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Original article

Design and synthesis of novel 2-(4-(2-(dimethylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)pyridines as potential antitumor agents

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ABSTRACT

New 2-(4-(2-(dimethylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)pyridine derivatives were synthesized and evaluated for their *in vitro* cytotoxicity against five cancer cell lines namely MKN-45, H460, HT-29, A549 and U87MG, as well as the normal cell line WI-38. Nearly all the compounds exhibited superior potency to sorafenib with a better selectivity towards the MKN-45, H460 and HT-29 cell lines. In addition, the enzymatic screening result demonstrated that the optimized compounds possessed potent Raf kinase inhibition as well as favorable enzyme selectivity. The most promising compound, **11f**, showed high levels of cytotoxicity against MKN-45, H460 and HT-29 cells with IC_{50} values of 51, 72 and 130 nM, respectively, which are 45.5, 30.4 and 27.8 folds higher than the corresponding IC_{50} values for sorafenib against these cell lines. Structure–activity relationships revealed that the dimethylaminoethyl group was crucial for high activity.

1. Introduction

Cancer is a group of diseases characterized by uncontrolled cell growth in a body, causing over 7 million deaths a year [1]. The current chemotherapeutics are far from satisfactory due to serious side effects and acquired drug resistance, which lead to an ongoing need to explore safer and more efficient antitumor agents [2,3]. Recently, small molecule inhibitors have attracted increasing levels of interest in cancer therapy, not only for their outstanding antitumor activity, but also for their diverse mechanisms of action exerted in different malignancies, which offer the possibility of minimizing relative toxicity and circumventing drug resistance [4–10].

Sorafenib is an orally available multikinase inhibitor which is active against Raf kinase by targeting the MAPKs signaling pathway, and shows a powerful inhibitory action on VEGFR and PDGFR [11,12]. It demonstrates significant inhibition across a broad spectrum of tumor types, and is considered to be a first-line therapy in patients with advanced renal and hepatocellular carcinomas [13]. In light of its satisfactory antitumor properties, as well as its proven clinical utility, scientists have paid much attention to the modification of sorafenib and have designed and synthesized a large number of related compounds. A series of benzimidazole-based compounds with general structure **I** (Fig. 1) was reported to exhibit excellent Raf kinase inhibition (0.001–0.15 μM) and to have favorable pharmacokinetic properties [14]. Further optimization on the methyl amide fragment by introducing a trifluoromethyl imidazole moiety led to the identification of RAF265, a potent inhibitor of Raf and VEGFR-2 kinases which is in phase I clinical trials for the treatment of metastatic melanoma [15,16].

The co-crystal structure of sorafenib in complex with B-Raf kinase revealed that the urea and pyridine moieties interact with amino acid residues (Asp593, Glu500 and Cys531) via hydrogen bonds, and the trifluoromethyl phenyl ring occupied the hydrophobic pocket [17,18]. Therefore, with the intention of identifying more potent analogs of novel chemotypes, we focused on modification of the pharmacophore of sorafenib while maintaining the interaction mode with the kinase domains.

Despite the high potency of sorafenib, its low level of bioavailability persuaded us to pursue a program to identify compounds

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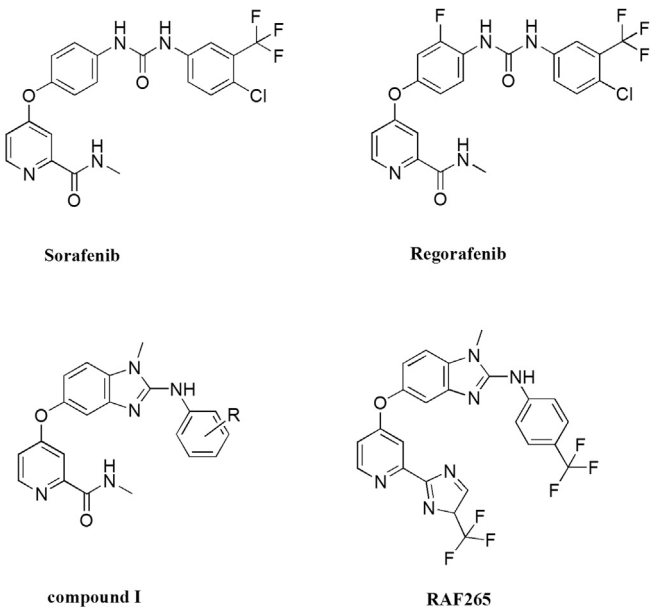


Fig. 1. Structures of sorafenib and its analogs.

with high potency combined with an improved physicochemical profile, especially higher levels of water-solubility [19–21]. As part of our overall design strategy, we inserted an additional imine group between the urea and phenyl ring, arguing that it should improve structure flexibility, which might be beneficial to biological activity and the solubility of the compounds [22,23]. Furthermore, triazole, a privileged moiety in modern drug design [24–26], replaced the methyl amide fragment to build a more drug-like skeleton (Fig. 2).

We started with a study of structure–activity relationships around the triazole moiety. We hypothesized that enhanced solubility could be achieved by introducing an ionogenic amino group on the triazole unit, and thus compounds **10a**–**10c**, with variations in the position of linkage and nature of substitution were prepared. Biological evaluation identified compound **10c**, which possessed a dimethylaminoethyl group at position-4 of triazole, as being of particular interest (comparable potency and superior physicochemical profile to sorafenib), and suggested that we should look more closely at 2-(4-(2-(dimethylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)pyridine derivatives. Therefore, our further research efforts focused on optimizing the skeleton of **10c**, with modifications to the terminal aryl moiety to find a substitution pattern which would improve the potency even further. Substituents varied in nature, steric-hindrance as well as electron property were introduced to yield compounds **11a**–**11x** and **12a**–**12c**. In this paper, we report the newly synthesized compounds and their *in vitro* antitumor potency, together with the results of a study of the mechanism of action.

2. Chemistry

The general route to synthesize the target compounds is described in Scheme 1. The starting material picolinic acid **1** was chlorinated with thionyl chloride, and then treated with methanol in an ice-bath to give the methyl ester **2** as a light-yellow solid [27]. Subsequently, a nucleophilic substitution of **2** with 2-fluoro-4-nitrophenol in refluxing chlorobenzene provided **3** in a moderate yield [28]. Aminolysis of **3** with ammonia in acetone led to the desired intermediate **4a**, which then reacted with *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) to afford **5a**. Intermediate **5b** was synthesized from **3** in a similar manner to that described for **5a**, but employing hydrazine hydrate instead of ammonia. The key intermediate **6a** was obtained by cyclization of **5a** with methylhydrazine in glacial acetic acid [29], while **6b** and **6c** were prepared by reaction of **5b** with appropriate amines (methylamine, *N*,*N*-dimethylethylenediamine) under the same conditions [30]. After reduction of the nitro compounds **6a**–**6c** with hydrazine hydrate in ethanol, the resultant amino derivatives **7a**–**7c** were further treated with phenyl chloroformate in acetone in the presence of anhydrous K_2CO_3 to give the corresponding intermediates **8a**–**8c** in good yields. In the following step, **8a**–**8c** were treated with hydrazine hydrate in refluxing 1,4-dioxane generating **9a**–**9c**, respectively. Finally, condensation of **9a**–**9c** with aromatic aldehydes in isopropanol in the presence of a catalytic amount of glacial acetic acid yielded compounds **10a**–**10c**, **11a**–**11x** and **12a**–**12c** [31].

The chemical structures of compounds **10a**–**10c**, **11a**–**11x** and **12a**–**12c** were confirmed by IR, NMR and mass spectra. 2D NOESY NMR spectra were recorded in order to determine configuration of the imino double bonds of these compounds. In the case of compound **11f**, a clear NOESY signal was observed between the proton of –NH–N= (δ 11.33 ppm, singlet) and the proton of –N=CH– (δ 8.43 ppm, singlet), indicating that compound **11f** has the *E*-configuration (Fig. 3 and supplementary data). Similarly, for compound **11g**, the NOE interaction between –NH–N= (δ 11.29 ppm, singlet) and –N=CH– (δ 8.36 ppm, singlet) confirmed that it was the *E*-isomer. The other related compounds were assigned the same *E*-configuration by analogy.

3. Results and discussion

3.1. In vitro cytotoxicity and structure–activity relationships

The cytotoxicity of compounds **10a**–**10c**, **11a**–**11x** and **12a**–**12c** were evaluated *in vitro* against five cancer cell lines, namely MKN-45 (human gastric cancer), H460 (human lung cancer), HT-29 (human colorectal cancer), A549 (human non-small-cell lung cancer) and U87MG (human glioblastoma), by the standard MTT assay. Sorafenib was used as the positive control. Potent compounds were further assessed for their safety towards the non-malignant cell line WI-38 (human fetal lung fibroblasts). The bioactivity data are presented as IC_{50} values in Tables 1 and 2. The IC_{50} values shown are the mean of the data generated by at least three replicated experiments.

The biological data presented in Table 1 suggested that the substitution pattern on the triazole moiety was closely related to antitumor potency and physicochemical profile. As shown in Table 1, compound **10a** that has a methyl substituent at position-1, was almost inactive against all the tested cancer cells and possessed a Clog*P* of 3.82 units. However, shifting the methyl to position-4 (**10b**) increased the potency significantly and decreased Clog*P* by 0.82 units, indicating a contribution derived from the position of the substituent. Furthermore, replacement of the methyl group with a dimethylaminoethyl group (**10c**) led to a 2.8–16.1-fold improvement of the antitumor activity, coupled with a comparable Clog*P* but a favorable reduction of Clog$D_{7.4}$ by 1.59 units, which probably means a better distribution of the compound in blood serum. Given these encouraging results, we retained the promising skeleton of **10c**, and modified the terminal aryl moiety with the aim of improving antitumor potency even further.

As shown in Table 2, most of the 2-(4-(2-(dimethylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)pyridine derivatives exhibited excellent activity, similar to or even higher than that of sorafenib against one or

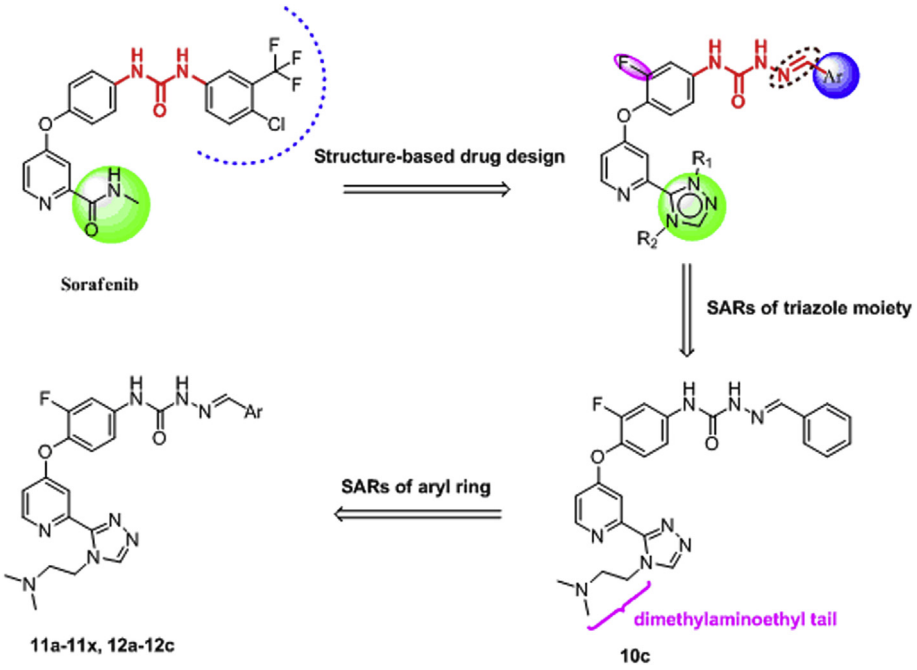


Fig. 2. Design strategy of the target compounds.

more cancer cell lines. Compounds **11a**–**11g**, **11j**, **11l**, **11s** and **12a**–**12c** displayed better efficacy than sorafenib against all the tested cancer cell lines with IC_{50} values ranging from 0.051 to 13.23 μM. Compound **11h** showed excellent levels of inhibition against MKN-45, H460, HT-29 and A549 cancer cells with IC_{50} values of 0.16, 0.35, 0.28 and 1.41 μM, respectively. Also noteworthy is that the potency of the compounds was generally more pronounced against the MKN-45, H460, HT-29 cells than against A549 and U87MG cells.

Further analysis clearly revealed that the potency of the compounds was extremely dependent on the electronic properties of the substituent(s) on the phenyl moiety. As a matter of fact, almost all the compounds (**11a**–**11h**, **11j**, **11l**) with electron-withdrawing groups (EWGs) displayed an enhanced potency in comparison to that of **10c** (R = Ph), whereas compounds (**11m**–**11s**, **11u**–**11x**) with electron-donating groups (EDGs) showed a comparative or decreased activity. It can be concluded that EWGs on the phenyl ring are more effective than EDGs. However, the introduction of EDGs at both the meta- and para-position appears to influence the inhibitory activity against the H460 and HT-29 cells, a case in point is that derivative **11r** possesses marked inhibition towards the H460 and HT-29 cells, exhibiting IC_{50} values of 0.057 and 0.12 μM, respectively. These results could be ascribed to the degree of electron density on the phenyl ring as well as the specificity of cancer cells.

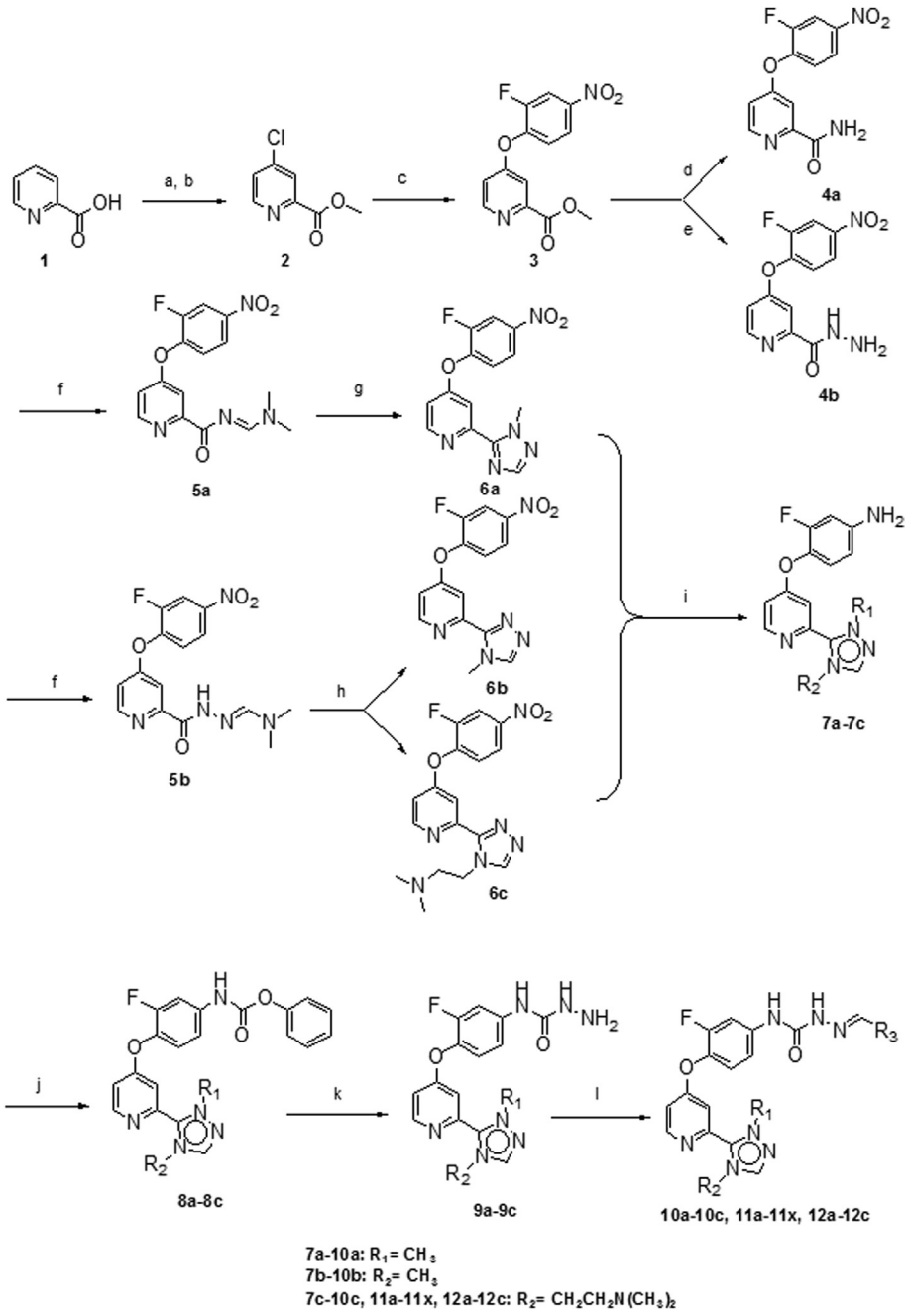
In addition, it is interesting to note that the nature of the halogen atoms on the phenyl ring plays an important role on antitumor activity. Compound **11a**, with a chlorine atom at the para-position, promoted the antitumor potency towards the H460 cells by 6.2 folds as compared to **11c**, which contains a fluorine at the same region, suggesting that chlorine is better tolerated than fluorine at the para-position. Further support for this conclusion can be drawn by comparing **11g** (R = 2,4-di-Cl-Ph; IC_{50} = 0.13 μM) with **11e** (R = 2-Cl-4-F-Ph, IC_{50} = 0.72 μM). Similarly, a comparison of **11i** (R = 3-F-4-OH-Ph) with **11j** (R = 3-Br-4-OH-Ph) in cytotoxic activity against H460 and HT-29 cells indicated that bromine is preferred in comparison to fluorine at the meta-position. In addition, the substitution position influenced the cytotoxicity of the compounds. As shown, the 2,3-dichloro-phenyl derivative **11f** showed outstanding activity towards MKN-45 cells, with an IC_{50} value of 0.051 μM, whereas the potency was reduced by 3.2 folds with the 2,6-dichloro-phenyl derivative **11h** (IC_{50} = 0.16 μM), and a further decrease in potency was observed with the 2,4-dichloro-phenyl derivative **11g** (IC_{50} = 0.75 μM).

Incorporation of bulky moieties such as bicyclic groups (**12a**–**12c**) maintained the potent activity, suggesting an insensitivity to the bulk of the aryl ring to a certain degree.

It is worth mentioning that most of the tested compounds were less toxic to human fibroblasts WI-38 in comparison to their effects on cancer cells, suggesting that the cancer cells were more sensitive to the newly synthesized compounds than the non-malignant cells. In particular, compound **11f** possessed a low level of toxicity towards WI-38 (IC_{50} = 6.13 μM), and the selectivity indexes for MKN-45 and H460 were 120.2 and 85.1, respectively, values which are 37 and 24.7 folds better than that of sorafenib.

3.2. *In vitro enzymatic assay*

In order to explore the mechanism of action of the newly synthesized compounds, analogs (**11b**, **11d**, **11f**, **11g**, **11r**, **11u** and **12c**) that had structural diversity and potent antitumor potency were screened in a panel of kinases (c-Met, VEGFR-2, B-Raf, Raf-1, PDGFRα). As shown in Table 3, five compounds (**11b**, **11d**, **11f**, **11g** and **12c**) exhibited significant inhibition against the two tested Raf kinase isoforms (B-Raf and Raf-1) at a concentration of 10 μM, while their effects on other tested kinases were much lower. These results implied that some compounds might be potential Raf kinase inhibitors, and had a favorable enzyme selectivity. In addition, the decreased affinity observed for **11r** and **11u** further confirmed SARs that the hydrophobic pocket was sensitive to the electronic properties of the substituent(s) on aryl fragment, with EWGs being well tolerated. However, we also noticed that all these analogs were less potent than sorafenib against Raf kinase. Therefore, we are currently carrying out more experiments to confirm the exact



Scheme 1. Reagents and conditions: (a) $SOCl_2$, NaBr, chlorobenzene, 50 °C, 30 min, 85 °C, 20 h; (b) MeOH, toluene, 0–15 °C, 1.5 h; (c) 2-fluoro-4-nitrophenol, chlorobenzene, reflux, 12 h; (d) $NH_3.H_2O$, acetone, 50 °C, 3 h; (e) $NH_2NH_2.H_2O$, MeCN, 25 °C, 3 h; (f) DMF-DMA, CH_2Cl_2, reflux, 2–3 h; (g) CH_3NHNH_2, HOAc, 90 °C, 3 h; (h) appropriate amine, HOAc, 90 °C, 3 h; (i) $NH_2NH_2.H_2O$, EtOH, reflux, 3–6 h; (j) phenyl chloroformate, K_2CO_3, acetone, 0–25 °C, 3 h; (k) $NH_2NH_2.H_2O$, dioxane, 90 °C, 3 h; (l) substituted aldehyde, isopropanol, reflux, 5–8 h.

targets to which these compounds bind and which leads to their interesting antitumor potency.

3.3. Binding model analysis

To elucidate the binding mode of this series of compounds with Raf kinase and to guide further SARs, a detailed docking analysis was performed on the most promising compound **11f**. The analysis was conducted using the AutoDock 4.0 protocol, and the crystal structure of wild type B-Raf kinase with sorafenib (PDB code: 1UWH) was obtained from the Protein Data Bank. Fig. 4 (left) shows the binding model overlay of the binding model of compound **11f** with sorafenib. It suggests that the two compounds act in a very similar way, with matching occupancy of the hydrophobic pocket and similar hydrogen-bond interactions. As shown in Fig. 4 (right), compound **11f** binds well to B-Raf kinase domain via four hydrogen bonds. The urea moiety of compound **11f** formed hydrogen-bonding interactions with residues Asp593 and Glu500 in the catalytic region, and the pyridine nitrogen atom interacted with residue Cys531 via a hydrogen bond. In addition, the active-site groove of 1UWH is occupied by the dimethylaminoethyl tail, which is surrounded by the residues Ile462, Val470 and Phe594.

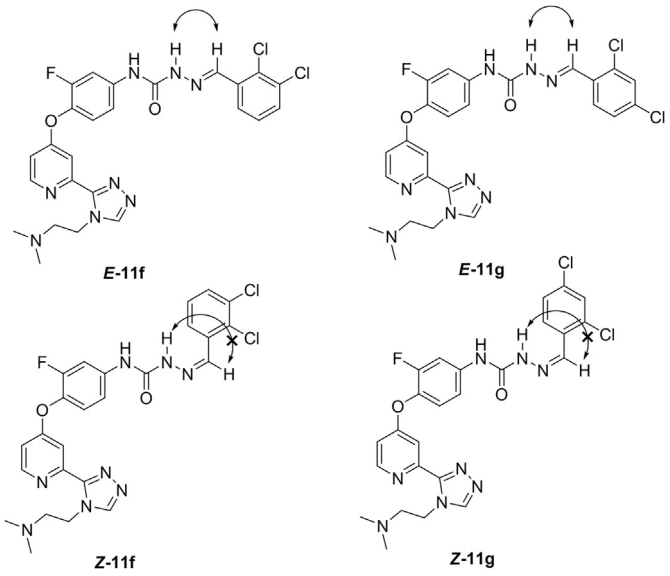


Fig. 3. NOESY effects of compounds **11f** and **11g**.

4. Conclusion

In this study, we have developed an interesting series of 2-(4-(2-(dimethylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)pyridine derivatives. Most compounds exhibited excellent cytotoxic activity towards MKN-45, H460 and HT-29 cancer cells, while showed much lower toxic effects towards human fibroblasts WI-38. Furthermore, some of the target compounds were evaluated as potential Raf kinase inhibitors with a satisfactory enzyme selectivity. An analysis of SARs revealed that the dimethylaminoethyl substituent on the triazole moiety is crucial for the observed activity, and also improves the physicochemical profile of the compounds. In addition, EWGs on the terminal aryl fragment can improve antitumor potency even further, and maintain favorable Raf kinase affinity. Taking the biological data together, compound **11f** has emerged as a promising candidate for further development of more selective Raf kinase inhibitors.

5. Experimental

5.1. Chemistry

Unless otherwise noted, all materials were obtained from commercially available sources and used without further purification. All melting points were determined on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. ^{1}H NMR (1D and 2D) and ^{13}C NMR spectroscopy were performed using Bruker ARX-300 or ARX-600 spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Mass spectra (MS) were determined in ESI mode on an Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). The IR spectra were recorded by means of the KBr pellet technique on a Bruker FTS 135 spectrometer.

5.1.1. Methyl 4-chloropicolinate (**2**)

To a solution of picolinic acid (150 g, 1.22 mol) and sodium bromide (20.1 g, 0.197 mol) in chlorobenzene (200 mL), thionyl chloride (355 mL, 4.88 mol) was added slowly at room temperature. The reaction mixture was stirred at 50 °C for 30 min, and heated to 85 °C and stirred for another 20 h. The completion of reaction was determined by TLC, at which point the solvent was removed under reduced pressure to give a brown oil, which was immediately dissolved in toluene (300 mL). Then anhydrous methanol (80 mL) was added dropwise in an ice-bath. The mixture was stirred at 15 °C for 1.5 h. The precipitate was filtered out and dissolved in CH_2Cl_2 (500 mL). The organic solution was washed with saturated aqueous K_2CO_3 (300 mL), dried over Na_2SO_4 and evaporated to give **2** as a light-yellow solid (158.5 g, yield 76.0%). M.p. 67–68 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (d, J = 5.2 Hz, 1H), 8.02 (d, J = 1.7 Hz, 1H), 7.78 (dd, J = 5.1, 1.6 Hz, 1H), 3.85 (s, 3H); ESI-MS m/z: 194.0 $[M+Na]^+$.

5.1.2. Methyl 4-(2-fluoro-4-nitrophenoxy)picolinate (**3**)

A solution of **2** (20 g, 0.117 mol) and 2-fluoro-4-nitrophenol (27.5 g, 0.175 mol) in chlorobenzene (400 mL) was stirred under reflux for 12 h. After cooling to room temperature, the mixture was concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (500 mL), and then washed with saturated aqueous K_2CO_3 (3 × 250 mL), followed by brine (200 mL). The organic phase were dried over Na_2SO_4 and concentrated to yield a gray solid, which was recrystallized from absolute ethanol to generate **3** as a pale-yellow solid (15.5 g, yield 45.4%). M.p. 86–88 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, J = 5.5 Hz, 1H), 8.19–8.17 (m, 1H), 8.16 (t, J = 3.0 Hz, 1H), 7.69 (d, J = 2.4 Hz, 1H), 7.40–7.34 (m, 1H), 7.11 (dd, J = 5.5, 2.5 Hz, 1H), 4.01 (s, 3H); ESI-MS m/z: 315.0 $[M+Na]^+$.

5.1.3. 4-(2-Fluoro-4-nitrophenoxy)picolinamide (**4a**)

Aqueous ammonia (30 mL) was added to a solution of **3** (2.92 g, 0.01 mol) in acetone (30 mL) at room temperature. The mixture was stirred at 50 °C for 3 h. The completion of the reaction was proved by TLC, and then poured into water. After stirring for 30 min, the precipitate was collected by filtration, and dried to provide **4a** as a white solid (2.09 g, yield 75.6%). M.p. 107–109 °C; ESI-MS m/z: 300.1 $[M+Na]^+$.

5.1.4. N-((dimethylamino)methylene)-4-(2-fluoro-4-nitrophenoxy) picolinamide (**5a**)

A mixture of **4a** (2.77 g, 0.01 mol) and DMF-DMA (3.58 g, 0.03 mol) in CH_2Cl_2 (20 mL) was stirred under reflux for 2 h. The solvent was removed under reduced pressure. The residue was washed with water, filtered, and dried to give **5a** as a light-yellow solid (2.84 g, yield 85.6%). M.p. 128–131 °C; ESI-MS m/z: 333.3 $[M+H]^+$.

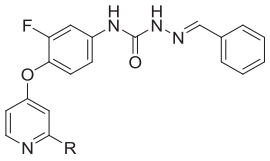
5.1.5. 4-(2-Fluoro-4-nitrophenoxy)-2-(1-methyl-1H-1,2,4-triazol-5-yl)pyridine (**6a**)

A mixture of **5a** (2 g, 6 mmol) and methylhydrazine (1.1 g, 24 mmol) in glacial acetic acid (20 mL) was heated to 90 °C and stirred for 3 h. The mixture was concentrated and then poured into ice water. After stirring for 30 min, the precipitate was collected by filtration, and then washed with water, subsequently dried to give **6a** as a light-yellow solid (0.98 g, yield 52.1%). M.p. 139–141 °C; ESI-MS m/z: 316.2 $[M+H]^+$.

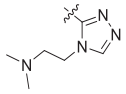
5.1.6. 4-(2-Fluoro-4-nitrophenoxy)picolinohydrazide (**4b**)

A mixture of **3** (14.6 g, 0.05 mol) and 80% hydrazine hydrate (70 mL) in MeCN (100 mL) was stirred at room temperature. TLC indicated that the reaction had gone to completion after 3 h. The mixture was diluted with CH_2Cl_2 (300 mL), and then washed with brine (3 × 100 mL). The organics were evaporated to furnish **4b** as a pale-yellow solid (9.97 g, yield 68.3%). M.p. 98–100 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.60 (d, J = 5.6 Hz, 1H), 8.47 (dd, J = 10.5, 2.7 Hz, 1H), 8.23 (m, 1H), 7.69 (t, J = 8.5 Hz, 1H), 7.53 (d,

Table 1
In vitro cytotoxicity, Clog*P* and Clog*D* values of compounds **10a**–**10c**.



10a-10c

Compd.	R_1	IC50 (μM)					Clog*P*[a]	Clog*D*[b]
		MKN-45	H460	HT-29	A549	U87MG		
10a		NA	52.23 ± 3.79	30.17 ± 2.35	NA	NA	3.82	3.82
10b		7.61 ± 1.91	15.73 ± 1.47	8.79 ± 0.69	7.56 ± 1.01	36.32 ± 4.39	3.00	3.00
10c		2.73 ± 0.76	1.78 ± 0.23	1.12 ± 0.27	1.86 ± 0.86	2.25 ± 0.75	3.02	1.41
Sorafenib		2.32 ± 0.25	2.19 ± 0.11	3.61 ± 0.36	1.92 ± 0.12	15.57 ± 1.78	4.34	4.34

NA: compound showing IC_{50} value > 100 μM.
[a] Clog*P* values, calculated using JChem_For_Excel_6.0.0.865.
[b] Clog*D* values, predicted at PH = 7.4.

J = 2.5 Hz, 1H), 7.34 (dd, *J* = 5.6, 2.6 Hz, 1H), 4.72 (s, 2H); ESI-MS *m*/*z*: 315.1 $[M+Na]^+$.

*5.1.7. N′-(4-(2-fluoro-4-nitrophenoxy)picolinoyl)-N,N-dimethylformohydrazonamide (**5b**)*

At room temperature, DMF-DMA (14.3 g, 0.12 mol) was added to **4b** (11.7 g, 0.04 mol) in CH_2Cl_2 (150 mL), the mixture was refluxed for 2 h until TLC showed the completion of the reaction. The mixture was evaporated under reduced pressure, and then washed with diethyl ether, subsequently dried to yield **5b** as an orange solid (9.84 g, yield 70.9%). M.p. 153–155 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.00 (s, 1H), 8.60 (d, *J* = 5.6 Hz, 1H), 8.47 (dd, *J* = 10.5, 2.6 Hz, 1H), 8.23 (d, *J* = 9.1 Hz, 1H), 8.06 (s, 1H), 7.69 (t, *J* = 8.6 Hz, 1H), 7.54 (d, *J* = 2.5 Hz, 1H), 7.33 (dd, *J* = 5.5, 2.6 Hz, 1H), 2.83 (s, 6H); ESI-MS *m*/*z*: 348.3 $[M+H]^+$.

*5.1.8. General procedure for preparation of compounds (**6b** and **6c**)*

Appropriate amine (0.06 mol) was added slowly to a stirred solution of **5b** (5.2 g, 0.015 mol) in glacial acetic acid (30 mL) at room temperature. The mixture was stirred at 90 °C for 3 h, and then concentrated under vacuum. The residue was poured into water, and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, and evaporated under reduced pressure to give the crude residue, which was purified by silica gel column chromatography using CH_2Cl_2/MeOH (100/1 to 100/10) as eluent to obtain the solids **6b** and **6c**.

*5.1.8.1. 4-(2-Fluoro-4-nitrophenoxy)-2-(4-methyl-4H-1,2,4-triazol-3-yl)pyridine (**6b**).* A light-yellow solid; Yield: 57%; M.p. 142–143 °C; ESI-MS *m*/*z*: 316.2 $[M+H]^+$.

*5.1.8.2. 2-(3-(4-(2-Fluoro-4-nitrophenoxy)pyridin-2-yl)-4H-1,2,4-triazol-4-yl)-N,N-dimethylethanamine (**6c**).* A yellow solid; Yield: 42%; M.p. 117–119 °C; ESI-MS *m*/*z*: 373.3 $[M+H]^+$.

*5.1.9. General procedure for preparation of compounds (**7a**–**7c**)*

At room temperature, to a suspension of nitro derivatives **6a**–**6c** (10 mmol), active carbon (0.036 g, 3 mmol) and $FeCl_3.6H_2O$ (0.41 g, 1.5 mmol) was added 80% hydrazine hydrate aqueous solution (6.25 mL, 0.1 mol) with vigorous agitation. Upon completion of addition, the mixture was stirred under reflux for 3–6 h until TLC showed the completion of the reaction. The reaction mixture was concentrated under vacuum, which was then dissolved in CH_2Cl_2, washed with brine, and evaporated to yield the solids **7a**–**7c**.

*5.1.9.1. 3-Fluoro-4-(2-(1-methyl-1H-1,2,4-triazol-5-yl)pyridin-4-yloxy)aniline (**7a**).* A gray solid; Yield: 63%; M.p. 150–153 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61 (d, *J* = 5.7 Hz, 1H), 7.99 (s, 1H), 7.48 (d, *J* = 2.4 Hz, 1H), 7.14–7.01 (m, 2H), 6.56 (dd, *J* = 13.2, 2.4 Hz, 1H), 6.47 (dd, *J* = 8.7, 2.1 Hz, 1H), 5.56 (s, 2H), 4.27 (s, 3H); ESI-MS *m*/*z*: 308.3 $[M+Na]^+$.

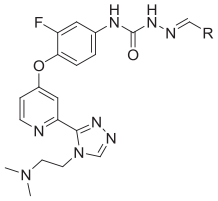
*5.1.9.2. 3-Fluoro-4-(2-(4-methyl-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)aniline (**7b**).* A light-yellow solid; Yield: 61%; M.p. 173–175 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (s, 1H), 8.58 (d, *J* = 5.7 Hz, 1H), 7.49 (d, *J* = 2.2 Hz, 1H), 7.11–7.01 (m, 2H), 6.56 (dd, *J* = 13.3, 2.3 Hz, 1H), 6.47 (dd, *J* = 8.8, 1.9 Hz, 1H), 5.68 (d, *J* = 56.8 Hz, 2H), 4.00 (s, 3H); ESI-MS *m*/*z*: 308.3 $[M+Na]^+$.

*5.1.9.3. 4-(2-(4-(2-(Dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluoroaniline (**7c**).* A light-yellow solid; Yield: 52%; M.p. 103–105 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.56 (d, *J* = 5.7 Hz, 1H), 7.49 (d, *J* = 2.4 Hz, 1H), 7.08 (m, 1H), 7.04 (m, 1H), 6.55 (dd, *J* = 13.2, 2.5 Hz, 1H), 6.46 (dd, *J* = 8.9, 2.4 Hz,1H), 5.54 (s, 2H) 4.61 (t, *J* = 6.3 Hz, 2H), 2.57 (t, *J* = 6.2 Hz, 2H), 2.13 (s, 6H); ESI-MS *m*/*z*: 343.4 $[M + H]^+$.

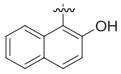
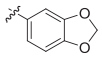
*5.1.10. General procedure for preparation of compounds (**8a**–**8c**)*

To a stirred, cooled to 0 °C solution of **7a**–**7c** (4 mmol) and K_2CO_3 (0.83 g, 6 mmol) in dry acetone (20 mL) was added phenyl chloroformate (0.81 g, 5.2 mmol). Upon completion of the addition, the reaction mixture was heated to room temperature and stirred for 3 h. The reaction mixture was quenched by addition of water and extracted with CH_2Cl_2, the combined organics were washed with brine, evaporated to give compounds **8a**–**8c**.

Table 2
In vitro cytotoxicity, Clog*P* and Clog*D* values of 2-(4-(2-(dimethylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)pyridine derivatives.



10c, 11a-11x, 12a-12c

Compd.	*R*	IC_{50} (μM)						Clog*P*[a]	Clog*D*[b]
		MKN-45	H460	HT-29	A549	U87MG	WI-38		
10c	Ph	2.73 ± 0.76	1.78 ± 0.23	1.12 ± 0.27	1.86 ± 0.86	2.25 ± 0.75	ND	3.02	1.41
11a	4-Cl-Ph	0.57 ± 0.01	0.32 ± 0.03	0.53 ± 0.02	0.78 ± 0.15	1.37 ± 0.07	1.87 ± 0.63	3.62	2.01
11b	3-Br-Ph	0.45 ± 0.03	0.52 ± 0.07	0.38 ± 0.06	0.97 ± 0.11	0.83 ± 0.13	4.23 ± 0.32	3.78	2.18
11c	4-F-Ph	1.03 ± 0.12	1.98 ± 0.08	1.37 ± 0.23	1.79 ± 0.31	2.31 ± 0.09	3.98 ± 1.17	3.16	1.56
11d	2,4-di-F-Ph	0.81 ± 0.03	1.93 ± 0.08	0.96 ± 0.13	1.87 ± 0.17	1.90 ± 0.37	5.03 ± 0.45	3.30	1.69
11e	2-Cl-4-F-Ph	0.87 ± 0.07	0.72 ± 0.12	0.51 ± 0.06	1.12 ± 0.04	1.21 ± 0.12	2.09 ± 0.33	3.76	2.15
11f	2,3-di-Cl-Ph	0.051 ± 0.022	0.072 ± 0.016	0.13 ± 0.09	1.09 ± 0.12	13.23 ± 1.67	6.13 ± 0.21	4.22	2.62
11g	2,4-di-Cl-Ph	0.75 ± 0.06	0.13 ± 0.07	0.39 ± 0.16	0.81 ± 0.15	2.33 ± 0.98	4.76 ± 0.55	4.22	2.62
11h	2,6-di-Cl-Ph	0.16 ± 0.03	0.35 ± 0.15	0.28 ± 0.09	1.41 ± 0.13	NA	3.32 ± 0.27	4.22	2.62
11i	3-F-4-OH-Ph	1.97 ± 0.27	2.62 ± 0.15	3.31 ± 0.93	13.37 ± 1.26	NA	ND	1.75	1.32
11j	3-Br-4-OH-Ph	1.86 ± 0.27	0.43 ± 0.17	0.45 ± 0.22	1.37 ± 0.11	12.38 ± 0.69	5.56 ± 0.72	2.25	1.97
11k	3,5-di-Br-4-OH-Ph	4.32 ± 0.52	3.76 ± 0.67	2.67 ± 0.85	NA	NA	ND	2.93	2.93
11l	2-NO_3-Ph	1.02 ± 0.06	1.27 ± 0.13	0.95 ± 0.21	1.20 ± 0.12	7.15 ± 0.71	6.86 ± 0.53	2.96	1.35
11m	3-OH-Ph	1.37 ± 0.32	0.78 ± 0.07	0.33 ± 0.16	NA	NA	ND	2.20	1.11
11n	4-OH-Ph	0.82 ± 0.19	1.78 ± 0.09	1.92 ± 0.32	3.56 ± 0.81	7.75 ± 0.85	ND	2.17	1.11
11o	2,4-di−OH−Ph	2.86 ± 0.67	11.32 ± 0.93	52.63 ± 5.27	12.71 ± 2.70	15.43 ± 1.26	ND	1.60	0.82
11p	3,4-di−OH−Ph	3.24 ± 0.13	1.93 ± 0.13	1.89 ± 0.07	NA	NA	ND	1.81	0.81
11q	3-OH-4-OCH_3-Ph	2.51 ± 0.12	0.62 ± 0.11	0.96 ± 0.12	NA	NA	ND	2.16	0.95
11r	4-OH-3-OCH_3-Ph	3.78 ± 0.32	0.057 ± 0.012	0.12 ± 0.02	NA	NA	5.32 ± 0.35	2.16	0.95
11s	4-OH-3,5-di−CH_3−Ph	1.51 ± 0.52	2.01 ± 0.06	1.22 ± 0.13	1.64 ± 0.25	7.71 ± 0.68	3.61 ± 0.68	3.38	2.13
11t	3,5-di-tert-butyl-2-OH-Ph	3.30 ± 0.23	0.59 ± 0.08	0.68 ± 0.15	0.98 ± 0.09	0.52 ± 0.07	2.35 ± 0.23	5.41	4.20
11u	2,4-di−OCH_3−Ph	1.68 ± 0.37	1.57 ± 0.058	1.78 ± 0.36	2.82 ± 0.12	12.63 ± 0.52	6.63 ± 1.17	2.70	1.09
11v	2,5-di−OCH_3−Ph	1.51 ± 0.28	2.93 ± 0.12	2.07 ± 0.079	1.95 ± 0.25	6.40 ± 0.73	ND	2.70	1.09
11w	2,3,4-tri−OCH_3−Ph	2.02 ± 0.17	1.79 ± 0.068	3.97 ± 0.72	6.68 ± 0.83	7.65 ± 0.82	ND	2.54	0.93
11x	3,4,5-tri−OCH_3−Ph	2.90 ± 0.10	2.77 ± 0.32	3.62 ± 0.32	NA	22.47 ± 3.32	ND	2.54	0.93
12a		0.79 ± 0.02	1.63 ± 0.37	1.30 ± 0.12	0.82 ± 0.22	0.69 ± 0.034	3.62 ± 0.37	3.11	1.51
12b		2.02 ± 0.12	0.89 ± 0.11	0.67 ± 0.06	0.88 ± 0.11	0.62 ± 0.06	3.25 ± 1.55	2.89	2.11
12c		1.97 ± 0.23	1.17 ± 0.15	0.47 ± 0.09	1.83 ± 0.13	0.72 ± 0.08	4.27 ± 0.69	2.64	1.03
Sorafenib		2.32 ± 0.25	2.19 ± 0.11	3.61 ± 0.36	1.92 ± 0.12	15.57 ± 1.78	7.54 ± 0.78	4.34	4.34

NA: compound showing IC_{50} value > 100 μM.
ND: not determined.
[a] Clog*P* values, calculated using JChem_For_Excel_6.0.0.865.
[b] Clog*D* values, predicted at PH = 7.4.

*5.1.10.1. Phenyl 3-fluoro-4-(2-(1-methyl-1H-1,2,4-triazol-5-yl)pyridin-4-yloxy)phenylcarbamate (**8a**).* A white solid; Yield: 59%; M.p. 164–166 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.66 (d, *J* = 5.7 Hz, 1H), 8.00 (s, 1H), 7.68 (dd, *J* = 12.8, 2.0 Hz, 1H), 7.51 (d, *J* = 2.5 Hz, 1H), 7.48 (d, *J* = 4.2 Hz, 1H), 7.47–7.43 (m, 3H), 7.27 (m 3H), 7.18 (dd, *J* = 5.7, 2.6 Hz, 1H), 4.27 (s, 3H); ESI-MS *m*/*z*: 406.2 $[M+H]^+$.

*5.1.10.2. Phenyl 3-fluoro-4-(2-(4-methyl-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)phenylcarbamate (**8b**).* A white solid; Yield: 77%; M.p. 198–200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.63 (m 2H), 7.68 (d, *J* = 12.5 Hz, 1H), 7.52 (m, 1H), 7.49–7.40 (m, 4H), 7.29 (m, 3H), 7.16 (dd, *J* = 5.6, 2.6 Hz, 1H), 4.00 (s, 3H); ESI-MS *m*/*z*: 428.2 $[M+Na]^+$.

*5.1.10.3. Phenyl 4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenylcarbamate (**8c**).* A white solid; Yield: 83%; M.p. 121–123 °C; ESI-MS *m*/*z*: 463.3 $[M+H]^+$.

*5.1.11. General procedure for preparation of compounds (**9a**–**9c**)*

To a solution of **8a**–**8c** (3 mmol) in dioxane, was added 80% hydrazine hydrate (0.38 mL, 6 mmol). After 3 h of vigorous stirring at 90 °C, the mixture was cooled to room temperature. The precipitate was collected by filtration, and dried to provide compounds **9a**–**9c**.

*5.1.11.1. N-(3-Fluoro-4-(2-(1-methyl-1H-1,2,4-triazol-5-yl)pyridin-4-yloxy)phenyl)hydrazinecarboxamide (**9a**).* A white solid; Yield: 71%; M.p. 210–213 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (s, 1H), 8.58

Table 3
Enzymatic screening of compounds **11b**, **11d**, **11f**, **11g**, **11r**, **11u** and **12c** *in vitro*.

Compd.	Enzyme inhibition (%)[a,b]				
	c-Met	VEGFR-2	B-Raf	Raf-1	PDGFRα
11b	7.8	1.1	72.3	66.1	13.2
11d	1.3	21.7	77.2	59.7	16.6
11f	2.2	12.8	82.6	71.1	5.9
11g	3.1	9.2	67.5	73.7	11.2
11r	10.2	33.2	56.6	52.3	3.7
11u	6.1	19.6	42.7	37.9	22.3
12c	5.8	7.3	70.5	61.6	12.3
Sorafenib	–	–	95.7	97.3	–
Foretinib	97.5	99.6	–	–	98.7

[a] Values were the average of two independent experiments, SD < 10%.
[b] Compounds tested at a concentration of 10 μM.

(d, J = 5.7 Hz, 1H), 7.93 (s, 1H), 7.79 (d, J = 13.6 Hz, 1H), 7.63 (s, 1H), 7.42 (m, 2H), 7.26 (t, J = 8.8 Hz, 1H), 7.10 (dd, J = 6.6, 3.0 Hz, 1H), 4.39 (s, 2H), 4.21 (s, 3H); ESI-MS m/z: 366.1 $[M+Na]^+$.

*5.1.11.2. N-(3-Fluoro-4-(2-(4-methyl-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)phenyl)hydrazinecarboxamide (**9b**).* A white solid; Yield: 62%; M.p. 196–197 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.61 (m, 2H), 7.85 (d, J = 13.5 Hz, 1H), 7.65 (s, 1H), 7.52 (d, J = 2.2 Hz, 1H), 7.45 (s, 1H), 7.32 (t, J = 9.0 Hz, 1H), 7.12 (dd, J = 5.7, 2.5 Hz, 1H), 4.54 (s, 2H), 4.00 (s, 3H); ESI-MS m/z: 344.2 $[M+H]^+$.

*5.1.11.3. N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl) pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**9c**).* A white solid; Yield: 81%; M.p. 207–210 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (s, 1H), 8.64 (s, 1H), 8.59 (d, J = 5.7 Hz, 1H), 7.85 (d, J = 13.6 Hz, 1H), 7.64 (s, 1H), 7.52 (d, J = 2.5, 1H), 7.45 (s, 1H), 7.33 (t, J = 9.1 Hz, 1H), 7.12 (dd, J = 2.6, 5.8 Hz, 1H), 4.62 (t, J = 6.0 Hz, 2H), 4.52 (s, 2H) 2.59 (t, J = 6.1 Hz, 2H), 2.14 (s, 6H); ESI-MS m/z: 423.2 $[M+Na]^+$.

*5.1.12. General procedure for the preparation of the target compounds (**10a**–**10c**, **11a**–**11x** and **12a**–**12c**)*

A mixture of **9a**–**9c** (1 mmol), appropriate aromatic aldehyde (1.2 mmol) and a catalytic amount of glacial acetic acid was refluxed in isopropanol (5 mL) for 5–8 h until TLC showed the completion of the reaction. After cooling to room temperature, the precipitate was filtered, and purified by silica gel column chromatography to afford compounds **10a**–**10c**, **11a**–**11x** and **12a**–**12c**.

*5.1.12.1. (E)-2-benzylidene-N-(3-fluoro-4-(2-(1-methyl-1H-1,2,4-triazol-5-yl)pyridin-4-yloxy)phenyl)hydrazinecarboxamide (**10a**).* A white solid; Yield: 57%; M.p. 152–155 °C; IR (KBr, cm^{-1}): 3389.7, 2920.2, 2850.6, 1686.1, 1593.8, 1510.7, 1491.2; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.94 (s, 1H), 9.24 (s, 1H), 8.66 (d, J = 5.7 Hz, 1H), 7.99 (s, 2H), 7.87 (d, J = 6.4 Hz, 3H), 7.65 (d, J = 10.0 Hz, 1H), 7.53 (d, J = 2.4 Hz, 1H), 7.43 (m, 4H), 7.18 (dd, J = 5.8, 2.5 Hz, 1H), 4.27 (s, 3H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.43, 154.98, 153.49, 152.54, 151.79, 151.10, 150.53, 149.89, 141.92, 139.13, 134.72, 134.55, 130.03, 129.06, 127.61, 124.01, 117.09, 112.44, 109.71, 109.04, 108.81, 25.95; ESI-MS m/z: 432.3 $[M+H]^+$; Anal. calcd. for $C_{22}H_{18}FN_7O_2$ (%): C, 61.25; H, 4.21; N, 22.73. Found (%): C, 61.30; H, 4.27; N, 22.70.

*5.1.12.2. (E)-2-Benzylidene-N-(3-fluoro-4-(2-(4-methyl-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)phenyl)hydrazinecarboxamide (**10b**).* A white solid; Yield: 62%; M.p. 147–149 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.99 (s, 1H), 9.29 (s, 1H), 8.65 (s, 2H), 8.01 (s, 1H), 7.88 (m, 3H), 7.67 (d, J = 7.9 Hz, 1H), 7.57 (s, 1H), 7.43 (m, 4H), 7.16 (s, 1H), 4.02 (s, 3H); ESI-MS m/z: 432.2 $[M+H]^+$; Anal. calcd. for $C_{22}H_{18}FN_7O_2$ (%): C, 61.25; H, 4.21; N, 22.73. Found (%): C, 61.27; H, 4.19; N, 22.77.

*5.1.12.3. (E)-2-benzylidene-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**10c**).* A light-yellow solid; Yield: 63%; M.p. 170–171 °C; IR (KBr, cm^{-1}): 3437.7, 2919.3, 2850.5, 1699.9, 1593.9, 1508.3; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.26 (s, 1H) 9.55 (s, 1H), 8.64 (s, 1H), 8.60 (d, J = 5.8 Hz, 1H), 8.01 (s, 1H), 7.91 (dd, J = 13.5, 2.7 Hz, 1H), 7.85 (d, J = 6.8 Hz, 2H), 7.62 (d, J = 9.3 Hz, 1H), 7.55 (d, J = 2.4 Hz, 1H), 7.47–7.37 (m, 4H), 7.13 (dd, J = 5.7, 2.1 Hz, 1H), 4.61 (t, J = 6.1 Hz, 2H), 2.57 (t, J = 6.3 Hz, 2H), 2.13 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.34, 156.62, 155.01, 153.47, 152.58, 151.73, 150.62, 150.06, 147.47, 141.89, 139.35, 137.67, 134.87, 129.93, 129.08, 127.51, 124.05, 116.84, 112.23, 109.23, 108.74, 59.25, 45.54 (2C), 43.85; ESI-MS m/z: 489.2 $[M+H]^+$; Anal. calcd. for $C_{25}H_{25}FN_8O_2$ (%): C, 61.47; H, 5.16; N, 22.94. Found (%): C, 61.41; H, 5.19; N, 23.03.

*5.1.12.4. (E)-2-(4-chlorobenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl) hydrazinecarboxamide (**11a**).* A white solid; Yield: 47%; M.p. 135–137 °C; IR (KBr, cm^{-1}): 3385.7, 2920.1, 2851.5, 1696.9, 1594.9, 1508.1; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.28 (s, 1H), 8.64 (s, 1H), 8.61 (d, J = 5.7 Hz, 1H), 7.97 (s, 1H), 7.94–7.88 (m, 3H), 7.64 (d, J = 8.5 Hz, 1H), 7.56 (d, J = 2.2 Hz, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.42 (t, J = 9.1 Hz, 1H), 7.15 (dd, J = 5.7, 2.5 Hz, 1H), 4.62 (t, J = 6.0 Hz, 2H), 2.59 (t, J = 6.2 Hz, 2H), 2.14 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.29, 154.56, 153.41, 152.94, 151.71, 150.57, 150.02, 147.50, 140.52, 139.04, 134.53, 134.41, 133.70, 129.26 (2C), 129.11 (2C), 124.04, 117.16, 112.28, 109.09, 59.15, 45.45 (2C), 43.73. ESI-MS m/z: 523.1 $[M+H]^+$; Anal. calcd. for $C_{25}H_{24}ClFN_8O_2$ (%): C, 57.42; H, 4.63; N, 21.43. Found (%): C, 57.46; H, 4.57; N, 21.50.

*5.1.12.5. (E)-2-(3-Bromobenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl) hydrazinecarboxamide (**11b**).* A white solid; Yield: 52%; M.p. 161–163 °C; IR (KBr, cm^{-1}): 3384.1, 2919.7, 2851.0, 1695.2, 1593.7, 1507.7; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.09 (s, 1H), 9.38 (s, 1H), 8.65 (s, 1H), 8.60 (d, J = 6.0 Hz, 1H), 8.19 (s, 1H), 7.99–7.85 (m, 2H), 7.78 (d, J = 7.5 Hz, 1H), 7.62–7.56 (m, 3H), 7.41 (m, 2H), 7.14 (s, 1H), 4.63 (s, 2H), 2.60 (s, 2H), 2.15 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.30, 154.97, 153.41, 152.54, 151.72, 150.61, 150.07, 147.47, 140.25, 139.04, 137.22, 134.68, 132.54, 131.18, 129.41, 127.05, 124.02, 122.71, 117.37, 112.27, 109.19, 59.25, 45.53 (2C), 43.85. ESI-MS m/z: 567.1 $[M+H]^+$; Anal. calcd. for $C_{25}H_{24}BrFN_8O_2$ (%): C, 52.92; H, 4.26; N, 19.75. Found (%): C, 53.01; H, 4.22; N, 19.81.

*5.1.12.6. (E)-N-(4-(2-(4-(2-(Dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-(4-fluorobenzylidene)hydrazinecarboxamide (**11c**).* A white solid; Yield: 35%; M.p. 122–123 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (s, 1H), 9.20 (s, 1H), 8.59 (s, 1H), 8.56 (d, J = 5.8 Hz, 1H), 7.94–7.83 (m, 4H), 7.59 (d, J = 9.0 Hz, 1H), 7.51 (d, J = 2.4 Hz, 1H), 7.37 (t, J = 9.0 Hz, 1H), 7.23 (t, J = 8.8 Hz, 2H), 7.10 (dd, J = 5.7, 2.4 Hz, 1H), 4.58 (t, J = 6.1 Hz, 2H), 2.55 (t, J = 6.1 Hz, 2H), 2.10 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.31, 162.08, 154.98, 153.48, 152.56, 151.73, 150.60, 150.05, 147.49, 140.72, 139.12, 134.58, 131.39, 129.82, 129.74, 124.04, 117.10, 116.19, 115.97, 112.29, 109.15, 59.20, 45.50 (2C), 43.79; ESI-MS m/z: 507.3 $[M+H]^+$; Anal. calcd. for $C_{25}H_{24}F_2N_8O_2$ (%): C, 59.28; H, 4.78; N, 22.12. Found (%): C, 59.32; H, 4.81; N, 22.09.

*5.1.12.7. (E)-2-(2,4-difluorobenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl) hydrazinecarboxamide (**11d**).* A white solid; Yield: 42%; M.p. 117–119 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.25 (s, 1H),

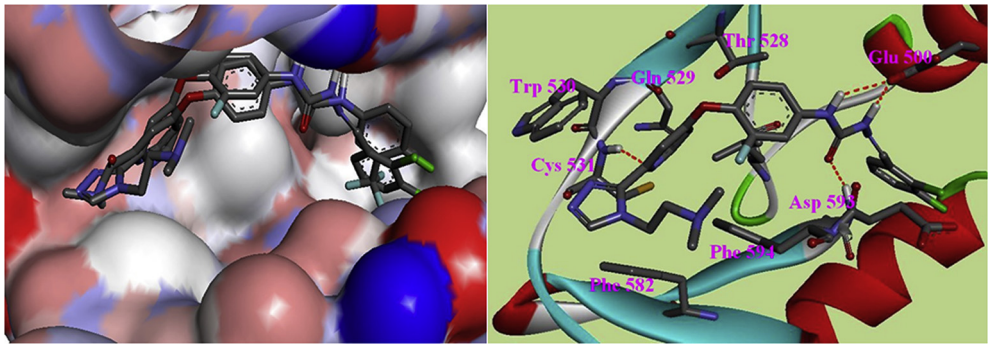


Fig. 4. Docking model of compound **11f** with wild-type B-Raf kinase (PDB code: 1UWH). Left: a representation of the overlap of docking model of 11f with sorafenib in protein surface. Right: a representation of the kinase with selected residues in stick model. The inhibitors were colored by atom type (grey = carbon/hydrogen, red = oxygen, blue = nitrogen, green = chlorine, cyan = fluorine). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

8.59 (s, 1H), 8.56 (d, J = 5.8 Hz, 1H), 8.36 (dd, J = 15.7, 8.8 Hz, 1H), 8.10 (s, 1H), 7.84 (dd, J = 13.2, 2.4 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.51 (d, J = 2.5 Hz, 1H), 7.37 (m, 1H), 7.32–7.25 (m, 1H), 7.17 (m, 1H), 7.10 (dd, J = 5.7, 2.5 Hz, 1H), 4.58 (t, J = 6.2 Hz, 2H), 2.55 (t, J = 6.0 Hz, 2H), 2.11 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.30, 154.99, 153.32, 152.56, 151.69, 150.60, 150.05, 147.46, 139.01, 134.70, 133.48, 128.94, 124.04, 119.15, 117.18, 112.85, 112.64, 112.28, 109.16, 108.92, 104.64, 59.16, 45.43 (2C), 43.76. ESI-MS m/z: 525.2 $[M+H]^+$; Anal. calcd. for $C_{25}H_{23}F_3N_8O_2$ (%): C, 57.25; H, 4.42; N, 21.36. Found (%): C, 57.17; H, 4.48; N, 21.40.

*5.1.12.8. (E)-2-(2-Chloro-4-fluorobenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**11e**).* A white solid; Yield: 51%; M.p. 150–152 °C; IR (KBr, cm^{-1}): 3392.5, 2919.3, 2850.8, 1695.6, 1595.9, 1507.7; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.28 (s, 1H), 8.59 (s, 1H), 8.56 (d, J = 5.7 Hz, 1H), 8.46–8.38 (m, 1H), 8.28 (s, 1H), 7.84 (dd, J = 13.4, 1.8 Hz, 1H), 7.58 (d, J = 8.5 Hz, 1H), 7.53–7.45 (m, 2H), 7.33 (m, 2H), 7.10 (dd, J = 5.7, 3.1 Hz, 1H), 4.58 (t, J = 6.0 Hz, 2H), 2.57 (t, J = 6.1 Hz, 2H), 2.11 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.28, 163.66, 162.00, 154.56, 153.29, 152.94, 151.71, 150.56, 150.01, 147.50, 138.95, 136.75, 134.69, 133.83, 129.79, 128.75, 124.06, 117.24, 115.62, 112.29, 109.08, 59.12, 45.42 (2C), 43.71; ESI-MS m/z: 541.1 $[M+H]^+$; Anal. calcd. for $C_{25}H_{23}ClF_2N_8O_2$ (%): C, 55.51; H, 4.29; N, 20.71. Found (%): C, 55.45; H, 4.33; N, 20.65.

*5.1.12.9. (E)-2-(2,3-Dichlorobenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl) hydrazinecarboxamide (**11f**).* A white solid; Yield: 42%; M.p. 136–142 °C; IR (KBr, cm^{-1}): 3442.8, 2920.8, 2851.5, 1701.7, 1596.1, 1508.3; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.33 (s, 1H), 9.42 (s, 1H), 8.67 (s, 1H), 8.62 (d, J = 5.3 Hz, 1H), 8.43 (s, 1H), 8.39 (d, J = 7.2 Hz, 1H), 7.91 (d, J = 12.8 Hz, 1H), 7.67 (dd, J = 27.0, 7.5 Hz, 2H), 7.56 (s, 1H), 7.49–7.40 (m, 2H), 7.16 (d, J = 2.8 Hz, 1H), 4.64 (t, J = 6.2 Hz, 2H), 2.63 (t, J = 6.3 Hz, 2H), 2.17 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.27, 154.56, 153.19, 152.93, 151.73, 150.56, 149.99, 147.51, 138.97, 137.44, 134.69, 134.39, 132.58, 131.47, 130.91, 128.61, 126.57, 124.07, 117.26, 112.29, 109.09, 59.05, 45.37 (2C), 43.66; ESI-MS m/z: 557.3 $[M+H]^+$; Anal. calcd. for $C_{25}H_{23}Cl_2FN_8O_2$ (%): C, 53.87; H, 4.16; N, 20.10. Found (%): C, 53.91; H, 4.13; N, 20.16.

*5.1.12.10. (E)-2-(2,4-dichlorobenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl) hydrazinecarboxamide (**11g**).* A white solid; Yield: 32%; M.p. 177–180 °C; IR (KBr, cm^{-1}): 3443.5, 2918.7, 2850.4, 1688.0, 1594.3, 1508.6; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.29 (s, 1H), 9.43 (s, 1H), 8.67 (s, 1H), 8.62 (d, J = 5.7 Hz, 1H), 8.44 (d, J = 8.6 Hz, 1H), 8.36 (s, 1H), 7.91 (dd, J = 13.3, 2.3 Hz, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.65 (d, J = 8.7 Hz, 1H), 7.57 (d, J = 2.5 Hz, 1H), 7.52 (dd, J = 8.6, 1.8 Hz, 1H), 7.43 (t, J = 9.0 Hz, 1H), 7.16 (dd, J = 5.7, 2.6 Hz, 1H), 4.64 (t, J = 6.2 Hz, 2H), 2.63 (t, J = 6.1 Hz, 2H), 2.17 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.27, 154.55, 153.20, 152.93, 151.72, 150.56, 149.99, 147.51, 139.00, 136.64, 134.95, 134.62, 133.75, 131.05, 129.61, 129.21, 128.08, 124.06, 117.24, 112.28, 109.10, 59.07, 45.38 (2C), 43.68; ESI-MS m/z: 557.3 $[M+H]^+$; Anal. calcd. for $C_{25}H_{23}Cl_2FN_8O_2$ (%): C, 53.87; H, 4.16; N, 20.10. Found (%): C, 53.92; H, 4.11; N, 20.16.

*5.1.12.11. (E)-2-(2,6-dichlorobenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl) hydrazinecarboxamide (**11h**).* A white solid; Yield: 47%; M.p. 138–140 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.33 (s, 1H), 9.18 (s, 1H), 8.66 (s, 1H), 8.60 (d, J = 5.1 Hz, 1H), 8.20 (s, 1H), 7.88 (d, J = 13.5 Hz, 1H), 7.58 (m, 4H), 7.48–7.36 (m, 2H), 7.13 (s, 1H), 4.63 (t, J = 6.1 Hz, 2H), 2.61 (t, J = 6.2 Hz, 2H), 2.16 (s, 6H); ESI-MS m/z: 557.2 $[M+H]^+$; Anal. calcd. for $C_{25}H_{23}Cl_2FN_8O_2$ (%): C, 53.87; H, 4.16; N, 20.10. Found (%): C, 53.90; H, 4.19; N, 20.02.

*5.1.12.12. (E)-N-(4-(2-(4-(2-(Dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-(3-fluoro-4-hydroxybenzylidene)hydrazinecarboxamide (**11i**).* A white solid; Yield: 45%; M.p. 125–128 °C; IR (KBr, cm^{-1}): 3368.9, 2919.9, 2850.7, 1685.9, 1593.9, 1510.3; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.81 (s, 1H), 10.28 (s, 1H), 9.19 (s, 1H), 8.65 (s, 1H), 8.61 (d, J = 5.4 Hz, 1H), 7.94–7.84 (m, 3H), 7.65 (d, J = 8.5 Hz, 1H), 7.56 (s, 1H), 7.41 (t, J = 9.2 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.15 (s, 1H), 6.98 (t, J = 8.6 Hz, 1H), 4.65 (s, 2H), 2.69 (s, 2H), 2.23 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) 165.32, 154.98, 153.45, 153.00, 151.73, 150.60, 150.05, 147.50, 141.14, 139.19, 134.53, 134.41, 126.57, 125.20, 124.00, 118.03, 117.04, 114.24, 114.04, 112.23, 109.21, 59.23, 45.52 (2C), 43.83; ESI-MS m/z: 523.1 $[M+H]^+$; Anal. calcd. for $C_{25}H_{24}F_2N_8O_3$ (%): C, 57.47; H, 4.63; N, 21.45. Found (%): C, 57.38; H, 4.55; N, 21.57.

*5.1.12.13. (E)-2-(3-Bromo-4-hydroxybenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**11j**).* A white solid; Yield: 37%; M.p. 111–113 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.76 (s, 1H), 10.66 (s, 1H), 9.22 (s, 1H), 8.69 (s, 1H), 8.62 (d, J = 5.7 Hz, 1H), 8.08 (m, 1H), 7.90 (m, 2H), 7.67–7.57 (m, 3H), 7.39 (t, J = 9.0 Hz, 1H), 7.15 (dd, J = 5.7, 2.4 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 4.73 (t, J = 6.1 Hz, 2H), 2.99 (t, J = 6.0 Hz, 2H), 2.43 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.42, 155.80, 153.49, 152.54, 151.79, 150.58, 149.74, 147.45, 140.74, 139.27, 134.48, 131.51, 128.76, 127.48, 123.99, 117.18, 116.65, 112.38, 110.47, 109.06, 108.83, 57.96, 44.38 (2C), 42.56; ESI-MS m/z:

583.3 $[M+H]^+$; Anal. calcd. for $C_{25}H_{24}BrFN_8O_3$ (%): C, 51.47; H, 4.15; N, 19.21. Found (%): C, 51.39; H, 4.21; N, 19.27.

*5.1.12.14. (E)-2-(3,5-Dibromo-4-hydroxybenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**11k**).* A white solid; Yield: 41%; M.p. 131–132 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.92 (s, 1H), 9.33 (s, 1H), 8.68 (s, 1H), 8.62 (d, J = 5.5 Hz, 1H), 8.08 (s, 2H), 7.91 (d, J = 13.2 Hz, 1H), 7.84 (s, 1H), 7.64 (m, 3H), 7.41 (t, J = 8.8 Hz, 1H), 7.16 (s, 1H), 4.71 (t, J = 6.6 Hz, 2H), 2.91 (t, J = 6.6 Hz, 2H), 2.38 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.33, 154.95, 154.62, 153.47, 152.52, 151.73, 150.60, 149.98, 147.47, 139.87, 139.25, 134.50, 131.12 (2C), 126.49, 123.97, 117.30, 113.30 (2C), 112.25, 109.18, 58.98, 45.31 (2C), 43.59; ESI-MS m/z: 661.1 $[M+H]^+$; Anal. calcd. for $C_{25}H_{23}Br_2FN_8O_3$ (%): C, 45.34; H, 3.50; N, 16.92. Found (%): C, 45.39; H, 3.61; N, 16.86.

*5.1.12.15. (E)-2-((2-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenylcarbamoyl)hydrazono)methyl)phenyl nitrate (**11l**).* A yellow solid; Yield: 42%; M.p. 125–127 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.29 (s, 1H), 9.35 (s, 1H), 8.65 (s, 1H), 8.61 (d, J = 5.6 Hz, 1H), 8.49 (d, J = 7.8 Hz, 1H), 8.42 (s, 1H), 8.06 (d, J = 8.3 Hz, 1H), 7.90 (d, J = 12.9 Hz, 1H), 7.79 (m, 1H), 7.63 (m, 2H), 7.57 (s, 1H), 7.42 (t, J = 8.8 Hz, 1H), 7.14 (dd, J = 6.0, 3.5 Hz, 1H), 4.64 (t, J = 5.9 Hz, 2H), 2.63 (t, J = 6.0 Hz, 2H), 2.17 (s, 6H); Anal. calcd. for $C_{25}H_{24}FN_9O_4$ (%): C, 56.28; H, 4.53; N, 23.63. Found (%): C, 56.17; H, 4.60; N, 23.71.

*5.1.12.16. (E)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-(3-hydroxybenzylidene)hydrazinecarboxamide (**11m**).* A white solid; Yield: 51%; M.p. 129–131 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.84 (s, 1H), 9.68 (s, 1H), 9.24 (s, 1H), 8.65 (s, 1H), 8.62 (d, J = 5.6 Hz, 1H), 7.90 (m, 2H), 7.63 (d, J = 8.6 Hz, 1H), 7.56 (s, 1H), 7.41 (t, J = 8.9 Hz, 1H), 7.26 (m, 3H), 7.16 (d, J = 3.1 Hz, 1H), 6.83 (d, J = 5.9 Hz, 1H), 4.64 (t, J = 6.6 Hz, 2H), 2.66 (t, J = 6.6 Hz, 2H), 2.19 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.31, 158.16, 155.00, 153.40, 152.57, 151.73, 150.61, 150.04, 147.49, 142.24, 139.32, 136.00, 134.40, 130.00, 124.05, 118.64, 117.25, 116.82, 113.89, 112.22, 109.21, 59.23, 45.52 (2C), 43.82; ESI-MS m/z: 505.1 $[M+H]^+$; Anal. calcd. for $C_{25}H_{25}FN_8O_3$ (%): C, 59.52; H, 4.99; N, 22.21. Found (%): C, 59.59; H, 4.93; N, 22.17.

*5.1.12.17. (E)-N-(4-(2-(4-(2-(Dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-(4-hydroxybenzylidene)hydrazinecarboxamide (**11n**).* A white solid; Yield: 33%; M.p. 151–152 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.68 (s, 1H), 9.83 (s, 1H), 9.13 (s, 1H), 8.68–8.54 (m, 2H), 7.91 (m, 2H), 7.73–7.61 (m, 3H), 7.56 (s, 1H), 7.39 (t, J = 9.0 Hz, 1H), 7.14 (s, 1H), 6.82 (d, J = 7.9 Hz, 2H), 4.62 (t, J = 6.0 Hz, 2H), 2.59 (t, J = 5.9 Hz, 2H), 2.14 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.33, 159.43, 154.99, 153.52, 152.56, 151.71, 150.60, 150.05, 147.49, 142.18, 139.29, 134.38, 129.31, 125.78, 124.02, 116.86, 115.93, 112.26, 109.17, 108.79, 108.57, 59.24, 45.53 (2C), 43.83; ESI-MS m/z: 505.2 $[M+H]^+$; Anal. calcd. for $C_{25}H_{25}FN_8O_3$ (%): C, 59.52; H, 4.99; N, 22.21. Found (%): C, 59.59; H, 5.07; N, 22.24.

*5.1.12.18. (E)-2-(2,4-Dihydroxybenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**11o**).* A white solid; Yield: 52%; M.p. 146–147 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.54 (s, 1H), 9.97 (s, 1H), 9.69 (s, 1H), 9.11 (s, 1H), 8.59 (s, 1H), 8.55 (d, J = 5.6 Hz, 1H), 8.13 (s, 1H), 7.84 (d, J = 13.4 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.52 (m, 2H), 7.34 (t, J = 9.0 Hz, 1H), 7.08 (dd, J = 6.0, 3.0 Hz, 1H), 6.27 (m, 2H), 4.57 (t, J = 6.0 Hz, 2H), 2.53 (t, J = 6.2 Hz, 2H), 2.08 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.36, 160.62, 158.60, 156.14, 155.07, 153.29, 152.64, 151.73, 150.62, 150.03, 147.49, 141.58, 139.80, 134.01, 129.41, 124.10, 116.19, 112.15, 109.28, 107.95, 102.96, 59.24, 45.53 (2C), 43.82; ESI-MS m/z: 521.4 $[M+H]^+$; Anal. calcd. for $C_{25}H_{25}FN_8O_4$ (%): C, 57.69; H, 4.84; N, 21.53. Found (%): C, 57.62; H, 4.90; N, 21.57.

*5.1.12.19. (E)-2-(3,4-Dihydroxybenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**11p**).* A white solid; Yield: 54%; M.p. 135–137 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.57 (s, 1H), 9.36 (s, 1H), 9.10 (s, 1H), 8.59 (s, 1H), 8.55 (d, J = 5.7 Hz, 2H), 7.91–7.74 (m, 2H), 7.61–7.49 (m, 2H), 7.35 (t, J = 9.0 Hz, 1H), 7.24 (s, 1H), 7.05 (m, 2H), 6.74 (d, J = 7.9 Hz, 1H), 4.57 (t, J = 6.7 Hz, 2H), 2.53 (t, J = 6.9 Hz, 2H), 2.09 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.33, 155.01, 153.49, 152.58, 151.72, 150.61, 150.04, 147.92, 147.48, 146.03, 142.70, 139.39, 134.31, 126.22, 124.03, 120.23, 116.69, 116.02, 114.29, 112.22, 109.21, 59.23, 45.52 (2C), 43.83; ESI-MS m/z: 521.3 $[M+H]^+$; Anal. calcd. for $C_{25}H_{25}FN_8O_4$ (%): C, 57.69; H, 4.84; N, 21.53. Found (%): C, 57.65; H, 4.78; N, 21.59.

*5.1.12.20. (E)-N-(4-(2-(4-(2-(Dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-(3-hydroxy-4-methoxybenzylidene)hydrazinecarboxamide (**11q**).* A white solid; Yield: 39%; M.p. 122–123 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.69 (s, 1H), 9.17 (s, 1H), 9.06 (s, 1H), 8.62 (m, 2H), 7.96–7.82 (m, 2H), 7.63 (d, J = 8.1 Hz, 1H), 7.56 (s, 1H), 7.38 (m, 2H), 7.14 (m, 2H), 6.96 (d, J = 8.2 Hz, 1H), 4.63 (t, J = 6.8 Hz, 2H), 3.82 (s, 3H), 2.64 (t, J = 6.7 Hz, 2H), 2.18 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.31, 154.59, 153.43, 152.97, 151.73, 150.58, 149.99, 149.70, 147.51, 147.13, 142.30, 139.37, 134.27, 127.66, 124.06, 120.16, 116.71, 113.58, 112.22, 112.11, 109.16, 59.14, 56.05, 45.45 (2C), 43.74; ESI-MS m/z: 535.3 $[M+H]^+$; Anal. calcd. for $C_{26}H_{27}FN_8O_4$ (%): C, 58.42; H, 5.09; N, 20.96. Found (%): C, 58.36; H, 5.15; N, 20.89.

*5.1.12.21. (E)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-(4-hydroxy-3-methoxybenzylidene)hydrazinecarboxamide (**11r**).* A white solid; Yield: 47%; M.p. 110–112 °C; IR (KBr, cm^{-1}): 3439.0, 2919.5, 2850.4, 1688.1, 1595.0, 1508.5; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.80 (s, 1H), 9.55 (s, 1H), 9.27 (s, 1H), 8.64 (s, 1H), 8.58 (d, J = 4.9 Hz, 1H), 7.90 (d, J = 13.3 Hz, 1H), 7.87 (s, 1H), 7.60 (d, J = 7.0 Hz, 1H), 7.50 (d, J = 31.2 Hz, 2H), 7.39 (t, J = 8.5 Hz, 1H), 7.12 (s, 2H), 6.83 (d, J = 7.7 Hz, 1H), 4.60 (t, J = 6.1 Hz, 2H), 3.84 (s, 3H), 2.57 (t, J = 6.3 Hz, 2H), 2.12 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.32, 154.59, 153.49, 152.97, 151.72, 150.58, 150.00, 149.00, 148.40, 147.51, 142.45, 139.29, 134.29, 126.14, 124.07, 122.03, 116.90, 115.78, 112.24, 110.32, 109.10, 59.14, 56.20, 45.45 (2C), 43.74; ESI-MS m/z: 535.3 $[M+H]^+$; Anal. calcd. for $C_{26}H_{27}FN_8O_4$ (%): C, 58.42; H, 5.09; N, 20.96. Found (%): C, 58.36; H, 5.13; N, 20.92.

*5.1.12.22. (E)-N-(4-(2-(4-(2-(Dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-(4-hydroxy-3,5-dimethylbenzylidene)hydrazinecarboxamide (**11s**).* A white solid; Yield: 42%; M.p. 134–137 °C; IR (KBr, cm^{-1}): 3380.3, 2919.0, 2850.6, 1688.6, 1595.1; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.66 (s, 1H), 9.12 (s, 1H), 8.63 (m, 2H), 8.60 (d, J = 5.9 Hz, 1H), 7.92 (d, J = 13.6 Hz, 1H), 7.83 (s, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.44–7.36 (m, 3H), 7.14 (dd, J = 6.0, 3.1 Hz, 1H), 4.62 (t, J = 6.3 Hz, 2H), 2.59 (t, J = 6.2 Hz, 2H), 2.21 (s, 6H), 2.14 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.33, 155.45, 154.58, 153.47, 152.96, 151.73, 150.58, 149.98, 147.51, 142.60, 139.37, 134.36, 127.88 (2C), 125.62, 124.88 (2C), 124.02, 116.89, 112.23, 109.13, 59.05, 45.37 (2C), 43.66, 17.08 (2C); ESI-MS m/z: 533.3 $[M+H]^+$; Anal. calcd. for $C_{27}H_{29}FN_8O_3$ (%): C, 60.89; H, 5.49; N, 21.04. Found (%): C, 60.82; H, 5.43; N, 21.09.

*5.1.12.23. (E)-2-(3,5-Di-tert-butyl-2-hydroxybenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**11t**).* A white solid; Yield: 45%. M.p. 113–116 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.23 (s, 1H) 10.63 (s, 1H), 9.43 (s, 1H), 8.63 (s, 1H), 8.60 (d, J = 5.7 Hz, 1H), 8.25 (s, 1H), 7.79 (d, J = 13.0 Hz, 1H), 7.56 (s, 1H), 7.40 (m, 2H), 7.28 (s, 1H), 7.22 (s, 1H), 7.13 (dd, J = 5.7, 2.4 Hz, 1H), 4.62 (t, J = 6.0 Hz, 2H), 2.61 (t, J = 6.2 Hz, 2H), 2.16 (s, 6H), 1.42 (s, 9H), 1.29 (s, 9H); ESI-MS m/z: 617.3 $[M+H]^+$; Anal. calcd. for $C_{33}H_{41}FN_8O_3$ (%): C, 64.27; H, 6.70; N, 18.17. Found (%): C, 64.31; H, 6.77; N, 18.13.

*5.1.12.24. (E)-2-(2,4-dimethoxybenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**11u**).* A white solid; Yield: 51%; M.p. 132–134 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.70 (s, 1H), 9.13 (s, 1H), 8.63 (s, 1H), 8.60 (d, J = 5.7 Hz, 1H), 8.25 (s, 1H), 8.12 (d, J = 8.3 Hz, 1H), 7.91 (dd, J = 13.5, 2.3 Hz, 1H), 7.64 (d, J = 9.0 Hz, 1H), 7.57 (d, J = 2.3 Hz, 1H), 7.39 (t, J = 9.1 Hz, 1H), 7.13 (dd, J = 5.7, 2.5 Hz, 1H), 6.61 (m, 2H), 4.62 (t, J = 6.3 Hz, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 2.59 (t, J = 6.3 Hz, 2H), 2.14 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.33, 162.49, 159.23, 153.50, 152.56, 151.69, 150.61, 150.05, 147.47, 139.30, 137.50, 134.40, 127.84, 123.98, 116.89, 115.55, 112.24, 109.19, 108.70, 106.72, 98.40, 59.22, 56.18, 55.86, 45.49 (2C), 43.81; ESI-MS m/z: 549.1 $[M+H]^+$; Anal. calcd. for $C_{27}H_{29}FN_8O_4$ (%): C, 59.12; H, 5.33; N, 20.43. Found (%): C, 59.18; H, 5.37; N, 20.39.

*5.1.12.25. (E)-2-(2,5-dimethoxybenzylidene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**11v**).* A white solid; Yield: 41%; M.p. 114–117 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (s, 1H), 9.28 (s, 1H), 8.67–8.56 (m, 2H), 8.33 (s, 1H), 7.92 (d, J = 13.0 Hz, 1H), 7.75 (s, 1H), 7.68–7.55 (m, 2H), 7.46–7.35 (m, 1H), 7.13 (s, 1H), 7.01 (m, 2H), 4.63 (t, J = 6.0 Hz, 2H), 3.81 (m, 6H), 2.59 (t, J = 6.1 Hz, 2H), 2.14 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d_6) δ 165.32, 154.98, 153.78, 153.43, 152.55, 152.33, 151.72, 150.60, 150.05, 147.50, 139.26, 137.27, 134.45, 124.04, 123.38, 117.04, 113.40, 112.24, 111.17, 109.16, 108.91, 59.23, 56.61, 56.07, 45.52 (2C), 43.82; ESI-MS m/z: 549.3 $[M+H]^+$; Anal. calcd. for $C_{27}H_{29}FN_8O_4$ (%): C, 59.12; H, 5.33; N, 20.43. Found (%): C, 59.17; H, 5.26; N, 20.49.

*5.1.12.26. (E)-N-(4-(2-(4-(2-(Dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-(2,3,4-trimethoxybenzylidene)hydrazinecarboxamide (**11w**).* A white solid; Yield: 35%; M.p. 136–139 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.77 (s, 1H), 9.16 (s, 1H), 8.64 (s, 1H), 8.61 (d, J = 5.8 Hz, 1H), 8.21 (s, 1H), 8.01–7.83 (m, 2H), 7.64 (d, J = 9.3 Hz, 1H), 7.58 (s, 1H), 7.41 (t, J = 9.0 Hz, 1H), 7.14 (dd, J = 5.4, 2.4 Hz, 1H), 6.91 (d, J = 8.9 Hz, 1H), 4.63 (t, J = 6.0 Hz, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H), 2.60 (t, J = 6.3 Hz, 2H), 2.15 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d6) δ 170.79, 165.32, 155.20, 153.41, 152.74, 151.71, 150.61, 150.06, 147.47, 141.93, 139.27, 137.62, 134.51, 134.39, 124.02, 121.66, 120.90, 116.90, 112.25, 109.19, 108.97, 62.21, 60.94, 60.21, 59.24, 45.51 (2C), 43.83; ESI-MS m/z: 579.2 $[M+H]^+$; Anal. calcd. for $C_{28}H_{31}FN_8O_5$ (%): C, 58.12; H, 5.40; N, 19.37. Found (%): C, 58.19; H, 5.37; N, 19.32.

*5.1.12.27. (E)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-(3,4,5-trimethoxybenzylidene)hydrazinecarboxamide (**11x**).* A white solid; Yield: 55%; M.p. 117–120 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.98 (s, 1H), 9.30 (s, 1H), 8.65 (m, 2H), 7.92 (m, 2H), 7.70–7.38 (m, 4H), 7.18 (s, 2H), 4.63 (s, 2H), 3.88 (s, 6H), 3.71 (s, 3H), 2.59 (s, 2H), 2.14 (s, 6H); ^{13}C NMR (600 MHz, DMSO-d6) δ 165.32, 155.01, 153.58 (2C), 153.44, 152.58, 151.72, 150.59, 150.04, 147.50, 141.87, 139.24, 139.17, 134.47, 130.28, 124.11, 117.10, 112.27, 109.09, 104.99 (2C), 60.57, 59.19, 56.53 (2C), 45.48 (2C), 43.79; ESI-MS m/z: 579.2 $[M+H]^+$; Anal. calcd. for $C_{28}H_{31}FN_8O_5$ (%): C, 58.12; H, 5.40; N, 19.37. Found (%): C, 58.16; H, 5.43; N, 19.40.

*5.1.12.28. (E)-2-((1H-Indol-3-yl)methylene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**12a**).* A white solid; Yield: 35%; M.p. 136–138 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.58 (s, 1H), 10.51 (s, 1H), 8.95 (s, 1H), 8.57 (m, 2H), 8.23 (m, 2H), 7.91–7.75 (m, 2H), 7.52 (s, 2H), 7.37 (m, 2H), 7.12 (m, 3H), 4.57 (t, J = 7.5 Hz, 2H), 2.53 (t, J = 7.3 Hz, 2H), 2.08 (s, 6H); ESI-MS m/z: 528.2 $[M+H]^+$; Anal. calcd. for $C_{27}H_{26}FN_9O_2$ (%): C, 61.47; H, 4.97; N, 23.90. Found (%): C, 61.09; H, 5.35; N, 23.37.

*5.1.12.29. (E)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)-2-((2-hydroxynaphthalen-1-yl)methylene)hydrazinecarboxamide (**12b**).* A yellow solid; Yield: 56%; M.p. 169–171 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.38 (s, 1H), 10.74 (s, 1H), 9.41 (s, 1H), 8.95 (s, 1H), 8.59 (s, 1H), 8.55 (d, J = 5.7 Hz, 1H), 8.39 (d, J = 7.5 Hz, 1H), 7.80 (m, 3H), 7.53 (m, 2H), 7.45 (d, J = 10.4 Hz, 1H), 7.35 (m, 2H), 7.18 (d, J = 8.8 Hz, 1H), 7.09 (s, 1H), 4.57 (t, J = 6.0 Hz, 2H), 2.53 (t, J = 6.1 Hz, 2H), 2.09 (s, 6H); ESI-MS m/z: 555.3 $[M+H]^+$; Anal. calcd. for $C_{29}H_{27}FN_8O_3$ (%): C, 62.81; H, 4.91; N, 20.21. Found (%): C, 62.96; H, 4.82; N, 20.33.

*5.1.12.30. (E)-2-(Benzo[d][1,3]dioxol-4-ylmethylene)-N-(4-(2-(4-(2-(dimethylamino)ethyl)-4H-1,2,4-triazol-3-yl)pyridin-4-yloxy)-3-fluorophenyl)hydrazinecarboxamide (**12c**).* A white solid; Yield: 40%; M.p. 143–145 °C; IR (KBr, cm^{-1}): 3428.7, 2918.8, 2850.6, 1691.3, 1594.9; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.75 (s, 1H), 9.13 (s, 1H), 8.58 (s, 1H), 8.56 (d, J = 5.6 Hz, 1H), 7.86 (m, 2H), 7.69 (s, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.51 (s, 1H), 7.36 (t, J = 8.5 Hz, 1H), 7.09 (d, J = 5.9 Hz, 2H), 6.91 (d, J = 7.1 Hz, 1H), 6.04 (s, 2H), 4.57 (t, J = 6.0 Hz, 2H), 2.53 (t, J = 5.9 Hz, 2H), 2.08 (s, 6H). ^{13}C NMR (600 MHz, DMSO-d_6) δ 164.96, 154.58, 153.10, 152.15, 151.36, 150.21, 149.57, 148.70, 148.06, 147.08, 141.29, 138.82, 134.13, 128.92, 123.60, 123.45, 116.75, 111.92, 108.75, 108.24, 105.53, 101.48, 58.43, 44.75 (2C), 43.02; ESI-MS m/z: 533.3 $[M+H]^+$; Anal. calcd. for $C_{26}H_{25}FN_8O_4$ (%): C, 58.64; H, 4.73; N, 21.04. Found (%): C, 58.70; H, 4.69; N, 21.23.

5.2. Pharmacology

5.2.1. MTT assay in vitro

The cytotoxic activities of compounds **10a**–**10c**, **11a**–**11x** and **12a**–**10c** were evaluated against MKN-45, H460, HT-29, A549 and U87MG cancer cell lines and WI-38 normal cell line by the standard MTT assay *in vitro*. Sorafenib was used as the positive control. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS).

Approximate 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The tested compounds were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL, and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of at least three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) sofeware.

5.2.2. Enzymatic assay

The kinase inhibitory measurements versus c-Met, VEGFR-2, B-Raf, Raf-1 and PDGFRα were evaluated using homogeneous time-

resolved fluorescence (HTRF) assay. All experiments were performed in NUNC 264706 white 384-well low-volume plates (Cat. 6007290, PerkinElmer).

In B-Raf and Raf-1 kinase assay, a mixture of peptide substrates, ATP, kinase, and diluted compound was added to the kinase reaction buffer (50 mM Hepes/NaOH pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 0.01% BRIJ-35) in each well. As a negative control, the tested compound was dissolved in 4% DMSO (v/v). The incubation reactions were performed at 25 °C for 1 h. All the results were read using Spectra M5(MD) instrument at 340 nm and 395 nm following the addition of 10 μL working solution diluted with EDTA and Tb anti pMAP2K1. In enzymatic assay against c-Met, VEGFR-2, PDGFRα, the solution of peptide substrates, ATP, appropriate kinase, and diluted compound was mixed with the kinase reaction buffer (250 mM HEPES pH 7.0, 0.1% NaN_3, 0.05% BSA, 0.5 mM orthovanadate, and 1 mM DTT), subsequently reacted at 25 °C for 20 min. As a negative control, the tested compound was dissolved in 2.5% DMSO (v/v). The HTRF readings were performed on ENVISION (Perkinelmer) instrument by the addition of 5 μL of Streptavidin-XL665 and 5 μL Tk Antibody europium Cryptate solution. The inhibition rate (%) was calculated using the following equation: % inhibition = 100 − [(Activity of enzyme with tested compounds − Min)/(Max − Min)] × 100. The results were expressed as percentage inhibition from two independent experiments.

5.2.3. Docking study

The docking study was performed using AutoDock 4.0. The model of the wild-type B-Raf kinase (1UWH) was converted to PDBQT format using AutoDock Tools (ADT) version 1.5.4 (http://mgltools.scripps.edu). All hydrogen was added using the Hydrogen module in ADT for B-Raf. Then, Kollman united atom partial charges were assigned for the receptor. All the torsion angles in the small molecules were freed to perform flexible docking. The Lamarckian genetic algorithm was used with a population size of 200 dockings and 25 million energy evaluations. The results were clustered according to the root-mean-square deviation (RMSD) criterion.

Conflict of interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.059.

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