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PII: S0968-0896(16)30075-X
DOI: http://dx.doi.org/10.1016/j.bmc.2016.02.003
Reference: BMC 12805

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 4 December 2015
Revised Date: 2 February 2016
Accepted Date: 3 February 2016

Please cite this article as: Zhai, X., Bao, G., Wang, L., Cheng, M., Zhao, M., Zhao, S., Zhou, H., Gong, P., Design, synthesis and biological evaluation of novel 4-phenoxy-6,7-disubstituted quinolines possessing (thio)semicarbazones as c-Met kinase inhibitors, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.02.003

Design, synthesis and biological evaluation of novel 4-phenoxy-6,7-disubstituted quinolines possessing (thio)semicarbazones as c-Met kinase inhibitors

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Abstract

In continuing our efforts to identify small molecules able to inhibit c-Met kinase, three series of novel 6,7-disubstituted-4-phenoxyquinoline derivatives (**23a-w**, **26a-d** and **30a-d**) bearing (thio)semicarbazone scaffold were designed, synthesized and evaluated for their cytotoxicity. The biological data revealed that most compounds exhibited moderate-to-excellent activity against HT-29, MKN-45, A549 cancer cell lines and relative poor potency toward MDA-MB-231 cell as well as hardly any cytotoxicity in normal PBL cell. Eleven compounds were further examined for their inhibitory activity against c-Met kinase and three compounds (**23h**, **23n** and **26a**) demonstrated good inhibitory activity. This work resulted in the discovery of a potent c-Met inhibitor **23n**, bearing 2-hydroxy-3-allylphenyl group at R^2 moiety, as a valuable lead molecule, which possessed remarkable cytotoxicity and high selectivity against A549 and HT-29 cell lines with IC_{50} values of 11 nM and 27 nM. Besides, it displayed excellent c-Met kinase inhibition on a single-digital nanomolar level (IC_{50} = 1.54 nM). Meanwhile, the results from preliminarily *in vivo* study reflected that compound **23n** showed promising overall PK profiles, consistent with the efficacy in both MKN-45 and HT-29 tumor xenograft mice model. These results clearly indicated that compound **23n** is a potent and highly selective c-Met inhibitor and its favorable *in vitro* and *in vivo* profiles warrant further investigation.

Keywords: Quinoline; (Thio)semicarbazone; Anti-tumor activity; c-Met kinase inhibitor; Pharmacokinetic profile; Xenograft mice model.

1. Introduction

c-Met, also referred to as the surface receptor of hepatocyte growth factor receptor (HGFR), is a heterodimeric transmembrane receptor tyrosine kinase, that mediates activation of several signaling pathways implicated in aggressive cancer phenotypes.[1-3] Aberrant c-Met/HGF signaling, resulting from MET genomic amplification, c-Met/HGF overexpression or c-Met mutations, has been identified in a variety of human malignances, and in many instances has been correlated with advanced disease stage and poor prognosis.[4-6] This emphasizes c-Met as an attractive therapeutic target, triggering a number of approaches to disrupt aberrant c-Met signaling.

Cabozantinib (**1**) (Fig. 1), a novel oral multi-kinase inhibitor mainly targeting on c-Met and vascular endothelial growth factor receptor 2 (VEGFR-2), was approved by U.S. FDA in November 2012 for the treatment of patients with progressive metastatic medullary thyroid cancer (MTC).[7] Recently, significant

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progress has been made on the development of c-Met inhibitors with several compounds reaching clinical trials, including Foretinib (2) (Fig. 1),[8] Kirin (3),[9] AM7 (4)[10] and AMG458 (6)[11] (Fig. 1) as well as several newly developed compounds bearing 6,7-disubstituted-4-phenoxyquinoline frameworks as the primary pharmacophoric scaffolds. The structure-activity relationships (SARs) of quinoline-based inhibitors indicated that quinoline pharmacophores and linkers between the phenyl ring A and part B were responsible for the interaction with the backbone of the corresponding kinase.[12,13] Furthermore, the part B of phenyl ring probably extended into the hydrophobic pocket formed by tyrosine residues to enhance the inhibitory activity.[14] By means of Discovery Studio 3.0,[15] the spatial distances (SD) of linkers in the representative inhibitors **2**, **4**, and **5** [1] were obtained as 6.327 Å (PDB: 3LQ8), 6.549 Å (PDB: 2RFN), 6.434 Å (PDB: 3CE3), respectively, which would be attribute to the fitting into the hydrophobic pocket of c-Met. What′s more, whether the structures of linkages were constrained (**4**, **5** and **6**) or not (**1**, **2**, and **3**), it was found that six chemical bonds space, which was characterized by the illustrated "**5 atoms regulation**" in our previous work,[16] were retained by summarizing the structures of a large number of quinoline based inhibitors.

As a continuation of our study towarding identification of potent c-Met inhibitors, we decide to take advantage of the widely-used 4-phenoxyquinoline skeleton as a privileged scaffold of protein tyrosine kinases (PTK) inhibitors and modify it with three key linkages, thereby designing three series of quinoline analogs. In light of our early study,[17,18] we revealed that semicarbazone moiety, a hybrid linkage derived from two fused pharmacophoric domains of diaryl urea from Sorafenib[19] and *N*-acylhydrazone from PAC-1[20] (Fig. 1), were also widely used as building blocks in several antitumor agents on account of the presence of hydrogen bond donors and acceptors as well as its flexible skeleton (Fig. 1). To our delight, examination from corresponding software indicated that whose SD value is approximate 6.496 Å, which is similar to that of compounds **2**, **4**, **5** and meet the "**5 atoms regulation**". Accordingly, incorporation of semicarbazone scaffold into 4-phenoxy-6,7-disubstituted quinoline artwork led to a series of hybrids **23a-w**. Furthermore, enlightened by the structural characteristics of **3** in Fig.1, compounds **26a-d** bearing thiosemicarbazones instead of semicarbazones were synthesized based on bioisosterism. Moreover, in order to identify the linkage between phenyl ring A and part B, compounds **30a-d** bearing reversed semicarbazone were prepared. In view of our previous works,[17,18] the potential 3-carbon tether at the 7-position of quinoline moiety was reserved, while the morpholino group was replaced by three other water-soluble substituents, including piperidin-1-yl, 4-methylpiperidin-1-yl and pyrrolidin-1-yl groups, to extend the SARs of the new designed compounds. Furthermore, various substituents (R^2) were introduced with the purpose of exploring the influence of substituents on antitumor activity by regulating the electronic or steric effect at the phenyl ring (moiety B). Herein, in this report we disclosed the synthesis and c-Met inhibition study of these three series of new analogues.

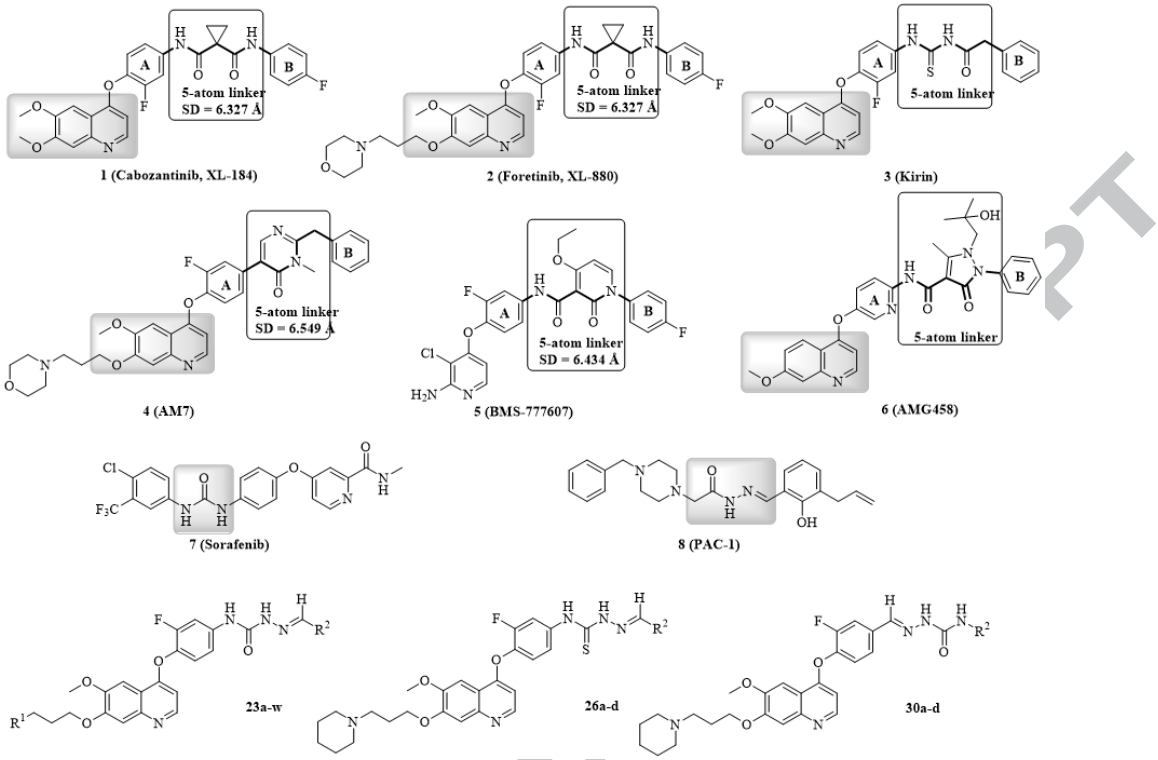
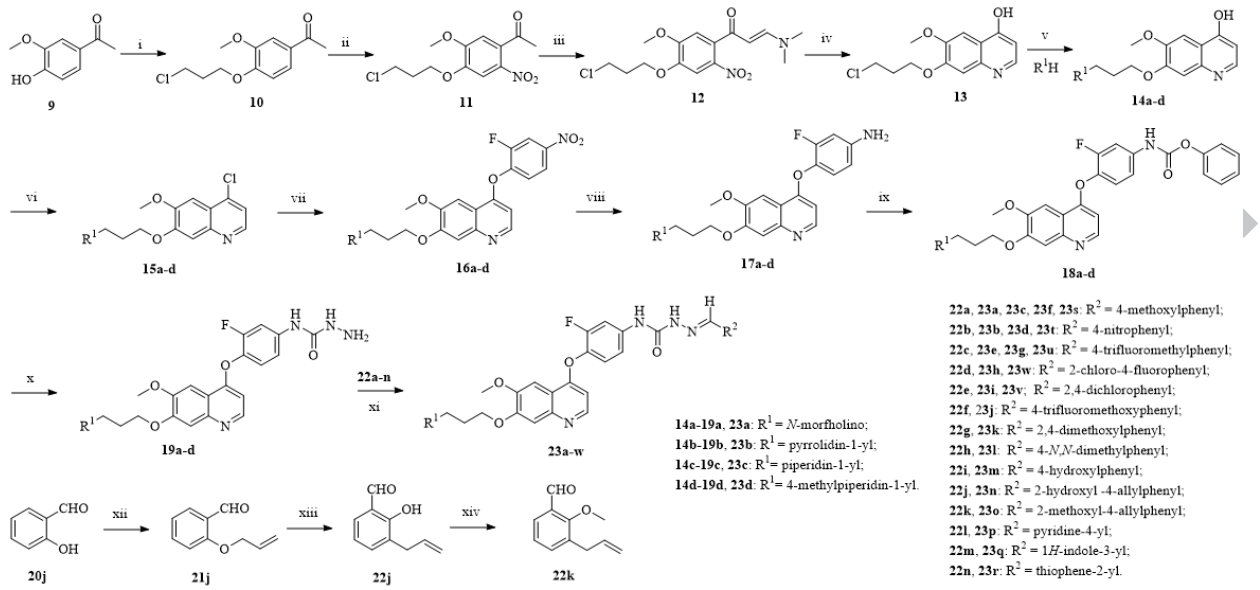


Fig. 1. The structures of small-molecular c-Met inhibitors bearing 6,7-disubstituted-4-phenoxyquinoline scaffold, Sorafenib, PAC-1 and the designed compounds.

2. Chemistry

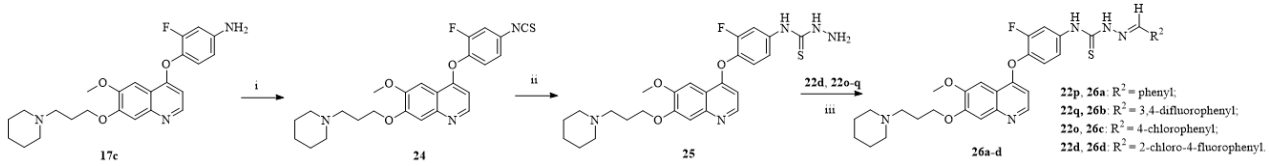
Compounds **23a-w** were synthesized according to the procedures outlined in Scheme 1. Commercially available 4-hydroxy-3-methoxyacetophenone **9** was alkylated with 1-bromo-3-chloropropane in the presence of potassium carbonate to provide **10**.[21] Nearly regioselective nitration with fuming nitric acid, subsequent aminomethylenation by means of modified Vilsmeier–Haack reagent *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) gave rise to **12**.[22] The intramolecular cyclization by iron powder and acetic acid proceeded smoothly to afford the quinolinol core **13**, which was subjected to a nucleophilic substitution with different amines (morpholine, piperidine, 4-methylpiperidine and pyrrolidine) to generate the desired intermediates **14a–d**. The resultant hydroxyl moieties **14a–d** were converted into the corresponding **15a–d** on exposure to phosphorus oxychloride. S_{N2} reactions of chloride in the **15a–d** with 2-fluoro-4-nitrophenol furnished **16a–d**, which were reduced by iron powder and concentrated hydrochloric acid in ethanol/water (9: 1 v/v) to provide anilines **17a–d**. Amide derivatives **18a–d** were introduced by further treatment with phenyl chloroformate **17a–d**. Subsequently, the key intermediate semicarbazides **19a–d** were available *via* hydrazinolysis of **18a–d** with 80% hydrazine monohydrate in refluxing 1,4-dioxane. The final products **23a-w** were obtained *via* the condensation of **19a–d** with appropriate aldehydes **22a-n** in isopropanol at 80 °C with catalytic amounts of acetic acid.[23]

In order to synthesis of aldehyde **22k**, a three-step reaction sequence was utilized. An initial alkylation of commercially available salicylaldehyde **20j** with 3-bromoprop-1-ene and potassium carbonate in *N*,*N*-dimethylformamide acquired **21j**. Subsequently, Claisen rearrangement delivered one desired aldehyde **22j** at 200 °C. Finally, **22j** was methylated with iodomethane in the darkness under basic condition using anhydrous potassium carbonate in dry acetone to bring out another target aldehyde **22k**.[24]



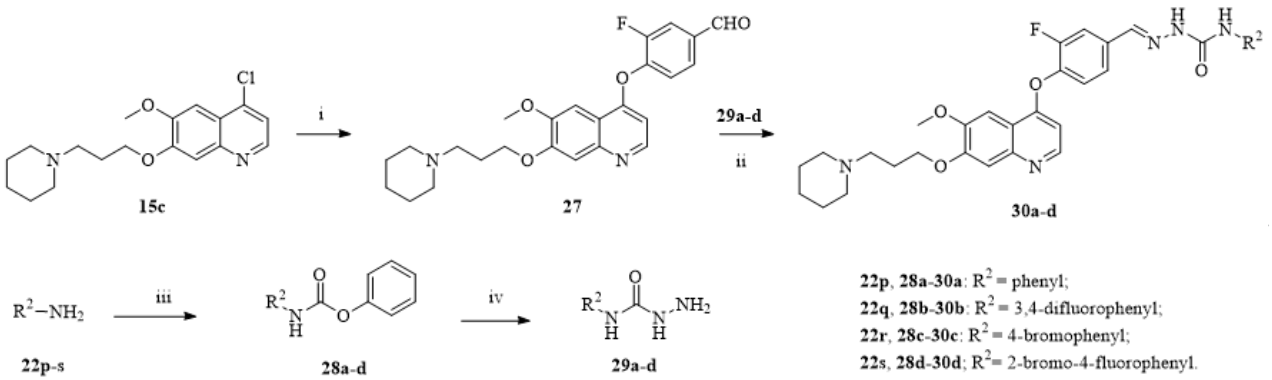
Scheme 1. Reagents and conditions: (i) 1-bromo-3-chloropropane, potassium carbonate, *N*,*N*-dimethylformamide, r.t.; (ii) fuming nitric acid, dichloromethane, 10 °C; (iii) *N*,*N*-dimethylformamide dimethyl acetal, toluene, reflux; (iv) iron powder, acetic acid, 80 °C; (v) amines, acetonitrile, reflux; (vi) phosphorus oxychloride, acetonitrile, reflux; (vii) 2-fluoro-4-nitrophenol, chlorobenzene, reflux; (viii) Fe (powder), conc. hydrochloric acid, ethanol/water (9: 1 v/v), reflux; (ix) phenylchloroformate, anhydrous potassium carbonate, dry acetone, 0 °C to r.t.; (x) 80% hydrazine monohydrate, 1,4-dioxane, reflux; (xi) aldehyde, acetic acid, 2-propanol, reflux; (xii) 3-bromoprop-1-ene, potassium carbonate, *N*,*N*-dimethylformamide, r.t.; (xiii) 200 °C; (xiv) iodomethane, potassium carbonate, acetone, reflux.

Scheme 2 depicts the sequence of reactions that lead to the construction of compounds **26a-d** utilizing **17c** as the starting material, which was obtained according to the reactions in Scheme 1. In the beginning, treatment of **17c** with thiophosgen in saturated sodium bicarbonate aqueous solution obtained isothiocyanate **24**,[25] which was then reacted with 80% hydrazine monohydrate in trichloromethane at room temperature to receive the key intermediate thiosemicarbazide **25**. The requisite benzylidenethiosemicarbazide derivatives **26a-d** were progressed as previously described reaction in Scheme 1.



Scheme 2. Reagents and conditions: (i) thiophosgen, salt. aq. sodium bicarbonate, trichloromethane, 0 °C to r.t.; (ii) 80% hydrazine hydrate, trichloromethane, r.t.; (iii) acetic acid (cat. amount), 2-propanol, reflux.

The preparation of analogues **30a-d** was illustrated in Scheme 3. Nucleophilic displacement of chloride group in the intermediate **15c** with 3-fluoro-4-hydroxybenzaldehyde afforded benzaldehyde **27**, which were condensed with semicarbazides **29a-d** in isopropanol to yield desired compounds **30a-d**. Herein, semicarbazides **29a-d** were installed from appropriate anilines **22p-s**, which were readily acylated by phenyl chloroformate in dry acetone with potassium carbonate to furnish **28a-d**. Subsequently, hydrazinolysis of **28a-d** with an excess of 80% hydrazine hydrate generated the intermediates **29a-d**.



Scheme 3. Reagents and conditions: (i) 3-fluoro-4-hydroxybenzaldehyde, chlorobenzene, reflux; (ii) acetic acid (cat. amount), 2-propanol, reflux. (iii) phenyl chloroformate, anhydrous potassium carbonate, dry acetone, 0 °C to r.t.; (iv) 80% hydrazine hydrate, 1,4-dioxane, reflux.

The chemical structures of obtained compounds were confirmed by ^{1}H NMR, NOESY NMR, ^{13}C NMR, and MS spectrum. All the target compounds might exist in the *E* or *Z* isomeric form due to the imine bonds formed in condensation procedures. Despite the possibility of the formation of two diastereoisomers, only the (*E*)-isomer was obtained from the experimental procedure adopted here. As for compounds **23a-w**, **26a-d** and **30a-d**, a detailed analysis of the ^{1}H NMR spectra revealed that only one –C***H***–N– signal existed for each compound. In addition, for the representative compound **23f**, the NOESY effects which resulted from the *E* isomer was observed between the proton of –N***H***–N– (H^1, δ 11.04 ppm, singlet) and the proton of –C**H**–N– (H^2, δ 8.49 ppm, singlet). This effect should not be observed in the (*Z*)-isomer due to the larger intramolecular distance of H^1 and H^2.[16, 17, 26, 27]

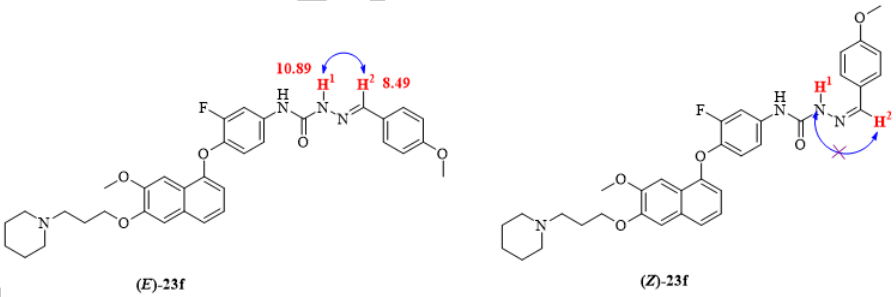


Fig. 2. The NOESY effects of representative compound **23f**.

3. Biology

3.1. HTRF kinase assay

The c-Met kinase activity was performed by homogeneous time-resolved fluorescence (HTRF) assays previously reported protocol.[28] In addition, the most promising compounds **23n** was evaluated against other six tyrosine kinases (Ron, KDR, PDGFR-α, c-kit, Flt-3 and EGFR) using the same screening method. Briefly, 20 μg/mL poly (Glu, Tyr) 4:1 (Sigma) was preloaded as a substrate in 384-well plates. Then 50 μL of 10 mM ATP (Invitrogen) solution diluted in kinase reaction buffer (50 mM HEPES, pH 7.0, 1 mM DTT, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 0.1% NaN_3) was added to each well. Various concentrations of compounds were diluted in 10 μL of 1% DMSO (v/v), with blank DMSO solution as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 39 μL of kinase reaction buffer solution. Reactions were

incubated for 30 min at 25 °C and stopped by the addition of 5 μL Streptavidin-XL665 and 5 μL Tk Antibody Cryptate working solution to all of wells. The plate was read by Envision (Perkinelmer) at 320 nm and 615 nm. The inhibition rate (%) was calculated using the following equation: % inhibition = 100-[(Activity of enzyme with tested compounds-Min)/(Max-Min)]-100 (Max: the observed enzyme activity measured in the presence of enzyme, substrates, and co-factors; Min: the observed enzyme activity in the presence of substrates, cofactors and in the absence of enzyme). Half-maximal inhibitory concentration (IC_{50}) values were calculated from the inhibition curves.

3.2. Cell proliferation assay

The anti-proliferativity of compounds **23a-w**, **26a-d** and **30a-d** were evaluated against human nonsmall-cell lung cancer cell (A549), human colorectal cancer cell (HT-29) and human gastric cancer cell (MKN-45), a c-Met less sensitive human breast cancer cell (MDA-MB-231) as well as a normal human peripheral blood lymphocyte cell (PBL) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay *in vitro*, taking Foretinib and PAC-1 as positive controls.[29] The cancer cells were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximately 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 test compounds were added to the culture medium at the indicated final concentrations and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a final concentration of 5 μg/mL and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL DMSO per each well, and the absorbency at 492 nm (for the absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as IC_{50} values were the mean ± SD and were calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

3.3. Pharmacokinetic (PK) parameters obtained in mice

To evaluate the bioavailability of compound **23n** in male mice, a single dose was administered as a solution in PEG 400/water (70:30) by either tail vein injection (iv, 20 mg/kg) or oral gavage (po, 35 mg/kg).[30] The mice were fasted overnight prior to dosing and fed 4 h postdose. A total of 18 mice were used in the study (n = 9 each for iv and po group). Three serum samples were collected from each mouse, the first two samples by retro-orbital bleed (~100 μL/20-25 g mouse) and the third sample by cardiac puncture. Blood samples were collected at 0.17, 0.5, 1, 3, 6, 8, 10, 12, and 24 h time points following iv dosing and at 0.25, 0.5, 1, 3, 6, 8, 10, 12, and 24 h following oral dosing. Blood samples were allowed to clot on ice and were centrifuged, and serum was harvested. Serum samples were stored at -20 °C until analysis. Concentrations of parent compound were later determined by LC/MS/MS. Composite serum concentration-time profiles were constructed for PK analysis.

3.4. In vivo antitumor efficacy studies

Initial antitumor efficacy studies were carried out *in vivo* to investigate the efficacy of desired compounds in HT-29 and MKN-45 xenograft nude mice models using Foretinib as positive control.[31] Two cell lines, HT-29 cell (1×10^7/100 μL PBS per mice) and MKN-45 cell (1×10^7/100 μL PBS per mice), were subcutaneously (s.c.) injected into the right flank of 7- to 8-week old male SCID mice or Balb/c nude mice. Cell numbers were confirmed by try pan blue staining prior to injection. When the average s.c. tumor volume reached 100 mm^3,

mice were randomly divided into various treatment and control groups (six mice per group). Tumor size was measured once every two days with a caliper (calculated volume = shortest diameter 2×longest diameter/2). Body weight, diet consumption and tumor size were recorded once every two days. After two or four weeks, mice were sacrificed and tumors were excised and stored at 80 °C until further analysis.

4. Results and discussion

4.1. In vitro cytotoxicity and structure activity relationships

The cytotoxicity of the target compounds **23a-w**, **26a-d** and **30a-d** were evaluated *in vitro* against three c-Met-addicted cancer cell lines including A549, HT-29, MKN-45, MDA-MB-231 and PBL cell lines by standard MTT assay taking Foretinib and PAC-1 as positive controls. The results were expressed as IC_{50} values and presented in Tables 1 and 2, as mean values of experiments performed in triplicate.

As illustrated in Tables 1 and 2, most compounds exhibited moderate-to-excellent cytotoxicity, with IC_{50} values ranging from 0.011 to 1.6 μM against c-Met-addicted A549, HT-29 and MKN-45 cell lines. Compared with Foretinib, three of them (**23h**, **30a**, **30d**) exhibited equivalent or even higher potency against A549 and HT-29 cell lines. Particularly compound **23n** displayed promising cytotoxicity with IC_{50} values of nanomolar level on A549, HT-29 and MKN-45 cells. As a general trend, the title compounds were more potent in A549 and HT-29 than they were in MKN-45 cells. By contrast, the compounds exhibited poor activity against MDA-MB-231 cell line and hardly any antitumor potency on PBL cells.

The SARs commenced by the introduction of a carbon tether, which contains different tertiary amines, off of the quinoline ring at 7-position was examined first. A three-carbon tether between-amines and the quinoline had previously been determined to be the optimal length for improving the potency; therefore, four kinds of amines at the end of this tether were examined. Of these amines, piperidinyl and 4-methylpiperidinyl derivatives **23f-g** and **23s-u** was proved to be superior to that of **23a-b** and **23c-e** bearing morpholino and pyrrolidinyl groups, respectively. Accordingly, piperidinyl and 4-methylpiperidinyl derivatives were further studied in the following work.

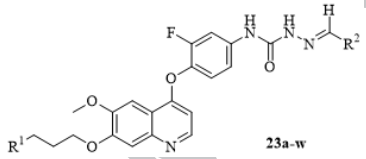
In terms of phenyl ring, the influence of substituents were explored either. The pharmacological data from Table 1 referred to substituted-benzene analogs disclosed that variations of R^2 made a moderate impact on the cytotoxicity. For compounds **23a-g** and **23s-u**, the presence of the electron-withdrawing groups such as nitro (**23b**, **23d** and **23t**) imparted enhanced activity. By contrast, the electron-donating groups such as methoxyl (**23a**, **23c**, **23f** and **23s**) weakened the biological activity slightly. While, compounds bearing bulky groups at 4-position, such as **23j** with 4-trifluoromethoxy group, **23l** possessing 4-dimethylamino group and **23e**, **23g**, **23u** bearing trifluoromethyl group, decreased or even vanished the antitumor potency against A549 and HT-29 cell lines, which suggested that the substitution at this position is fairly sensitive to a substituent's size.

Based on earlier study, we rationalized modifications of the phenyl group could modulate the π-stacking interactions with corresponding receptors allowing for increasing potency. However, compared with piperidinyl analog **21b** bearing phenyl group (IC_{50} = 0.16 μM (A549), 0.18 μM (HT-29) and 0.58 μM (MKN-45)) [17], incorporation of heterocyclic groups, such as the thiophen-2-yl, pyridine-4-yl and 1*H*-indol-2-yl groups in compounds **23p**, **23q** and **23r**, led to 1.10- to 5.25-folds decrease in antitumor tendency against A459, HT-29 and MKN-45 cells. Fortunately, compound **23n** bearing 2-hydroxy-3-allylphenyl group, the optimal pharmacophore of PAC-1,[20,32] was screening out displaying nanomolar level inhibition in cell-based assay with IC_{50} values of 11, 27 and 39 nM against A549, HT-29 and MKN-45 cancer cell lines, which were 15.4, 9.6 and 1.7 folds more active than that of Foretinib (0.17, 0.26 and 0.023 μM), respectively. The dramatic boost in

tendency might be ascribe to the introduction of 2-hydroxy-3-allyl group conduced to conjugation on corresponding receptors necessary for activity. This hypothesis could be confirmed by comparing **23n** with its methylation compound **23o**, as **23o** caused significant loss of potency on all three c-Met-addicted cancer cell lines.

Attention was turned to focus on linkage between A and B moiety in the subsequent efforts. Initially, the replacement of semicarbazone with thiosemicarbazone was carried out to investigate the effects of the oxygen atom. Although thiosemicarbazone derivatives **26a-d** displayed a slightly boost in the subsequent kinase-biochemical assays (Table 3), their activity against tested cancer cells decreased somewhat, which corroborated the results from date **23h** (0.13, 0.26, 0.27 and 0.79 μM toward tested cell lines) *vs*. **26d** (0.23, 0.42, 0.82 and 1.4 μM against tested lines). In the following work, a new series of derivatives **30a-d** conjugated with reversed semicarbazone were synthesized to identify the linkage. The pharmacological data revealed that **30a-d** exhibited elevated tendency than **26a-d** against all the tested cell lines in comparison **26a-b** with **30a-b**, especially for **30d**, which showed excellent activity 0.098, 0.11 and 0.14 μM against A549, HT-29 and MKN-45 cancer cell lines, respectively.

Table 1 Structures and cytotoxicity of the target compounds **23a-w** against A549, HT-29, MKN-45 and MDA-MB-231 cancer cell lines *in vitro*.



23a-w

Compd	R^1	R^2	IC_{50} [a] (μM)				
			A549	HT-29	MKN-45	MDA-MB-231	PBL
23a	*N*-morpholino	4-methoxylphenyl	ND [b]	0.90±0.13	0.77±0.05	1.9±0.25	17.25±2.62
23b	*N*-morpholino	4-nitrophenyl	ND	0.71±0.02	0.75±0.03	1.7±0.14	ND
23c	pyrrolidin-1-yl	4-methoxylphenyl	0.68±0.06	0.69±0.05	0.75±0.01	1.3±0.11	26.48±2.51
23d	pyrrolidin-1-yl	4-nitrophenyl	0.46±0.10	0.31±0.05	0.47±0.07	0.97±0.12	ND
23e	pyrrolidin-1-yl	4-trifluoromethylphenyl	0.79±0.10	0. 81±0.12	1.3±0.21	1.8±0.25	ND
23f	piperidin-1-yl	4-methoxylphenyl	0.54±0.12	0.59±0.09	0.66±0.05	0.84±0.11	125.56±8.35
23g	piperidin-1-yl	4-trifluoromethylphenyl	0.66±0.08	0.66±0.10	0.63±0.08	1.1±0.15	ND
23h	piperidin-1-yl	2-chloro-4-fluorophenyl	0.13±0.02	0.26±0.01	0.27±0.07	0.79±0.17	ND
23i	piperidin-1-yl	2,4-dichlorophenyl	ND	0.37±0.05	0.46±0.07	0.75±0.06	ND
23j	piperidin-1-yl	4-trifluoromethoxyphen	0.59±0.05	0.51±0.08	0.63±0.08	0.82±0.12	ND
23k	piperidin-1-yl	2,4-dimethoxylphenyl	1.4±0.10	1.6±0.32	1.99±0.35	ND	ND
23l	piperidin-1-yl	4-*N*,*N*-dimethylphenyl	ND	1.0±0.31	0.87±0.12	1.4±0.17	ND
23m	piperidin-1-yl	4-hydroxylphenyl	0.78±0.10	0.47±0.05	0.85±0.09	0.79±0.08	ND
23n	piperidin-1-yl	2-hydroxyl	0.011±0.001	0.027±0.002	0.039±0.004	0.44±0.10	106.91±7.28
23o	piperidin-1-yl	2-methoxyl-4-allylpheny	0.25±0.04	0.46±0.07	0.67±0.11	0.79±0.11	ND
23p	piperidin-1-yl	pyridine-4-yl	0.84±0.10	0.94±0.12	0.98±0.17	1.4±0.11	ND
23q	piperidin-1-yl	1*H*-indole-3-yl	0.78±0.08	0.82±0.09	0.83±0.05	0.98±0.12	ND
23r	piperidin-1-yl	thiophene-2-yl	0.79±0.06	0.77±0.05	0.74±0.07	1.26±0.15	68.68±4.12
23s	4-methylpiperidin-1-	4-methoxylphenyl	0.43±0.09	0.61±0.12	0.47±0.08	1.0±0.21	23.02±2.64
23t	4-methylpiperidin-1-	4-nitrophenyl	0.38±0.03	0.23±0.02	0.42±0.09	0.87±0.14	ND
23u	4-methylpiperidin-1-	4-trifluoromethylphenyl	0.65±0.07	0.74±0.13	1.0±0.23	1.3±0.13	ND
23v	4-methylpiperidin-1-	2,4-dichlorophenyl	ND	0.47±0.10	0.35±0.07	0.91±0.12	ND
23w	4-methylpiperidin-1-	2-chloro-4-fluorophenyl	0.19±0.01	0.44±0.04	0.53±0.03	1.5±0.17	79.49±6.65
Foretini	-	-	0.17±0.02 [c]	0.26±0.03 [d]	0.023±0.004 [e]	ND	ND
PAC-1	-	-	ND	0.83±0.07	0.82±0.13	4.6±0.33	123.81±9.64

[a] The average of three independent experiments;

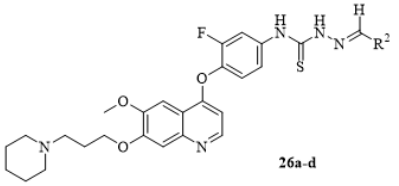
[b] ND: not determined;

[c] Reported value is 0.029 mM;[24]

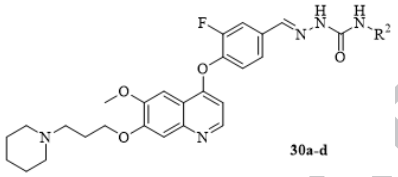
[d] Reported value is 0.165 mM;[24]

[e] Reported value is 0.008 mM.[25]

Table 2 Structures and cytotoxicity of compounds **26a-d** and **30a-d** against A549, HT-29, MKN-45 and MDA-MB-231 cancer cell lines *in vitro*.



26a-d



30a-d

Compd.	R^2	IC_{50} [a] (μM)				
		A549	HT-29	MKN-45	MDA-MB-231	PBL
26a	phenyl	0.33±0.02	0.21±0.02	0.71±0.08	1.2±0.17	78.36±6.54
26b	3,4-difluorophenyl	ND	0.61±0.10	0.74±0.15	1.4±0.15	ND
26c	4-chlorophenyl	ND	0.19±0.05	0.50±0.10	1.3±0.12	ND
26d	2-chloro-4-fluorophenyl	0.23±0.02	0.42±0.09	0.82±0.21	1.4±0.15	34.12±2.72
30a	phenyl	0.28±0.02	0.19±0.03	0.18±0.02	1.2±0.13	25.47±2.32
30b	3,4-difluorophenyl	ND	0.49±0.08	0.32±0.05	0.96±0.12	ND
30c	4-bromophenyl	ND	0.27±0.07	0.060±0.004	0.80±0.11	ND
30d	2-bromo-4-fluorophenyl	0.098±0.01	0.11±0.01	0.14±0.01	1.0±0.13	36.43±2.86
Foretinib	-	0.17±0.02	0.26±0.03	0.023±0.004	ND	ND
PAC-1	-	ND	0.83±0.07	0.82±0.13	4.6±0.33	123.81±9.64

[a] The average of three independent experiments.

4.2. In vitro enzymatic assays and selectivity

The c-Met kinase assays of eleven selected analogues (**23a**, **23c**, **23f**, **23n**, **23r**, **23s**, **23w**, **26a**, **26d**, **30a**, **30d**) were performed by HTRF assay. Served as the positive control, Foretinib was determined simultaneously. As shown in Table 3, all the eleven tested compounds exhibited c-Met enzymatic potency, reflecting that the inhibition of c-Met is a candidate underlying mechanism for the antitumor effect. In particular, compound **23n** was distinguished with an IC_{50} value of 1.54 nM, which was comparable to that of Foretinib (IC_{50} = 1.16 nM).

Table 3 c-Met kinase activity of the selected compounds and foretinib *in vitro*.

Compd.	IC_{50} [a] (nM)	Compd.	IC_{50} [a] (nM)
23a	56.42±4.36	**23w**	26.26±2.43
23c	72.33±7.63	**26a**	8.92±1.13
23f	13.51±1.15	**26d**	16.83±1.62
23n	1.54±0.12	**30a**	62.82±5.84
23r	54.25±5.26	**30d**	121.42±9.26
23s	15.67±1.28	**Foretinib**[b]	1.16±0.17

[a] Data presented is the mean±SD value of three independent determinations;

[b] Used as positive control, reported value is 4 nM.[24]

Then we were prompted to investigate their specifical inhibition against c-Met kinase, the optimal compound **23n** was screened against six selected kinases inhibited by most of reported quinoline-based multi-targeted kinase inhibitors further, which are potential biological targets for the designed compounds. As shown in Table 4, compound **23n** inhibited c-Met family member Ron kinase either, with IC_{50} value of 14.5 nM. In contrast to its high potency against c-Met, compound **23n** barely inhibited the kinase activity of other tested tyrosine kinases.

It exhibited preferable selectivity versus KDR (IC_{50} = 210.2 nM), PDGFR-α (IC_{50} = 212.3 nM), c-kit (IC_{50} = 162.5 nM), Flt-3 (IC_{50} = 366.7 nM), and no inhibition activity against EGFR (IC_{50} > 100 μM).

Furthermore, although **23n** exhibited similar c-Met kinase inhibition to the Foretinib, the results of c-Met kinase assay in Table 3 showed that most of compounds were less active than Foretinib. It suggested that the compounds might act through some other mechanisms rather than only by inhibiting c-Met and Ron. Further studies on the mechanisms of these compounds are in progress.

Table 4 Inhibition of tyrosine kinases by compound **23n**.

Compd.	Kinases IC_{50} [a] in nM					
	Ron	KDR	PDGFR-α	c-kit	Flt-3	EGFR
23n	14.5	210.2	212.3	162.5	366.7	>100 000

[a] Data presented is the mean value of three independent determinations.

4.3. PK profiles of the selected compounds

Before assessing the *in vivo* pharmacological activity, the metabolic stability and PK properties were evaluated first. The PK parameters of selected analogues in mice by either iv (20 mg/kg) or po (35 mg/kg) are compiled in Table 5. **23h** and **30d** were metabolically unstable and rapidly cleared *in vivo*. For **26a**, its T_{max} value is relatively longer than others. Delightedly, differing from their similar enzymatic and cellular potency *in vitro*, compound **23n** demonstrated remarkable potency in its PK properties, which was confirmed by single oral dose administration. It displayed a longer half-lives ($t_{1/2}$, 13.75 ± 1.74 h) and mean residence times ($MRT_{0\rightarrow\infty}$, 23.41 ± 2.33 h), favorable clearance (CL_z/F, 2.23 ± 0.55 L/h/kg), and so on. Given the promising overall PK profiles and structural novelty, compound **23n** was selected as the lead for the subsequent evaluation *in vivo*.

Table 5 The pharmacokinetic profiles of Selected Compounds

Compd.	$MRT_{0\rightarrow\infty}$ (h)[a]	$AUC_{0\rightarrow\infty}$ (mg·h /L)[b]	Cl_z/F (L/h/kg)[c]	V_z/F (L/kg)[d]	$T_{1/2z}$ (h)[e]	C_{max} (μg/L)[f]	T_{max} (h)[g]
23h	13.18 ± 2.26	0.91 ± 0.19	80.02 ± 17.81	370.98 ± 194.58	3.30 ± 0.26	0.078 ± 0.011	0.75 ± 0.29
23n	23.41 ± 2.33	16.50 ± 4.54	2.23 ± 0.55	43.88 ± 10.04	13.75 ± 1.74	0.68 ± 0.19	6.00 ± 1.63
26a	24.28 ± 6.23	7.34 ± 2.69	3.92 ± 1.55	107.09 ± 30.62	13.48 ± 4.35	0.087 ± 0.022	12.00 ± 1.68
30d	14.77 ± 1.78	1.21 ± 0.19	30.21 ± 4.46	346.22 ± 10.59	5.07 ± 1.31	0.073 ± 0.012	7.33 ± 1.16

Values are presented as the mean ± SD in three independent experiments.

Dosed in DMSO: Cremophor EL: 0.9% saline (20: 20: 60 (v/v/v));

[a] Mean residence time;

[b] Area under the plasma concentration-time curve for 0-∞ h after dosing;

[c] Clearance (statistical moment parameter);

[d] Apparent volume of distribution (statistical moment parameter);

[e] Half-lives;

[f] Maximum plasma concentration after oral dosing;

[g] Time to reach C_{max}.

4.4. In vivo antitumor activity

On the basis of its encouraging PK profiles, **23n** was advanced for *in vivo* efficacy studies in HT-29 and MKN-45 xenograft nude mice models taking Foretinib as reference drug. It was found that when administered orally twice daily (BID) for 24 days at a dose of 50 mg/kg, **23n** significantly inhibited tumor growth of 70.18% ($p < 0.001$) *vs*. control group in MKN-45 xenograft mice model (Fig. 3A). In order to further confirm its tumor growth arrest *in vivo*, the test to another well-accepted c-Met-dependent HT-29 xenograft mice model was extended. At the same dosing regimens, **23n** shrank tumor growth significantly, and the inhibition ratio was 65.86% ($p < 0.01$) in a 27-day study, compared with the vehicle control (Fig. 3B). Meanwhile, Foretinib exhibited equivalent or better suppression than **23n** with tumor growth inhibitory rate of 78.23% and 73.68% on MKN-45 and HT-29 xenograft mice model at the same dosage without any significant differences, respectively. Furthermore, it was well tolerated with no overt signs of toxicity or changes in body weight in two xenograft models (Fig. 3C and 3D).

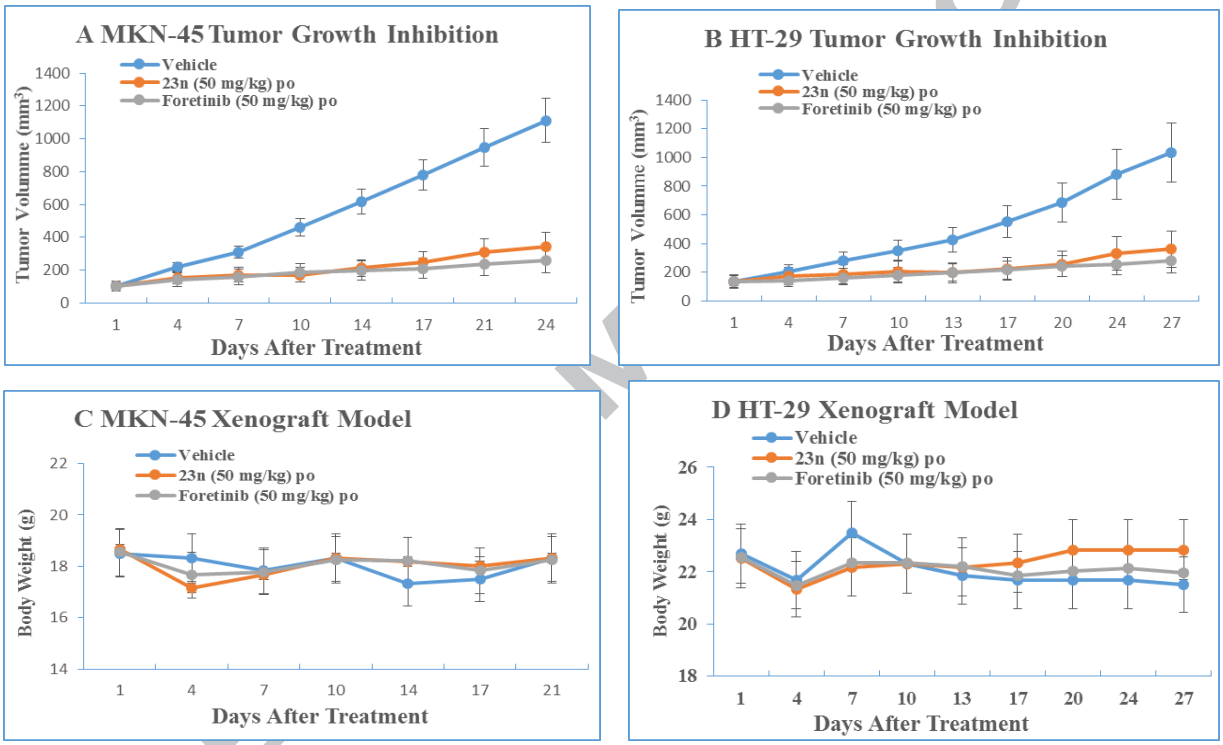


Fig. 3. Effects of compound **23n** and Foretinib in the MKN-45 (A) and HT-29 (B) xenograft models (mean tumor volume in mm^3 ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$, *vs*. vehicle control group). Tumor-bearing Balb/c nude mice were randomly divided into groups in which 6 mice were contained per group. While the tumor volume reached 100 mm^3, **23n** and Foretinib were administrated po (BID) at a dose of 50 mg/kg or vehicle alone for 24 or 27 days. (C) and (D) shown are the average body weights for **23n**-treated, Foretinib-treated mice groups and vehicle groups in the MKN-45 and HT-29 xenograft models, respectively.

5. Conclusions

In summary, thirty-one novel quinoline-based selective c-Met kinase inhibitors (**23a-w**, **26a-d** and **30a-d**) were designed and synthesized. Most compounds displayed moderate-to-excellent activity against HT-29, MKN-45, A549 cancer cell lines and relative poor potent toward MDA-MB-231 cell as well as hardly any cytotoxicity to normal PBL cell. The exploration of preliminary SARs indicated that the hybridization of quinoline pharmacophore and semicarbazone as a privileged structure is of great importance for the good antiproliferative potency and 4-methylpiperidin-1-yl was favorable for the better tendency. Besides, the identification of inhibitor **23n,** bearing 2-hydroxy-3-allylphenyl group at R^2 moiety, as a valuable lead molecule,

which possessed excellent c-Met kinase inhibition on a single-digital nanomolar level (IC_{50} = 1.54 nM). Moreover, it displayed remarkable cytotoxicity and high selectivity against A549 and HT-29 cell lines with IC_{50} values of 11 nM and 27 nM. Meanwhile, the results from preliminarily *in vivo* study reflected that compound **23n** showed promising overall PK profiles and shrank tumor growth significantly in MKN-45 and HT-29 xenograft mice model. In conclusion, compound **23n** is worth further studying as a new potential antitumor agent owing to its pronounced *in vitro* and *in vivo* antitumor activity. Further studies on structural optimization and the mechanisms are currently underway and will be reported in the future.

6. Experimental

6.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were generated on a Bruker ARX-300 or ARX-600 spectrometers (Bruker Bioscience, Billerica, MA, USA). Column chromatography was carried out on silica gel (200-300 mesh). Mass spectra (MS) were taken in ESI mode on 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).

6.2. 4-(3-Chloropropoxy)-3-methoxyacetophenone (10)

To a solution of 4-hydroxy-3-methoxy-acetophenone (6.00 g, 36.1 mmol) in DMF (25 mL) was added K_2CO_3 and 1-bromo-3-chloropropane (6.98 g, 50.6 mmol). The reaction mixture was then stirred at r.t. for 10 h. The mixture was then poured into cold water (100 mL) with vigorously agitating, and the resulting precipitate was filtered off, washed with water, and dried under vacuum to afford the title compound **10** (8.27 g) as a white solid, yield: 93.8%. m.p.: 61-63 °C. MS (ESI) *m/z*: 242.2, 244.1 $[M+H]^+$.

6.3. 4-(3-Chloropropoxy)-5-methoxy-2-nitroacetophenone (11)

A stirred solution of **10** (2.00 g, 8.22 mmol) in CH_2Cl_2 (10 mL) was cooled to 10 °C, and fuming nitric acid (95%, 1.30 g, 20.6 mmol) was added at a rate such that the temperature did not exceed 10 °C. The reaction mixture was allowed to stir at 10 °C for 2 h, then transferred with stirring to cold water (20 mL). After 15 min, the organic layer was separated and the aqueous layer washed with additional CH_2Cl_2 (10 mL). The organic portions were combined and washed with 10% w/w aqueous sodium bicarbonate solution (2×10 mL), then water (10 mL), then concentrated under reduced pressure to afford crude **11**, which was recrystallized from 90% ethanol to afford **11** (1.91 g) as a light yellow solid, yield: 80.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 6.76 (s, 1H), 4.26 (t, J = 6.0 Hz, 2H), 3.96 (s, 3H), 3.77 (t, J = 6.6 Hz, 2H), 2.50 (s, 3H), 2.29-2.37 (m, 2H). m.p.: 60-61 °C. MS (ESI) *m/z*: 287.0, 289.0 $[M+H]^+$.

6.4. 1-(4-(3-Chloropropoxy)-5-methoxy-2-nitrophenyl)-3-(dimethylamino)prop-2-en-1-one (12)

DMF-DMA (4.15 g, 34.8 mmol) was added to a solution of **11** (2.00 g, 6.95 mmol) in toluene (10 mL). The reaction was heated to 110 °C until TLC showed the completion of the reaction. After cooling to 10 °C, the resultant solid was collected by filtration, washed with toluene (5 mL) and then dried under vacuum to yield **12** (1.84 g) as a yellow solid, yield: 75.8%. m.p.: 117-119 °C. MS (ESI) *m/z*: 342.2, 344.1 $[M+H]^+$.

6.5. 6-Methyloxy-7-(4-(3-chloropropoxy))-4-quiolin-ol (13)

Fe (powder, 1.23 g, 22.0 mmol) was added to a solution of **12** (1.50 g, 4.40 mmol) in acetic acid (12 mL) at 40 °C, then the mixture was stirred at 80 °C with vigorous agitation for 2 h. The hot solution was filtered through celite and washed with hot acetic acid. The combined filtrate was cooled to 20 °C, and the resultant solid was collected by filtration which was recrystallized from acetic acid (6 mL) and washed with water to afford **13** (0.820 g) as a light yellow solid , yield: 65.0%. m.p.: 232-234 °C. MS (ESI) *m/z*: 267.0, 269.0 $[M+H]^+$.

6.6. General procedure for preparation of 4-hydroxyquinolines (14a–d)

To a stirring solution of **13** (10.0 g, 37.4 mmol) in acetonitrile (100 mL) at 25 °C was added appropriate secondary amines (0.374 mol), and the resulting reaction mixture was heated to 80 °C for 8 h, then cooled to room temperature. Approximately 60 mL acetonitrile was removed under reduced pressure. The residue was cooled to 10 °C, and the resultant precipitate was filtered, washed with ethyl acetate (35 mL) to give the corresponding 4-hydroxyquinolines **14a-d**.

6.6.1. 6-Methyloxy-7-(4-(3-morpholino-propoxy))-4-quiolinol (14a)

Yellow solid, yield: 93.9%. m.p.: 187-189 °C. MS (ESI) *m/z*: 319.3 $[M+H]^+$.

6.6.2. 6-Methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinolin-4-ol (14b)

Yellow solid, yield: 84.5%. m.p.: 167-169 °C. MS (ESI) *m/z*: 303.4 $[M+H]^+$.

6.6.3. 6-Methyloxy-7-(4-(3-(piperidin-1-yl)propoxy))-4-quiolin-ol (14c)

White solid, yield: 88.7%. m.p.: 176-77 °C. MS (ESI) *m/z*: 317.3 $[M+H]^+$.

6.6.4. 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinolin-4-ol (14d)

Sallow solid, yield: 87.9%. m.p.: 182-184 °C. MS (ESI) *m/z*: 331.4 $[M+H]^+$.

6.7. General procedure for preparation of 4-chloroquinolines (15a–d)

A solution of an appropriate dry 4-hydroxyquinoline (20.0 mmol) and $POCl_3$ (40 mL) in dry acetonitrile (40 mL) was refluxed on an oil-bath for approximate 6 h. After cooling to ambient temperature, the contents were concentrated under reduced pressure and the pale residue was poured into ice-water (100 mL) with vigorous agitation. The solution was treated with concentrated ammonium hydroxide to achieve pH to 8 while maintaining the temperature below 20 °C, and the mixture was extracted with CH_2Cl_2 (320 mL). The combined organic layer was washed with brine, then water, and dried over anhydrous Na_2SO_4, concentrated under reduced pressure to give corresponding 4-chloroquinolines **15a–d**.

6.7.1. 4-Chloro-6-methyloxy-7-(4-(3-morpholino-propoxy))quinoline (15a)

White solid, yield: 82.4%. m.p.: 173-176 °C. MS (ESI) *m/z*: 336.2, 338.2 $[M+H]^+$.

6.7.2. 4-Chloro-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinoline (15b)

White solid, yield: 83.1%. m.p.: 172-174 °C. MS (ESI) *m/z*: 321.4 $[M+H]^+$.

6.7.3. 4-Chloro-6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinoline (15c)

White solid, yield: 85.9%. m.p.: 187-188 °C. MS (ESI) *m/z*: 335.5 $[M+H]^+$.

6.7.4. 4-Chloro-6-methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinoline (15d)

White solid, yield: 81.4%. m.p.: 179-181 °C. MS (ESI) *m/z*: 349.3 $[M+H]^+$.

6.8. General procedure for preparation of nitro compounds (16a-d)

A stirring mixture of an appropriate **15a-d** (20.0 mmol) and 2-fluoro-4-nitrophenol (24.1 mmol) in chlorobenzene (40 mL) was heated to 140 °C for about 20 h. The reaction was considered complete when less than 5% starting material remained. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure to yield a pale solid. The solid was dissolved in CH_2Cl_2 (80 mL), and washed with saturated K_2CO_3 aqueous solution (2×20 mL), then water (20 mL), and dried over anhydrous Na_2SO_4, concentrated under reduced pressure to afford a wheat solid, which was recrystallized from anhydrous ethanol (30 mL) to yield corresponding **16a–d**.

6.8.1. 4-(2-Fluoro-4-nitrophenoxy)-6-methoxy-7-(3-morpholino-propoxy) quinoline (16a)

Light yellow solid, yield: 62.0%. m.p.: 135-136 °C; MS (ESI) *m/z* (%): 458.1 $[M+H]^+$.

6.8.2. 4-(2-Fluoro-4-nitrophenoxy)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy) quinoline (16b)

Yellow solid, yield: 76.3%. m.p.: 140-141 °C; MS (ESI) *m/z* (%): 442.2 $[M+H]^+$.

6.8.3. 4-(2-Fluoro-4-nitrophenoxy)-6-methoxy-7-(3-(piperidin-1-yl)propoxy) quinoline (16c)

Yellowish-white solid, yield: 80.4%. m.p.: 137-138 °C; MS (ESI) *m/z* (%): 456.4 $[M+H]^+$.

6.8.4. 4-(2-Fluoro-4-nitrophenoxy)-6-methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinoline (16d)

Yellow solid; yield: 78.7%. m.p.: 133-134 °C; MS (ESI) *m/z* (%): 470.6 $[M+H]^+$.

6.9. General procedure for preparation of anilines (17a-d)

A mixture of **16a-d** (20.0 mmol), Fe (powder, 0.200 mol) and concentrated hydrochloric acid (36.5%, 1.00 mL) in ethanol/water (100 mL, 9:1 v/v) was refluxed with vigorous agitation for 2 h. The hot solution was filtered through celite and the filter cake was washed with hot ethanol. The combined filtrate was concentrated under reduced pressure to afford a yellow solid, which was recrystallized from anhydrous ethanol (60 mL) to yield corresponding anilines **17a-d**.

6.9.1. 3-Fluoro-4-(6-methoxy-7-(3-morpholinopropoxy)quinolin-4-yloxy) aniline (17a)

White solid, yield: 92.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, J = 5.1 Hz, 1H), 7.58 (s, 1H), 7.44 (s, 1H), 7.04 (t, J = 8.4 Hz, 1H), 6.54-6.59 (dd, J_1 = 12.0 Hz, J_2 = 2.4 Hz, 1H), 6.49-6.52 (m, 1H), 6.42 (d, J = 5.1 Hz, 1H), 4.27 (t, J = 6.6 Hz, 2H), 4.04 (s, 3H), 3.77-3.88 (br, 2H), 3.74 (t, J = 4.5 Hz, 4H), 2.60 (t, J = 6.9 Hz, 2H), 2.49-2.52 (m, 4H), 2.10-2.19 (m, 2H). m.p.: 217-218 °C. MS (ESI) *m/z*: 427.1 $[M+H]^+$.

6.9.2. 3-Fluoro-4-(6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinolin-4-yloxy) aniline (17b)

Light yellow solid, yield: 72.3%. m.p.: 208-209 °C. MS (ESI) *m/z*: 412.5 $[M+H]^+$.

6.9.3. 3-Fluoro-4-(6-methoxy-7-(3-(piperdine-1-yl)propoxy)quinolin-4-yloxy) aniline (17c)

Gray solid, yield: 85.5%. m.p.: 196-197 °C. MS (ESI) *m/z*: 426.3 $[M+H]^+$.

6.9.4. 3-Fluoro-4-(6-methoxy-7-(3-(4-methylpiperdine-1-yl)propoxy)quinolin-4-yloxy)aniline (17d)

White solid, yield: 77.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, J = 5.3 Hz, 1H), 7.58 (s, 1H), 7.41 (s, 1H), 7.03 (t, J = 8.7 Hz, 1H), 6.56 (dd, J_1 = 11.8, J_2 = 2.6 Hz, 1H), 6.50 (dd, J_1 = 9.0, J_2 = 2.9 Hz, 1H), 6.39 (dd, J = 5.3, 0.8 Hz, 1H), 4.25 (t, J = 6.7 Hz, 2H), 4.03 (s, 3H), 3.82 (s, 2H), 2.94 (m, 2H), 2.57 (m, 2H), 2.15 (m, 2H), 1.98 (t, J = 10.9 Hz, 2H), 1.63 (d, J = 10.4 Hz, 2H), 1.28 (m, 3H), 0.93 (d, J = 6.0 Hz, 3H). m.p.: 193-194 °C. MS (ESI) *m/z*: 440.2 $[M+H]^+$.

6.10. General procedure for preparation of phenylcarbamates (18a-d)

To the mixture of **17a-d** (10.0 mmol) and anhydrous K_2CO_3 (2.76 g, 20.0 mmol) in dry acetone (60 mL), phenyl chloroformate (1.88 mL, 15.0 mmol) was added dropwise while maintaining the temperature between 0 °C and 5 °C. After the addition was completed, the mixture was warmed to room temperature for another 3 h, and the solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL), and washed with water (3×10 mL), dried over anhydrous Na_2SO_4, concentrated under reduced pressure to afford corresponding phenylcarbamates **18a–d**, which were immediately used in the next step without further purification.

6.10.1. Phenyl 3-fluoro-4-(6-methoxy-7-(3-morpholino-propoxy)-quinolin-4-yloxy)phenylcarbamate (18a)

Yellow oil, yield: 90.2%. m.p.: 219–221 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.44 (d, J = 5.1 Hz, 1H), 7.64-7.68 (m, 1H), 7.49 (s, 1H), 7.39-7.44 (m, 4H), 7.36 (s, 1H), 7.20-7.27 (m, 3H), 6.41 (d, J = 5.1 Hz, 1H), 4.17 (t, J = 6.3 Hz, 2H), 3.91 (s, 3H), 3.52–3.65 (br, 4H), 2.52-2.62 (br, 4H), 2.31-2.43 (br, 2H), 1.89-2.06 (m, 2H). MS (ESI) *m/z*: 548.3, 549.3 $[M+H]^+$.

6.10.2. Phenyl 3-fluoro-4-(6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy))-quinolin-4-yloxy) phenylcarbamate (18b)

Yellow oil, yield: 85.0%. m.p.: 204-206 °C. MS (ESI) *m/z*: 532.2 $[M+H]^+$.

6.10.3. Phenyl 3-fluoro-4-(6-methoxy-7-(3-(piperdine-1-yl)propoxy))-quinolin-4-yloxy) phenylcarbamate (18c)

Light yellow oil, yield: 83.1%. m.p.: 210-212 °C. MS (ESI) *m/z*: 546.4 $[M+H]^+$.

6.10.4. Phenyl 3-fluoro-4-(6-methoxy-7-(3-(4-methylpiperdine-1-yl)propoxy))-quinolin-4-yloxy) phenylcarbamate (18d)

Yellow oil, yield: 82.3%. m.p.: 226-228 °C. MS (ESI) *m/z*: 560.3 $[M+H]^+$.

6.11.1. N^1-(3-Fluoro-4-(6-methoxy-7-(3-morpholinopropoxy)quinolin-4-yloxy)phenyl)semicarbazide (19a)

A mixture of **18a** (4.40 g, 8.01 mmol) and 80% hydrazine monohydrate (10 mL) in 1,4-dioxane (20 mL) was refluxed overnight with vigorous agitation. Most of the solvent was evaporated under reduced pressure when white solid appeared. After cooling to 10 °C, the resulting precipitate was filtered off, washed with water, and dried under vacuum to afford the title compound **19a** (1.80 g) as a white solid, yield: 46.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.47 (d, J = 5.4 Hz, 1H), 7.83-7.88 (m, 1H), 7.63 (s, 1H), 7.53 (s, 1H), 7.44-7.48 (m, 1H), 7.40 (s, 1H), 7.33 (t, J = 9.0 Hz, 1H), 6.43 (d, J = 5.4 Hz, 1H), 4.32-4.59 (br, 2H), 4.20 (t, J = 6.6 Hz, 2H), 3.95 (s, 3H), 3.58-3.61 (m, 4H), 2.45-2.48 (m, 2H), 2.33-2.44 (br, 4H), 1.94-2.03 (m, 2H). m.p.: 188-190 °C. MS (ESI) *m/z*: 485.3 $[M+H]^+$.

6.11.2. N^1-(3-Fluoro-4-(6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinolin-4-yloxy)phenyl)semicarbazide

(19b)

White solid, yield: 43.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.47 (d, J = 5.1 Hz, 1H), 7.83-7.87 (m, 1H), 7.63 (s, 1H), 7.53 (s, 1H), 7.44-7.47 (m, 1H), 7.38 (s, 1H), 7.33 (t, *J* = 9.0 Hz, 1H), 6.42 (d, *J* = 5.1 Hz, 1H), 4.29-4.57 (br, 2H), 4.20 (t, *J* = 6.3 Hz, 2H), 3.95 (s, 3H), 2.58 (t, *J* = 6.3 Hz, 2H), 2.46 (s, 4H), 1.94-2.03 (m, 2H), 1.70 (s, 4H). m.p.: 177-178 °C. MS (ESI) *m/z*: 470.3 $[M+H]^+$.

6.11.3. N^1-(3-Fluoro-4-(6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yloxy)phenyl)semicarbazide (19c)

The intermediate **19c**, which was purified by silica gel column chromatography (eluent, CH_2Cl_2/ MeOH/ Et_3N = 100:1:1 to 100:10:1), was obtained from **18c** by the general procedure as a white solid, yield: 39.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.47 (d, *J* = 5.1 Hz, 1H), 7.82-7.87 (m, 1H), 7.60 (s, 1H), 7.53 (s, 1H), 7.41–7.49 (m, 1H), 7.38 (s, 1H), 7.32 (t, *J* = 9.0 Hz, 1H), 6.43 (d, *J* = 5.1 Hz, 1H), 4.30-4.52 (br, 2H), 4.18 (t, *J* = 6.3 Hz, 2H), 3.95 (s, 3H), 2.39-2.47 (m, 6H), 1.91-2.01 (m, 2H), 1.45–1.58 (m, 4H), 1.33-1.44 (m, 2H). m.p.: 181-182 °C. MS (ESI) *m/z*: 484.3 $[M+H]^+$.

6.11.4. N^1-(3-Fluoro-4-(6-methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinolin-4-yloxy)phenyl)semicarbazide (19d)

The intermediate **19d**, which was purified by silica gel column chromatography (eluent, CH_2Cl_2/ MeOH/ Et_3N = 100:1:1 to 100:10:1), was obtained from **18d** by the general procedure as a white solid, yield: 38.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.47 (d, *J* = 5.1 Hz, 1H), 7.82-7.86 (m, 1H), 7.58 (s, 1H), 7.41 (s, 1H), 7.03 (t, *J* = 8.7 Hz, 1H), 6.54-6.59 (dd, J_1 = 12.0 Hz, J_2 = 2.7 Hz, 1H), 6.48-6.52 (m, 1H), 6.41 (d, *J* = 5.1 Hz, 1H), 4.32-4.49 (br, 2H), 4.25 (t, *J* = 6.6 Hz, 2H), 4.03 (s, 3H), 2.89-2.98 (m 2H), 2.57 (m, 2H), 2.11-2.20 (m, 2H), 1.94-2.02 (m, 2H), 1.58-1.68 (m, 2H), 1.21-1.36 (m, 3H), 0.94 (d, *J* = 6.0 Hz, 3H). m.p.: 191-193 °C. MS (ESI) *m/z*: 498.2 $[M+H]^+$.

6.12. General procedure for the synthesis of semicarbazones (23a-w)

To a solution of **19a-d** (0.200 g, 0.414 mmol) in isopropanol (2 mL), 1.1 equiv. of aldehydes or ketones and acetic acid (1 drop) were added, and the mixture was refluxed for 5-6 h until TLC showed the completion of the reaction. After cooling to room temperature, the resultant precipitate was filtered and dried under vacuum.

6.12.1. (*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-morpholinopropoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-methoxybenzylidene)semicarbazide (23a)

White solid (88.5%); m.p.: 221-223 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (s, 1H), 9.19 (s, 1H), 8.51 (d, *J* = 5.1 Hz, 1H), 7.90-7.96 (m, 2H), 7.83 (d, *J* = 9.0 Hz, 2H), 7.64-7.68 (m, 1H), 7.58 (s, 1H), 7.46 (s, 1H), 7.41 (t, *J* = 9.0 Hz, 1H), 7.02 (d, *J* = 8.7 Hz, 2H), 6.50 (d, *J* = 5.1 Hz, 1H), 4.27 (t, *J* = 6.0 Hz, 2H), 3.97 (s, 3H), 3.81 (s, 3H), 3.71-3.79 (br, 4H), 2.94-3.19 (br, 6H), 2.15-2.24 (m, 2H); MS (ESI) *m/z*: 604.2 $[M+H]^+$. Anal. calcd. for $C_{32}H_{34}FN_5O_6$ (%): C, 63.67; H, 5.68; N, 11.60. Found (%): C, 63.62; H, 5.70; N, 11.58.

6.12.2. (*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-morpholinopropoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-nitrobenzylidene)semicarbazide (23b)

Yellow solid (92.4%); m.p.: 208-209 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.27 (s, 1H), 8.50 (d, *J* = 5.4 Hz, 1H), 8.01-8.04 (m, 3H), 7.89-7.94 (dd, J_1 = 13.2 Hz, J_2 = 2.4 Hz, 1H), 7.63-7.66 (m, 1H), 7.55 (s,

1H), 7.39-7.45 (m, 4H), 6.47 (d, *J* = 5.1 Hz, 1H), 4.21 (t, *J* = 6.3 Hz, 2H), 3.96 (s, 3H), 3.60 (s, 4H), 2.29-2.50 (br, 6H), 1.95-2.04 (m, 2H); MS (ESI) *m/z*: 619.4 $[M+H]^+$. Anal. calcd. for $C_{31}H_{31}FN_6O_7$ (%): C, 60.19; H, 5.05; N, 13.59. Found (%): C, 60.17; H, 5.07; N, 13.61.

6.12.3.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-methoxybenzylidene)semicarbazide (23c)

White solid (79.6%); m.p.: 217-196 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (s, 1H), 9.18 (s, 1H), 8.49 (d, *J* = 5.1 Hz, 1H), 7.94-7.96 (m, 1H), 7.86-7.91 (m, 1H), 7.83 (d, *J* = 8.7 Hz, 2H), 7.64-7.67 (m, 1H), 7.55 (s, 1H), 7.37-7.43 (m, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 6.47 (d, *J* = 5.1 Hz, 1H), 4.21 (t, *J* = 6.6 Hz, 2H), 3.96 (s, 3H), 3.81 (s, 3H), 2.61 (t, *J* = 7.2 Hz, 2H), 2.41-2.48 (br, 4H), 1.95-2.04 (m, 2H), 1.66-1.75 (m, 4H). MS (ESI) *m/z*: 588.2 $[M+H]^+$. Anal. calcd. for $C_{32}H_{34}FN_5O_5$ (%): C, 65.40; H, 5.83; N, 11.92. Found (%): C, 65.42; H, 5.85; N, 11.90.

6.12.4.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-nitrobenzylidene)semicarbazide (23d)

Yellow solid (84.8%); m.p.: 201-203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 9.40 (s, 1H), 8.50 (d, *J* = 5.4 Hz, 1H), 8.16 (d, *J* = 8.7 Hz, 1H), 8.01 (s, 1H), 7.98 (d, *J* = 8.7 Hz, 1H), 7.89-7.94 (dd, J_1 = 13.5 Hz, J_2 = 2.4 Hz, 1H), 7.63-7.68 (m, 1H), 7.55 (s, 1H), 7.40-7.46 (m, 2H), 6.47 (d, *J* = 5.4 Hz, 1H), 4.21 (t, *J* = 6.6 Hz, 2H), 3.96 (s, 3H), 2.62 (t, *J* = 6.3 Hz, 2H), 2.51-2.58 (m, 4H), 1.96-2.05 (m, 2H), 1.71 (s, 4H); MS (ESI) *m/z*: 603.3 $[M+H]^+$. Anal. calcd. for $C_{31}H_{31}FN_6O_6$ (%): C, 61.79; H, 5.19; N, 13.95. Found (%): C, 61.82; H, 5.21; N, 13.93.

6.12.5.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-(trifluoromethyl)benzylidene)semicarbazide (23e)

Yellow solid (77.9%); m.p.: 228-230 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.05 (s, 1H), 9.31 (s, 1H), 8.49 (d, *J* = 5.1 Hz, 1H), 8.01-8.03 (m, 3H), 7.89-7.94 (dd, J_1 = 13.2 Hz, J_2 = 2.1 Hz, 1H), 7.63-7.66 (m, 1H), 7.55 (s, 1H), 7.39-7.45 (m, 4H), 6.47 (d, *J* = 5.1 Hz, 1H), 4.22 (t, *J* = 6.3 Hz, 2H), 3.96 (s, 3H), 2.69 (t, *J* = 6.9 Hz, 2H), 2.59 (s, 4H), 1.98-2.07 (m, 2H), 1.74 (s, 4H); MS (ESI) *m/z*: 626.4 $[M+H]^+$. Anal. calcd. for $C_{32}H_{31}F_4N_5O_4$ (%): C, 61.43; H, 4.99; N, 11.19. Found (%): C, 61.45; H, 4.95; N, 11.21.

6.12.6.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-methoxybenzylidene)semicarbazide (23f)

White solid (82.1%); m.p.: 219-221 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 9.17-9.44 (br, 1H), 8.49 (d, *J* = 5.4 Hz, 1H), 7.89-7.95 (m, 2H), 7.79-7.83 (m, 2H), 7.62-7.66 (m, 1H), 7.55 (s, 1H), 7.37-7.43 (m, 2H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.46 (d, *J* = 5.4 Hz, 1H), 4.19 (t, *J* = 6.3 Hz, 2H), 3.96 (s, 3H), 3.81 (s, 3H), 2.43 (t, *J* = 7.2 Hz, 2H), 2.30-2.39 (br, 4H), 1.92-2.01 (m, 2H), 1.48-1.55 (m, 4H), 1.36-1.43 (m, 2H); MS (ESI) *m/z*: 602.5 $[M+H]^+$. Anal. calcd. for $C_{33}H_{36}FN_5O_5$ (%): C, 65.88; H, 6.03; N, 11.64. Found (%): C, 65.90; H, 6.05; N, 11.66.

6.12.7.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-(trifluoromethyl)benzylidene)semicarbazide (23g)

White solid (79.5%); m.p.: 235-237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.27 (s, 1H), 8.50 (d, J = 5.4 Hz, 1H), 8.04 (s, 1H), 8.01 (d, J = 1.5 Hz, 2H), 7.94 (dd, J_1 = 13.2 Hz, J_2 = 2.4 Hz, 1H), 7.63-7.66 (m, 1H), 7.55 (s, 1H), 7.39-7.45 (m, 4H), 6.47 (d, J = 5.1 Hz), 4.19 (t, J = 6.6 Hz, 2H), 3.96 (s, 3H), 2.44 (t, J = 7.2 Hz, 2H), 2.36 (s, 4H), 1.91-2.01 (m, 2H), 1.48-1.55 (m, 4H), 1.34-1.42 (m, 2H). MS (ESI) *m/z*: 656.5 $[M+H]^+$. Anal. calcd. for $C_{33}H_{33}F_4N_5O_4$ (%): C, 61.97; H, 5.20; N, 10.95. Found (%): C, 61.93; H, 5.23; N, 10.91.

6.12.8.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(2-chloro-4-fluorobenzylidene)-semicarbazide (23h)

White solid (84.7%); m.p.: 213-215 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1H), 9.33 (s, 1H), 8.45-8.49 (m, 2H), 8.34 (s, 1H), 7.88-7.92 (dd, J_1 = 13.5 Hz, J_2 = 2.1 Hz, 1H), 7.63-7.66 (m, 1H), 7.52-7.55 (m, 2H), 7.40-7.46 (m, 2H), 7.32-7.38 (m, 1H), 6.47 (d, J = 5.1 Hz, 1H), 4.19 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 2.43 (t, J = 6.9 Hz, 2H), 2.27-2.40 (br, 4H), 1.91-2.01 (m, 2H), 1.44-1.53 (m, 4H), 1.31-1.43 (m, 2H); MS (ESI) *m/z*: 624.2 $[M+H]^+$. Anal. calcd. for $C_{32}H_{32}ClF_2N_5O_4$ (%): C, 61.59; H, 5.17; N, 11.22. Found (%): C, 61.63; H, 5.20; N, 11.20.

6.12.9.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(2,4-dichlorobenzylidene)- semicarbazide (23i)

White solid (89.5%); m.p.: 204-206 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s, 1H), 9.33 (s, 1H), 8.49 (d, J = 5.4 Hz, 1H), 8.44 (d, J = 8.7 Hz, 1H), 8.34 (s, 1H), 7.87-7.92 (dd, J_1 = 13.5 Hz, J_2 = 2.4 Hz, 1H), 7.70 (d, J = 2.1 Hz, 1H), 7.62-7.65 (m, 1H), 7.54 (s, 1H), 7.50-7.54 (dd, J_1 = 8.7 Hz, J_2 = 2.1 Hz, 1H), 7.40-7.45 (m, 2H), 6.47 (d, J = 5.4 Hz, 1H), 4.19 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 2.44 (t, J = 6.9 Hz, 2H), 2.36 (s, 4H), 1.92-2.01 (m, 2H), 1.48-1.55 (m, 4H), 1.37-1.42 (m, 2H); MS (ESI) *m/z*: 640.3 $[M+H]^+$. Anal. calcd. for $C_{32}H_{32}Cl_2FN_5O_4$ (%): C, 60.00; H, 5.04; N, 10.93. Found (%): C, 60.03; H, 5.01; N, 10.95.

6.12.10.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-(trifluoromethoxy)benzylidene)semicarbazide (23j)

White solid (75.4%); m.p.: 239-241 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.05 (s, 1H), 9.31 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H), 8.00-8.05 (m, 3H), 7.89-7.94 (d, J = 13.5 Hz, 1H), 7.63-7.66 (m, 1H), 7.55 (s, 1H), 7.40-7.44 (m, 4H), 6.47 (d, J = 5.1 Hz, 1H), 4.19 (t, J = 6.0 Hz, 2H), 3.96 (s, 3H), 2.43-2.48 (m, 2H), 2.40 (s, 4H), 1.91-2.03 (m, 2H), 1,46-1.56 (m, 4H), 1.34-1.45 (m, 2H); MS (ESI) *m/z*: 656.2 $[M+H]^+$. Anal. calcd. for $C_{33}H_{33}F_4N_5O_5$ (%): C, 60.45; H, 5.07; N, 10.68. Found (%): C, 60.47; H, 5.03; N, 10.71.

6.12.11.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(2,4-dimethoxybenzylidene)semicarbazide (23k)

White solid (82.0%); m.p.: 227-228 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 1H), 9.74 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H), 8.24 (s, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.89-7.95 (dd, J_1 = 13.5 Hz, J_2 = 2.4 Hz, 1H), 7.63-7.66 (m, 1H), 7.54 (s, 1H), 7.36-7.42 (m, 2H), 6.59-6.62 (m, 2H), 6.46 (d, J = 5.1 Hz, 1H), 4.19 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 2.44 (t, J = 6.9 Hz, 2H), 2.37 (s, 4H), 1.92-2.01 (m, 2H), 1.48-1.55 (m,

4H), 1.35-1.45 (m, 2H); MS (ESI) *m/z*: 632.3 $[M+H]^+$. Anal. calcd. for $C_{34}H_{38}FN_5O_6$ (%): C, 64.65; H, 6.06; N, 11.09. Found (%): C, 64.65; H, 6.04; N, 11.11.

6.12.12.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-(dimethylamino)benzylidene)-semicarbazide (23l)

White solid (68.9%); m.p.: 226-229 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 9.11 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H), 7.91-7.96 (m, 1H), 7.86 (s, 1H), 7.61-7.68 (m, 3H), 7.54 (s, 1H), 7.36-7.42 (m, 2H), 6.75 (d, J = 8.7 Hz, 2H), 6.46 (d, J = 5.1 Hz, 1H), 4.19 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 2.97 (s, 6H), 2.43 (t, J = 6.9 Hz, 2H), 2.35 (s, 4H), 1.91-2.01 (m, 2H), 1.47-1.55 (m, 4H), 1.33-1.44 (m, 2H); MS (ESI) *m/z*: 615.2 $[M+H]^+$. Anal. calcd. for $C_{34}H_{39}FN_6O_4$ (%): C, 66.43; H, 6.40; N, 13.67.. Found (%): C, 66.45; H, 6.40; N, 13.63.

6.12.13.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-hydroxybenzylidene)semicarbazide (23m)

White solid (60.1%); m.p.: 201-203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H), 9.21 (s, 1H), 8.49 (d, J = 5.4 Hz, 1H), 7.90-7.95 (m, 2H), 7.62-7.69 (m, 3H), 7.55 (s, 1H), 7.36-7.42 (m, 2H), 6.86 (d, J = 8.7 Hz, 2H), 6.47 (d, J = 5.1 Hz, 1H), 4.20 (t, J = 6.0 Hz, 2H), 3.96 (s, 3H), 2.53-2.62 (br, 2H), 2.38-2.48 (br, 4H), 1.96-2.06 (m, 2H), 1.48-1.62(m, 4H), 1.34-1.45 (m, 2H); MS (ESI) *m/z*: 588.4 $[M+H]^+$. Anal. calcd. for $C_{32}H_{34}FN_5O_5$ (%): C, 65.40; H, 5.83; N, 11.92. Found (%): C, 65.36; H, 5.86; N, 11.93.

6.12.14.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(3-allyl-2-hydroxybenzylidene)semicarbazide (23n)

White solid (64.1%); m.p.: 197-198 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H), 10.44-10.79 (br, 1H), 9.63 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H), 8.26 (s, 1H), 7.80-7.85 (dd, J_1 = 13.5 Hz, J_2 = 2.1 Hz, 1H), 7.54 (s, 1H), 7.47-7.51 (m, 1H), 7.37-7.43 (m, 3H), 7.16 (d, J = 7.2 Hz, 1H), 6.88 (t, J = 7.5 Hz, 1H), 6.47 (d, J = 5.4 Hz, 1H), 5.93-6.06 (m, 1H), 5.02-5.10 (m, 2H), 4.19 (t, J = 5.7 Hz, 2H), 3.96 (s, 3H), 3.41 (d, J = 6.6 Hz, 2H), 2.45-2.49 (m, 2H), 2.41 (s, 4H), 1.94-2.03 (m, 2H), 1.49-1.56 (m, 4H), 1.34-1.45 (m, 2H); MS (ESI) *m/z*: 628.4 $[M+H]^+$. Anal. calcd. for $C_{35}H_{38}FN_5O_5$ (%): C, 66.97; H, 6.10; N, 11.16. Found (%): C, 66.95; H, 6.12; N, 11.14.

6.12.15.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(3-allyl-2-methoxybenzylidene)-semicarbazide (23o)

White solid (74.4%); m.p.: 213-216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H), 9.21 (s, 1H), 8.49 (d, J = 5.4 Hz, 1H), 8.28 (s, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.90-7.95 (dd, J_1 = 13.5 Hz, J_2 = 2.1 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.55 (s, 1H), 7.38-7.44 (m, 2H), 7.24-7.27 (m, 1H), 7.15-7.20 (m, 1H), 6.47 (d, J = 5.4 Hz, 1H), 5.93-6.06 (m, 1H), 5.07-5.12 (m, 2H), 4.19 (t, J = 6.6 Hz, 2H), 3.96 (s, 3H), 3.73 (s, 3H), 3.42 (d, J = 5.7 Hz, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.36 (s, 4H), 1.92-2.01 (m, 2H), 1.45-1.55 (m, 4H), 1.30-1.45 (m, 2H); MS (ESI) *m/z*: 642.7 $[M+H]^+$. Anal. calcd. for $C_{36}H_{40}FN_5O_5$ (%): C, 67.38; H, 6.28; N, 10.91. Found (%): C, 67.41; H, 6.30; N, 10.88.

6.12.16.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(pyridin-4-ylm

ethylene)semicarbazide (23p)

White solid (72.1%); m.p.: 211-212 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 6.0 Hz, 2H), 8.48 (d, J = 5.1 Hz, 1H), 8.03 (s, 1H), 7.84-7.90 (dd, J_1 = 13.5 Hz, J_2 = 2.1 Hz, 1H), 7.67 (d, J = 5.1 Hz, 2H), 7.54 (s, 1H), 7.47-7.52 (m, 1H), 7.34-7.40 (m, 2H), 6.46 (d, J = 5.1 Hz, 1H), 4.19 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 2.43 (t, J = 7.2 Hz, 2H), 2.35 (s, 4H), 1.92-2.01 (m, 2H), 1.48-1.55 (m, 4H), 1.33-1.43 (m, 2H); MS (ESI) *m/z*: 573.2 $[M+H]^+$. Anal. calcd. for $C_{31}H_{33}FN_6O_4$ (%): C, 65.02; H, 5.81; N, 14.68. Found (%): C, 65.00; H, 5.84; N, 14.65.

6.12.17.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-((1H-indol-2-yl)methylene)semicarbazide (23q)

White solid (62.6%); m.p.: 239-242 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.56 (s, 1H), 10.52 (s, 1H), 8.92 (s, 1H), 8.49 (d, J = 5.4 Hz, 1H), 8.24-8.27 (m, 1H), 8.22 (s, 1H), 7.91-7.97 (dd, J_1 = 13.2 Hz, J_2 = 2.1 Hz, 1H), 7.84 (d, J = 2.7 Hz, 1H), 7.60 (m, 1H), 7.55 (s, 1H), 7.39-7.46 (m, 3H), 7.15-7.24 (m, 2H), 6.48 (d, J = 5.4 Hz, 1H), 4.19 (t, J = 6.3 Hz, 2H), 3.97 (s, 3H), 2.44 (t, J = 7.2 Hz, 2H), 2.36 (s, 4H), 1.92-2.01 (m, 2H), 1.48-1.55 (m, 4H), 1.35-1.44 (m, 2H); MS (ESI) *m/z*: 611.6 $[M+H]^+$. Anal. calcd. for $C_{34}H_{35}FN_6O_4$ (%): C, 66.87; H, 5.78; N, 13.76. Found (%): C, 66.85; H, 5.81; N, 13.74.

6.12.18.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(thiophen-2-ylmethylene)semicarbazide (23r)

White solid (61.2%); m.p.: 189-191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 9.10 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H), 8.20 (s, 1H), 7.85-7.92 (dd, J_1 = 13.2 Hz, J_2 = 2.4 Hz, 1H), 7.66 (d, J = 5.1 Hz, 1H), 7.55-7.58 (m, 2H), 7.46 (m, 1H), 7.36-7.49 (m, 2H), 7.12-7.14 (m, 1H), 6.47 (d, J = 5.4 Hz, 1H), 4.21 (t, J = 6.3 Hz, 1H), 3.96 (s, 3H), 2.53-2.74 (br , 6H), 1.96-2.11 (m, 2H), 1.51-1.65 (m, 4H), 1.36-1.49 (m, 2H); MS (ESI) *m/z*: 578.2 $[M+H]^+$. Anal. calcd. for $C_{30}H_{32}FN_5O_4S$ (%): C, 62.38; H, 5.58; N, 12.12. Found (%): C, 62.36; H, 5.61; N, 12.10.

6.12.19.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-methoxybenzylidene)semicarbazide (23s)

White solid (82.1%); m.p.: 220-222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 9.13 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H), 7.91-7.96 (dd, J_1 = 13.5 Hz, J_2 = 2.1 Hz, 1H), 7.86 (s, 1H), 7.68 (d, J = 8.7 Hz, 3H), 7.56 (s, 1H), 7.36-7.42 (m, 2H), 6.75 (d, J = 8.7 Hz, 2H), 6.47 (d, J = 5.1 Hz, 1H), 4.19 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 3.81 (s, 3H), 2.87 (m, 2H), 2.45 (t, J = 6.6 Hz, 2H), 1.85-2.01 (m, 4H), 1.56-1.60 (m, 2H), 1.24-1.41 (m, 1H), 1.08-1.21 (m, 2H), 0.90 (d, J = 6.6 Hz, 3H); (ESI) *m/z*: 616.7 $[M+H]^+$. Anal. calcd. for $C_{34}H_{38}FN_5O_5$ (%): C, 66.33; H, 6.22; N, 11.37. Found (%): C, 66.35; H, 6.20; N, 11.35.

6.12.20.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-nitrobenzylidene)semicarbazide (23t)

Yellow solid (87.9%); m.p.: 204-207 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.26 (s, 1H), 9.39 (s, 1H), 8.50 (d, J = 5.1 Hz, 1H), 8.29 (d, J = 8.7 Hz, 2H), 8.17 (d, J = 8.7 Hz, 2H), 8.09 (s, 1H), 7.89-7.94 (dd, J_1 = 13.2 Hz, J_2 = 2.1 Hz, 1H), 7.64-7.67 (m, 1H), 7.55 (s, 1H), 7.40-7.47 (m, 2H), 6.47 (d, J = 5.4 Hz, 1H), 4.19 (t, J = 6.0 Hz, 2H), 3.96 (s, 3H), 2.78-3.02 (m, 2H), 2.53-2.61 (m, 2H), 1.97-2.01 (m, 4H), 1.57-1.61 (m, 2H), 1.28-1.45 (br,

1H), 1.05-1.22 (m, 2H), 0.90 (d, *J* = 6.3 Hz, 3H); MS (ESI) *m/z*: 631.8 $[M+H]^+$. Anal. calcd. for $C_{33}H_{35}FN_6O_6$ (%): C, 62.85; H, 5.59; N, 13.33. Found (%): C, 62.83; H, 5.59; N, 13.31.

6.12.21.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-(trifluoromethyl)benzylidene)semicarbazide (23u)

White solid (79.1%); m.p.: 241-243 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40-11.73 (br, 1H), 9.61-9.91 (br, 1H), 8.48 (d, *J* = 5.4 Hz, 1H), 8.08-8.14 (m, 3H), 7.91-7.96 (m, 1H), 7.73-7.81 (m, 2H), 7.63-7.67 (m, 1H), 7.59 (s, 1H), 7.38-7.47 (m, 2H), 6.48 (d, *J* = 5.1 Hz, 1H), 4.21 (t, *J* = 4.8 Hz, 2H), 3.97 (s, 3H), 2.98-3.05 (m, 2H), 2.61-2.65 (m, 2H), 1.92-2.09 (m, 4H), 1.60-1.65 (m, 2H), 1.32-1.49 (br, 1H), 1.20-1.31 (m, 2H), 0.91 (d, *J* = 6.3 Hz, 3H); (ESI) *m/z*: 654.5 $[M+H]^+$. Anal. calcd. for $C_{34}H_{35}F_4N_5O_4$ (%): C, 62.47; H, 5.40; N, 10.71. Found (%): C, 62.45; H, 5.42; N, 10.68.

6.12.22.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(2,4-dichlorobenzylidene)-semicarbazide (23v)

White solid (82.1%); m.p.: 213-215 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 9.33 (s, 1H), 8.49 (d, *J* = 5.4 Hz, 1H), 8.44 (d, *J* = 8.7 Hz, 1H), 8.34 (s, 1H), 7.87-7.92 (dd, J_1 = 13.5 Hz, J_2 = 2.4 Hz, 1H), 7.70 (d, *J* = 2.1 Hz, 1H), 7.62-7.65 (m, 1H), 7.54 (s, 1H), 7.50-7.53 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H), 7.39-7.45 (m, 2H), 6.47 (d, *J* = 5.1 Hz, 1H), 4.19 (t, *J* = 6.3 Hz, 2H), 3.96 (s, 3H), 2.87 (m, 2H), 2.45 (t, *J* = 6.6 Hz, 2H), 1.85-2.01 (m, 4H), 1.56-1.60 (m, 2H), 1.24-1.41 (m, 1H), 1.08-1.21 (m, 2H), 0.90 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.9, 154.7, 153.2, 152.4, 150.0, 149.3, 146.8, 138.7, 136.6, 135.5, 135.0, 133.8, 131.0, 129.6, 129.2, 128.1, 124.2, 117.1, 114.9, 109.0, 108.9, 102.4, 99.5, 67.2, 56.2, 55.2, 53.9 (2C), 34.4 (2C), 30.8 , 26.6, 22.3; MS (ESI) *m/z*: 654.4 $[M+H]^+$. Anal. calcd. for $C_{33}H_{34}Cl_2FN_5O_4$ (%): C, 60.55; H, 5.24; N, 10.70. Found (%): C, 60.53; H, 5.20; N, 10.72.

6.12.23.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(2-chloro-4-fluorobenzylidene)-semicarbazide (23w)

White solid (92.0%); m.p.: 218-219 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12(s, 1H), 9.30 (s, 1H), 8.44-8.49 (m, 2H), 8.34 (s, 1H), 7.87-7.93 (dd, J_1 = 13.5 Hz, J_2 = 2.4 Hz, 1H), 7.62-7.65 (m, 1H), 7.54 (s, 1H), 7.50-7.54 (m, 1H), 7.37-7.45 (m, 2H), 7.31-7.36 (m, 1H), 6.47 (d, *J* = 5.4 Hz, 1H), 4.19 (t, *J* = 6.3 Hz, 2H), 3.96 (s, 3H), 2.84-2.88 (m, 2H), 2.46 (t, *J* = 6.6 Hz, 2H), 1.86-2.00 (m, 4H), 1.55-1.60 (m, 2H), 1.24-1.41 (m, 1H), 1.08-1.21 (m, 2H), 0.90 (d, *J* = 6.6 Hz, 3H); MS (ESI) *m/z*: 638.4 $[M+H]^+$. Anal. calcd. for $C_{33}H_{34}ClF_2N_5O_4$ (%): C, 62.11; H, 5.37; N, 10.98. Found (%): C, 62.13; H, 5.33; N, 11.02.

6.13.

4-(4-(7-(3-(Piperidin-1-yl)propoxy)-6-methoxyquinolin-4-yloxy)-3-fluorophenyl)thiosemicarbazide (25)

Intermediate **17c** (5.00 g, 11.7 mmol) was dissolved in $CHCl_3$ (50 mL) and sat. aq. $NaHCO_3$ (50 mL) was added. The resulting biphasic solution was cooled to 0 °C and thiophosgene (2.03 g, 17.6 mmol) was then carefully added via syringe. After the addition was completed, the reaction was allowed to warm to room temperature and stirred for 6 h. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ 20 mL for twice. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford intermediate **24** (4.82 g, 87.2%) as a yellow oil which was used in the next step as soon as

possible without further purification.

To a solution of **24** (4.80 g, 10.2 mmol) in $CHCl_3$ (40 mL) was added 80% hydrazine monohydrate (40 mL), and the biphasic solution was vigorously stirred for 3 h at room temperature. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ 10 mL for twice. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to get a brown oil which was purified by silica gel column chromatography (eluent, CH_2Cl_2/ MeOH/ Et_3N = 100: 2: 1 to 100: 10: 1) to afford **25** (2.84 g) as a yellow solid, yield: 53.2%. m.p.: 105-109 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1H), 8.47 (d, J = 5.1 Hz, 1H), 7.80-7.86 (m, 1H), 7.58 (s, 1H), 7.52 (s, 1H), 7.40-7.49 (m, 1H), 7.40 (s, 1H), 7.31 (t, J = 9.0 Hz, 1H), 6.46 (d, J = 5.1 Hz, 1H), 4.32-4.48 (br, 2H), 4.19 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 2.39-2.47 (m, 6H), 1.91-2.03 (m, 2H), 1.43-1.57 (m, 4H), 1.31-1.43 (m, 2H). MS (ESI) *m/z*: 500.4 $[M+H]^+$.

6.14. General procedure for the synthesis of thiosemicarbazide (26a-d)

To a solution of **44** (0.200 g, 0.404 mmol) in isopropanol (2 mL), 1.1 equiv. of appropriate aldehyde and acetic acid (1 drop) were added, and the mixture was refluxed for 6-7 h until TLC showed the completion of the reaction. After cooling to 0 °C, the resultant precipitate was filtered and dried under vacuum.

6.14.1.
(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-benzylidene-thiosemicarbazide (26a)

Yellow solid (78.4%); m.p.: 121-123 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 10.27 (s, 1H), 8.53 (d, J = 5.4 Hz, 1H), 8.21 (s, 1H), 7.92-7.97 (m, 3H), 7.62-7.65 (m, 1H), 7.56 (s, 1H), 7.44-7.50 (m, 4H), 7.42 (s, 1H), 6.48 (d, J = 5.4 Hz, 1H), 4.21 (t, J = 6.3 Hz, 2H), 3.97 (s, 3H), 2.53-2.74 (m, 6H), 1.96-2.11 (m, 2H), 1.51-1.64 (m, 4H), 1.37-1.48 (m, 2H); MS (ESI) *m/z*: 588.6 $[M+H]^+$. Anal. calcd. for $C_{32}H_{34}FN_5O_3S$ (%): C, 65.40; H, 5.83; N, 11.92. Found (%): C, 65.38; H, 5.85; N, 11.88.

6.14.2.
(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(3,4-difluorobenzylidene)-thiosemicarbazide (26b)

Yellow solid (79.4%); m.p.: 137-138 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02-12.29 (br, 1H), 10.37 (s, 1H), 8.53 (d, J = 5.1 Hz, 1H), 8.16 (s, 1H), 7.91 (d, J = 12.6 Hz, 1H), 7.47-7.71 (m, 6H), 7.42 (s, 1H), 6.47 (d, J = 5.1 Hz, 1H), 4.21 (t, J = 6.0 Hz, 2H), 3.97 (s, 3H), 2.55-2.69 (m, 6H), 2.01-2.07 (m, 2H), 1.48-1.65 (m, 4H), 1.31-1.47 (m, 2H); MS (ESI) *m/z*: 624.2 $[M+H]^+$. Anal. calcd. for $C_{32}H_{32}F_3N_5O_3S$ (%): C, 61.62; H, 5.17; N, 11.23. Found (%): C, 61.64; H, 5.18; N, 11.20.

6.14.3.
(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(4-chlorobenzylidene)-thiosemicarbazide (26c)

Yellow solid (82.7%); m.p.: 128-130 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96-12.17 (br, 1H), 10.23-10.43 (br, 1H), 8.53 (d, J = 5.1 Hz, 1H), 8.18 (s, 1H), 7.99 (d, J = 8.4 Hz, 2H), 7.89-7.94 (dd, J_1 = 12.6 Hz, J_2 = 2.4 Hz, 1H), 7.58-7.62 (m, 1H), 7.46-7.55 (m, 4H), 7.41 (s, 1H), 6.47 (d, J = 5.4 Hz, 1H), 4.19 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 2.43 (t, J = 7.2 Hz, 2H), 2.27-2.39 (br, 4H), 1.92-2.01 (m, 2H), 1.45-1.58 (m, 2H), 1.34-1.45 (m, 2H); MS (ESI) *m/z*: 622.3 $[M+H]^+$. Anal. calcd. for $C_{32}H_{33}ClFN_5O_3S$ (%): C, 61.78; H, 5.35; N, 11.26. Found (%): C, 61.80; H, 5.32; N, 11.24.

6.14.4.

(*E*)-N^1-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)phenyl)-N^4-(2-chloro-4-fluorobenzylidene)-thiosemicarbazide (26d)

Yellow solid (77.3%); m.p.: 136-138 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12-12.29 (br, 1H), 10.39 (s, 1H), 8.51-8.59 (m, 3H), 7.88-7.92 (dd, J_1 = 12.6 Hz, J_2 = 1.8 Hz, 1H), 7.59-7.62 (m, 3H), 7.50 (t, J = 9.6 Hz, 1H), 7.41 (s, 1H), 7.33-7.39 (m, 1H), 6.47 (d, J = 5.1 Hz, 1H), 4.20 (t, J = 6.3 Hz, 2H), 3.97 (s, 3H), 2.45 (t, J = 7.2 Hz, 2H), 2.23-2.43 (br, 4H), 1.93-2.02 (m, 2H), 1.45-1.59 (m, 4H), 1.32-1.45 (m, 2H); MS (ESI) *m/z*: 640.2 $[M+H]^+$. Anal. calcd. for $C_{32}H_{32}ClF_2N_5O_3S$ (%): C, 60.04; H, 5.04; N, 10.94. Found (%): C, 60.07; H, 5.02; N, 10.98.

6.15. General procedure for the synthesis of semicarbazides (29a-d)

To the mixture of variant amines (50.0 mmol) and K_2CO_3 (13.8 g, 0.100 mol) in dry acetone (50 mL), phenyl chloroformate (75.0 mmol) was added dropwisely while maintaining the temperature between 0 and 5 °C. After the addition was completed, the mixture was warmed to room temperature for another 3 h, and the solvent was evaporated under reduced pressure. The residue was washed with water 50 mL for thrice, filtered, and dried under vacuum to afford corresponding carbamates **29a-d**.

A mixture of an appropriate carbamate **29a-d** (20.0 mmol) and 80% hydrazine hydrate (10 mL) in 1,4-dioxane (10 mL) was refluxed overnight with vigorous agitation. After cooling to room temperature, the resulting precipitate was filtered-off, washed with water, and dried under vacuum to afford corresponding semicarbazides **30a-d**.

6.15.1. *N*-Phenylhydrazinecarboxamide (29a)

White solid (77.6%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 7.52 (d, J = 7.5 Hz, 2H), 7.36 (s, 1H), 7.22 (t, J = 7.5 Hz, 2H), 6.91 (t, J = 7.5 Hz, 1H), 4.33 (s, 2H). MS (ESI) *m/z*: 152.2 $[M+H]^+$.

6.15.2. *N*-(3,4-Difluorophenyl)hydrazinecarboxamide (29b)

White solid (87.1%); MS (ESI) *m/z*: 188.0 $[M+H]^+$.

6.15.3. *N*-(4-Bromophenyl)hydrazinecarboxamide (29c)

White solid (86.9%); MS (ESI) *m/z*: 230.2, 232.1 $[M+H]^+$.

6.15.4. *N*-(2-Bromo-4-fluorophenyl)hydrazinecarboxamide (29d)

White solid (81.9%); MS (ESI) *m/z*: 248.1, 250.3 $[M+H]^+$.

6.16. 3-Fluoro-4-(6-methoxy-7-(3-(piperidin-1-yl)propoxy)-quinolin-4-yloxy)benzaldehyde (27)

A solution of 4-chloroquinoline (20.0 mmol) and 3-fluoro-4-hydroxybenzaldehyde (3.40 g, 24.0 mmol) in chlorobenzene (35 mL) was refluxed for 17 h. The reaction was considered complete when < 5% starting material remained. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure to yield a pale solid. The solid was dissolved in CH_2Cl_2 (60 mL), and washed with saturated K_2CO_3 aqueous solution 20 mL for twice, then water (20 mL), and dried over anhydrous Na_2SO_4, concentrated under reduced pressure to afford brown solid which was purified by silica gel column chromatography (eluent, CH_2Cl_2/MeOH/Et_3N = 100:2:1 to 100:5:1) to afford corresponding benzaldehydes **27**. White solid (41.9%); m.p.: 159-161 °C; MS (ESI) *m/z*: 439.3, 440.3 $[M+H]^+$.

6.17. General procedure for the synthesis of compounds (30a-d)

To a solution of **30a-d** (0.500 mmol) in isopropanol (3 mL), 1.1 equiv. of appropriate **15a–r** and one drop of acetic acid were added, and the mixture was refluxed for 4-6 h until TLC showed the completion of the reaction. After cooling to room temperature, the resultant precipitate was filtered and dried under vacuum to give the corresponding compounds.

6.17.1.
(*E*)-2-(3-Fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)benzylidene)-*N*-phenylsemicarbazide (30a)

White solid (84.3%); m.p.: 202-204 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 9.00 (s, 1H), 8.51 (d, J = 5.4 Hz, 1H), 8.20-8.24 (d, J = 11.7 Hz, 1H), 8.00 (s, 1H), 7.70-7.73 (m, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.47-7.53 (m, 2H), 7.42 (s, 1H), 7.31 (t, J = 8.1 Hz, 2H), 7.03 (t, J = 7.2 Hz, 1H), 6.53 (d, J = 5.4 Hz, 1H), 4.22 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 3.50-3.66 (br, 4H), 2.27-2.48 (m, 6H), 1.91-2.04 (m, 2H); MS (ESI) *m/z*: 572.4 $[M+H]^+$. Anal. calcd. for $C_{32}H_{34}FN_5O_4$ (%): C, 67.24; H, 6.00; N, 12.25. Found (%): C, 67.26; H, 6.03; N, 12.22.

6.17.2.
(*E*)-N^1-(3,4-Difluorophenyl)-N^4-(3-fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)benzylidene)semicarbazide (30b)

White solid (82.6%); m.p.: 219-221 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.21 (s, 1H), 8.94 (s, 1H), 8.52 (d, J = 5.1 Hz, 1H), 8.05 (s, 1H), 7.92-8.02 (m, 2H), 7.69-7.73 (m, 1H), 7.64-7.68 (dd, J_1 = 8.4, J_2 = 3.0 Hz, 1H), 7.49-7.54 (m, 2H), 7.45 (s, 1H), 7.26-7.33 (m, 1H), 6.57 (d, J = 5.4 Hz, 1H), 4.26 (t, J = 5.7 Hz, 2H), 3.96 (s, 3H), 2.75-3.22 (m, 6H), 2.15-2.31 (m, 2H), 1.67-1.83 (m, 4H), 1.42-1.60 (br, 2H); MS (ESI) *m/z*: 608.2 $[M+H]^+$. Anal. calcd. for $C_{32}H_{32}F_3N_5O_4$ (%): C, 63.25; H, 5.31; N, 11.53. Found (%): C, 63.22; H, 5.33; N, 11.51.

6.17.3.
(*E*)-N^1-(4-Bromophenyl)-N^4-(3-fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)benzylidene)semicarbazide (30c)

White solid (86.9%); m.p.: 193-196 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01 (s, 1H), 9.15 (s, 1H), 8.52 (d, J = 5.4 Hz, 1H), 8.19-8.23 (dd, J_1 = 12.3 Hz, J_2 = 1.5 Hz, 1H), 8.01 (s, 1H), 7.73 (m, 1H), 7.68 (d, J = 9.0 Hz, 1H), 7.45-7.55 (m, 5H), 6.54 (d, J = 5.1 Hz, 1H), 4.25 (t, J = 6.0 Hz, 2H), 3.96 (s, 3H), 2.75-3.06 (br, 6H), 2.13-2.23 (m, 2H), 1.64-1.79 (m, 4H), 1.44-1.55 (br, 2H); MS (ESI) *m/z*: 650.3 $[M+H]^+$. Anal. calcd. for $C_{32}H_{33}BrFN_5O_4$ (%): C, 59.08; H, 5.11; N, 10.77. Found (%): C, 59.06; H, 5.13; N, 10.73.

6.17.4.
(*E*)-N^1-(2-Bromo-4-fluorophenyl)-N^4-(3-fluoro-4-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinolin-4-yl)oxy)benzylidene)semicarbazide (30d)

White solid (86.6%); m.p.: 206-208 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 9.31 (s, 1H), 8.50 (d, J = 5.4 Hz, 1H), 8.45 (d, J = 6.3 Hz, 1H), 8.01 (s, 1H), 7.88-7.93 (dd, J_1 = 13.0 Hz, J_2 = 1.8 Hz, 1H), 7.63-7.66 (m, 1H), 7.55 (s, 1H), 7.51-7.54 (m, 1H), 7.39-7.49 (m, 2H), 7.32-7.38 (m, 1H), 6.47 (d, J = 5.4 Hz, 1H), 4.21 (t, J = 6.3 Hz, 2H), 3.96 (s, 3H), 3.58-3.61 (m, 4H), 2.44-2.48 (m, 2H), 2.33-2.44 (br, 4H), 1.95-2.03 (m, 2H); MS (ESI) *m/z*: 668.3 $[M+H]^+$. Anal. calcd. for $C_{32}H_{32}BrF_2N_5O_4$ (%): C, 57.49; H, 4.82; N, 10.48. Found (%): C, 57.51; H, 4.80; N, 10.51.

Acknowledgments

This work was supported by Liaoning Baiqianwan Talents Program (No. 2013921042), Program for Liaoning Excellent Talents in University (No. LR2014030) and the Special Scientific Research Fund of Shenyang Pharmaceutical University (No. ZCJJ2014401).

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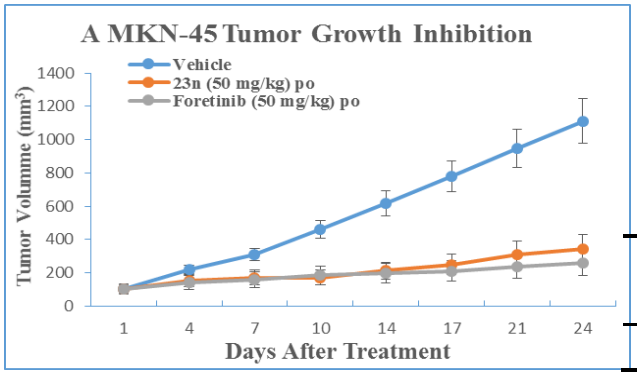
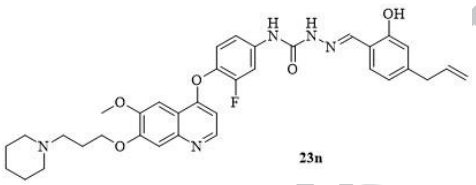
Graphical Abstract





Compd.	IC_{50} (nM)			
	A549	HT-29	MKN-45	c-Met
23n	11	27	39	1.54

Three series of novel 6, 7-disubstituted-4-phenoxyquinoline derivatives bearing semicarbazone scaffold were synthesized and evaluated for their cytotoxicity. Compound **23n** was further examined for its c-Met kinase activity *in vitro* as well as pharmacokinetic profiles and antitumor efficacy *in vivo*.