-2,′−6,′-CH), 129.13 (2C, Ar-2,′−6,′-CH), 128.55 (1C, 4,−CH), 127.60 (2C, Ar-3,′,5′−CH), 127.81 (1C, Ar-4,′-CH), 126.35 (1C, 6,−CH), 114.91 (2C, Ar-3,′,5′−CH), 110.31 (1C, 7,′−CH), 56.05 (1C, 4,′-OCH3), 42.89 (2H, s, 1,′-N-CH2Ph), 22.62 (1C, 7-CH3). HRMS (ES+): m/z calcld for C26H22N7O2S [M + H]+, 496.1566; found, 496.1563.
adhesive tape. The toxicity of each compound on the eggs was examined on the 11th day by candling (Galal et al., 2016).49

4.2.2.2. Testing the Effect of Synthesized compounds on HET-CAM Angiogenesis. The experiment was carried out as follows: the optimum nontoxic concentration of each compound (40 or 100 μM) was added on the filter paper disc. Fertilized eggs were incubated for 9 days at the same conditions and divided into the groups of three eggs. Each compound (40 μM) dissolved in 40 μL of 40% DMSO in saline was added dropwise on the filter paper disc. A control group was used where 40 μL of 40% DMSO in saline was used.

4.2.2.3. Quantitative Assessment of Angiogenesis on HET-CAM Using Image Analysis. The effect of the test materials on CAM angiogenesis was examined on the 11th day. An angiography was taken for every sampled egg. The examination was carried out using computerized image analysis to count newly formed small blood vessels in the circle of 100 mm² area representing a standard area for all the angiographs where the blood vessels were analyzed (Galal et al., 2016).49 The procedures were carried out for each test group, and the data were collected, tabulated, and statistically analyzed using a one-way ANOVA test, followed by Dunnett’s multiple comparison test.

4.2.3. VEGFR-2, PDGFR, and C-Met Kinase Inhibitory Assays. The design and protocol for these assays for the selected compounds 6b were designed according to the reported procedures.18,19,38

4.3. Molecular Modeling Study. Molecular docking study was conducted using Accelrys software (Discovery Studio 4) for compound 6b against VEGFR-2 (PDB code: 4AGD) and c-MET kinase (PDB code: 3ZZE). The 3D protein structures were retrieved from PDB19 and then prepared for docking by adding missing loops, removing water molecules, and adding the missing hydrogens. The co-crystallized ligand and compound 6b were drawn and prepared by preparing ligand protocol to generate a 3D structure and modeled using the CHARMM force field with full potential. Docking simulations were run using CDOCKER, and the top 10 docking poses would be finally saved. The docking process was verified by the alignment of the co-crystallized ligand and docked ligand to calculate rmsd values which were 0.95 and 0.87 Å, respectively. The docked poses were ranked according to their CDOCKER interaction energy, and the top pose was chosen for the analysis of interactions for each compound.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02038.

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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
This work was supported in part by the Center of Drug Research and Development (CDRD), Faculty of Pharmacy, The British University in Egypt. In addition, it was supported in part by the start-up fund by Texas A&M Health Sciences Center (to H.I.A.; grant number: 121500-35558).

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