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Research paper

Design, synthesis and biological evaluation of novel 4-arylaminopyrimidine derivatives possessing a hydrazone moiety as dual inhibitors of L1196M ALK and ROS1

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ABSTRACT

A series of 4-arylaminopyrimidine derivatives possessing a hydrazone moiety were designed, synthesized and evaluated for their biological activity. Most compounds exhibited moderate to excellent cytotoxic activity against ALK-addicted KARPAS299 and ROS1-addicted HCC78, while also showing much less potent activity against A549, H460 and HT-29, whose growth were not dependent on ALK and/or ROS1, as compared with crizotinib and ceritinib. The most promising compound, **7b**, showed high antiproliferative effects on ALK-addicted KARPAS299 and ROS1-addicted HCC78 cell lines with IC_{50} of 20 nM and 28 nM, respectively, but showed no inhibitory activity against A549, H460 and HT-29. The enzymatic assay identified **7b** as a potent and selective ALK and ROS1 dual inhibitor with IC_{50} of 2.5 nM and 2.7 nM, respectively. It also exhibited good inhibitory activity against the L1196M ALK with an IC_{50} value of 67 nM.

1. Introduction

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK), structurally belonging to the insulin receptor superfamily. The precise physiological role of full-length wild-type ALK in mammals is enigmatic [1]. By contrast, the constitutively active ALK has been identified to be involved in the initiation and progression of various cancers, such as anaplastic large cell lymphoma (ALCL), inflammatory myofibroblastic tumor (IMT), diffuse large B-cell lymphoma (DLBCL), and non-small cell lung cancer (NSCLC), among others [2]. It is noteworthy that the echinoderm microtubule-associated protein-like-4(EML4)-ALK fusion gene has been identified to be the driving force in approximately 5% of NSCLC patients, making it a promising molecular target for the treatment of NSCLC [1]. Crizotinib, the first-generation ALK inhibitor, was approved by FDA in 2011 for the treatment of patients with locally advanced or metastatic ALK-positive NSCLC [3,4]. Despite the remarkable clinical benefit achieved by crizotinib, clinically acquired resistance remains a serious challenge. In this case, the leucine^{1196} → methionine^{1196} (L1196M), identified as the "gate-keeper" mutation, is most frequently detected [5]. Ceritinib and lectinib, two second-generation ALK inhibitors which could overcome crizotinib-resistant issues, were approved in 2014.

Proto-oncogene tyrosine-protein kinase ROS (ROS1) is one of the last two orphan RTKs and the sole member of the ROS1 RTK family [6]. Among human tissues, the highest level of *ROS1* is expressed in the lungs, and its normal functions have not yet been fully identified [7]. However, dysregulation of ROS1, as a result of *ROS1* gene fusion, has been identified as another clinically actionable oncogenic driver mutation in approximately 1.4% of NSCLCs [8–10]. Although rare, the evidence supports ROS1 fusions as a promising therapeutic target in a subset of NSCLC patients [11]. Currently, only a series of pyrazole derivatives have been developed by S. H. Lee as selective and potent ROS kinase inhibitors [12–14].

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ALK and ROS1 share a 49% amino acid sequence homology in the kinase domains and a 77% identity at the adenosine triphosphate (ATP)-binding site [15]. Given the high homology of ROS1 and ALK, some ALK inhibitors, including crizotinib, AP26113 and TAE-684, were tested efficacious against ROS1-positive cell lines and tumors [16–18]. In a phase I trial (NCT00585195), crizotinib demonstrated marked antitumor activity in patients with advanced NSCLC, harboring ROS1 rearrangements with a response rate of 57% and a disease control rate of 79% at 8 weeks [19]. The success of crizotinib as an ALK and ROS1 dual inhibitor validates the pursuit of novel L1196M ALK and ROS1 dual inhibitors.

Recently, 4-arylaminopyrimidine derivatives have become an important class of L1196M ALK inhibitors, such as ceritinib, A and NVP-TAE684 (Fig. 1) [20–22]. The co-crystal structure of the ALK catalytic domain in complex with ceritinib reveals that the Cl moiety of ceritinib can interact with the Met in L1196M. This interaction may make up for the loss of the contact between Cl and the Leu side chain in wild-type ALK [23]. From this molecular model and SAR analysis, ceritinib forms hydrogen bond interactions onto the backbone of Met_{1199} *via* the pyrimidine [11]. The isopropylsulfonyl group in the pyrimidin-2-yl aminobenzamide moiety significantly increased ALK potency and repositioned the selectivity from c-Met to ALK [21]. This structure analysis indicates that 4-(2-(isopropylsulfonyl)aryl)aminopyrimidine nuclei are critical for potent activity and selectivity.

The hydrazone moiety has been widely applied in drug design due to its ability to act as a hydrogen bond donator and acceptor. Additionally, this functional group can impart a degree of flexibility to a chemical structure [24,25]. The beneficial properties of hydrazones prompted us to insert this moiety into ceritinib in order to design L1196M ALK and ROS1 dual kinase inhibitors (Fig. 2). Several kinds of aromatic groups, indicated by Ar (**6a-e**), were introduced to identify a better scaffold. In addition, various substituents at the R_1 position (**6f-x**) were added to the terminal phenyl ring to explore the electronic and steric effects. Further modifications were performed by introducing methyl groups at the R_2 position (**7a-h**) of the hydrazone moiety.

In the current study, a series of 4-arylaminopyrimidine derivatives possessing a hydrazone moiety were designed and synthesized. Compounds were then assayed for their anti-proliferative activity *in vitro* against five cancer cell lines: KARPAS299, HCC78, A549, H460 and HT-29. Based on the results anti-proliferative results, eight compounds were selected for further *in vitro* enzymatic, inhibitory studies.

2. Chemistry

Compounds were designed by a synthetic route illustrated in Scheme 1. Substitution of commercially available 2-fluoronitrobenzene with isopropyl mercaptan provided intermediate **1**, which was oxidized by hydrogen peroxide to afford **2** as a white solid. Intermediate **2** was then subjected to reduction by hydrazine hydrate in the presence of activated carbon and ferric chloride in EtOH to yield **3**. A regioselective condensation of **3** with the 4-position of 2,4,5-trichloropyrimidine in the presence of NaH in DMF provided intermediate **4**, which was substituted with hydrazine hydrate to lead to the key intermediate **5**. **5** was condensed with an appropriate aromatic aldehyde or ketone to afford target compounds with good yields.

The chemical structures of the target compounds were confirmed by infrared (IR), nuclear magnetic resonance (NMR), mass spectrometry (MS) and elemental analysis. The configuration of the imino double bonds in the target compounds was determined by nuclear overhauser spectroscopy (NOESY). Taking compound **6f** as an example, a clear NOESY signal was observed between the proton of =N–N**H**– (δ 11.37 ppm, singlet) and the proton of –C**H**=N– (δ 8.14 ppm, singlet), which existed only in the *E* isomer due to the appropriate intramolecular H–H distance (see Supporting Information). Thus, the target compounds were confirmed as the *E* isomer.

3. Results and discussion

3.1. In vitro cytotoxic activities and structure-activity relationships

The MTT colorimetric assay was used to measure the cytotoxic activities of compounds **6a-x** and **7a-h** in KARPAS299, HCC78, A549, H460 and HT-29 cell lines. Since our goal was to discover novel dual inhibitors of ALK and ROS1, ALK and ROS1 inhibition were evaluated in a cellular context by measuring the proliferation of the KARPAS299 cell line expressing the NPM-ALK fusion protein [19] and the HCC78 cell line which expresses the SLC34A2-ROS1 fusion protein [14]. Tumor cell lines A549 (an EGFR-positive human NSCL cell line), H460 (a large cell lung cancer cell line) and HT-29 (a human colon cancer cell line), whose growth was not dependent on ALK and/or ROS1, were used to test the potential off-target effects. Crizotinib and ceritinib were used as the positive controls. These cytotoxic experiments performed in triplicate and are presented as IC_{50} values in Tables 1 and 2.

As a general trend, most of the compounds exhibited moderate to excellent cytotoxic activities against ALK-addicted KARPAS299 cells. Additionally, a similar trend was observed with the ROS1-addicted HCC78 cell lines. Furthermore, these compounds showed little potency against A549, H460 and HT-29 cells, which suggests that these compounds are selective for ALK and ROS1. The most promising compound, **7b**, displayed significant activity against KARPAS299 and HCC78, with respective IC_{50} values of 20 nM and IC_{50} = 28 nM. **7b** was approximately 2.4- and 11.1-fold more active than that of crizotinib (IC_{50} = 68 nM against KARPAS299, IC_{50} = 340 nM against HCC78), respectively, and was approximately as potent as ceritinib (IC_{50} = 27 nM against KARPAS299, IC_{50} = 18 nM against HCC78). Meanwhile, **7b** showed no inhibitory activity against A549, H460 and HT-29.

In order to preliminarily define a structure affinity relationship, a small set of compounds (**6a-e**) with different aryl groups were synthesized and evaluated for their cytotoxic activity. The results

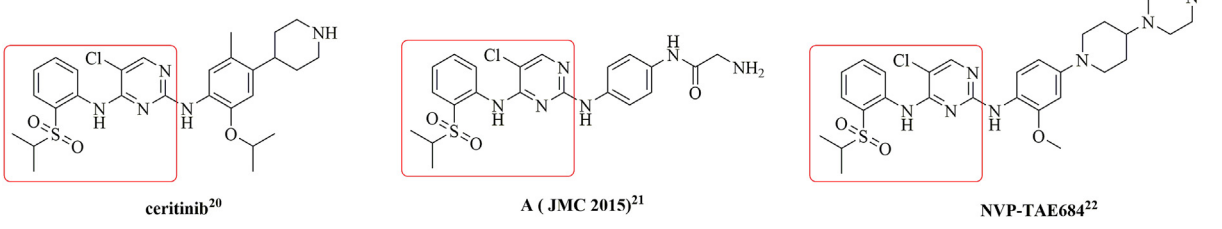


Fig. 1. Structures of representative L1196M ALK inhibitors containing 4-arylaminopyrimidine nuclei.

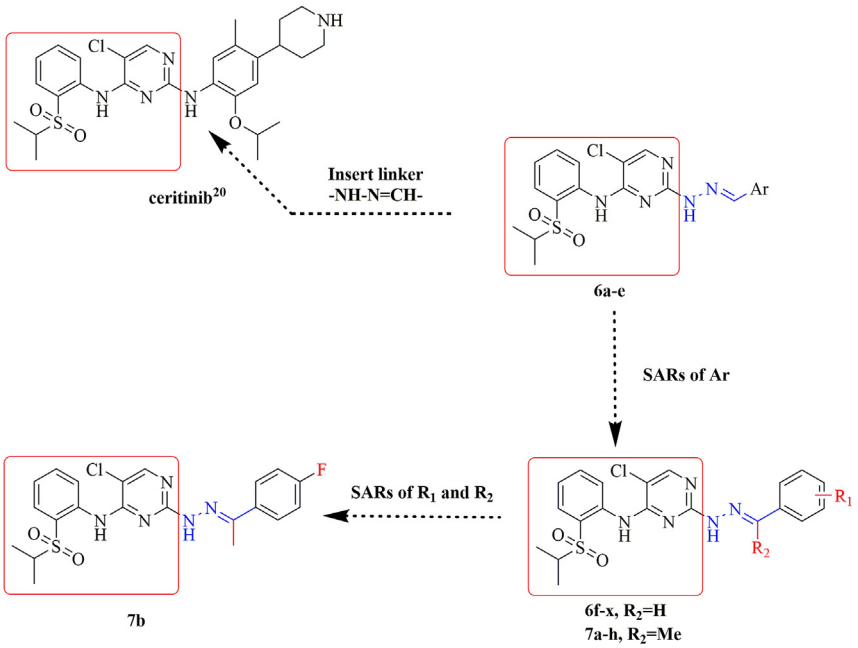
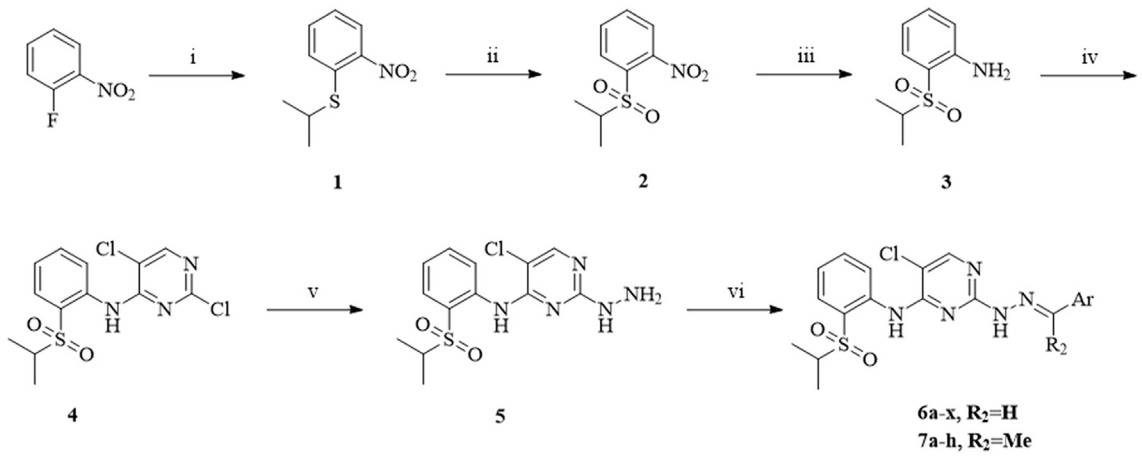
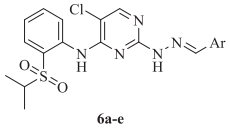


Fig. 2. Design strategies for the novel 4-arylaminopyrimidine derivatives possessing a hydrazone moiety.



Scheme 1. Reagents and conditions: (i) isopropyl mercaptan, K_2CO_3, DMF, 110 °C; (ii) H_2O_2, AcOH, 80 °C; (iii) hydrazine hydrate, C, $FeCl_3$, EtOH, reflux; (iv) 2,4,5-trichloropyrimidine, NaH, DMF, r.t; (v) hydrazine hydrate, EtOH, r.t; (vi) appropriate aromatic aldehyde or ketone, EtOH, reflux.

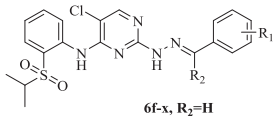
Table 1
Cytotoxicity of **6a**-**e** against KARPAS299, HCC78, A549, H460 and HT-29 cell lines *in vitro*.



Compd.	Ar	IC_{50}[a] (μmol/L) ± SD				
		KARPAS299	HCC78	A549	H460	HT-29
6a	Ph	0.25 ± 0.030	0.47 ± 0.060	1.23 ± 0.26	1.48 ± 0.19	1.32 ± 0.097
6b	Pyridin-4-yl	0.87 ± 0.10	0.82 ± 0.090	1.42 ± 0.16	1.13 ± 0.14	0.98 ± 0.082
6c	Pyridin-3-yl	0.93 ± 0.13	0.97 ± 0.12	1.15 ± 0.13	1.53 ± 0.20	0.83 ± 0.060
6d	Thiophen-2-yl	0.91 ± 0.15	0.88 ± 0.11	0.47 ± 0.031	0.57 ± 0.042	0.88 ± 0.092
6e	Furan-2-yl	0.75 ± 0.11	2.52 ± 0.20	1.94 ± 0.14	1.03 ± 0.089	3.10 ± 0.25

[a] Values are the means of at least three independent experiments.

Table 2
Cytotoxicity of **6f-e** and **7a-h** against KARPAS299, HCC78, A549, H460 and HT-29 cell lines *in vitro*.



6f-x, R_2=H
7a-h,R_2=Me

Compd.	R_1	IC_{50}[a] (μmol/L) ± SD				
		KARPAS299	HCC78	A549	H460	HT-29
6f	4-F	0.016 ± 0.0013	0.019 ± 0.0013	1.40 ± 0.16	1.30 ± 0.12	1.76 ± 0.28
6g	4-Cl	1.32 ± 0.11	1.70 ± 0.13	2.93 ± 0.30	2.90 ± 0.13	8.19 ± 0.69
6h	4-Br	1.44 ± 0.16	0.95 ± 0.080	1.31 ± 0.18	3.13 ± 0.28	2.30 ± 0.10
6i	4-NO_2	2.97 ± 0.23	1.98 ± 0.16	9.25 ± 0.40	>10	1.72 ± 0.26
6j	4-CH_3	1.62 ± 0.13	1.73 ± 0.13	4.42 ± 0.50	1.65 ± 0.18	>10
6k	4-CF_3	0.035 ± 0.0022	0.082 ± 0.0060	0.94 ± 0.038	>10	1.12 ± 0.22
6l	4-OCH_3	0.028 ± 0.0030	0.020 ± 0.0018	0.58 ± 0.050	1.32 ± 0.15	1.13 ± 0.25
6m	4-OCF_3	0.030 ± 0.0028	0.031 ± 0.0023	0.98 ± 0.048	1.20 ± 0.13	1.93 ± 0.23
6n	4-CN	0.34 ± 0.028	0.27 ± 0.019	2.51 ± 0.17	2.83 ± 0.20	2.96 ± 0.14
6o	4-SCH_3	0.28 ± 0.015	0.21 ± 0.035	0.88 ± 0.058	0.32 ± 0.058	0.98 ± 0.053
6p	4-SO_2CH_3	0.24 ± 0.026	0.31 ± 0.046	1.33 ± 0.10	2.02 ± 0.13	1.10 ± 0.050
6q	2-OCH_3	6.93 ± 0.58	4.16 ± 0.38	7.04 ± 0.83	>10	>10
6r	3-OCH_3	0.022 ± 0.0012	0.044 ± 0.0032	1.25 ± 0.25	2.23 ± 0.14	5.57 ± 0.29
6s	2-F	0.45 ± 0.034	0.36 ± 0.029	>10	1.23 ± 0.11	1.42 ± 0.21
6t	3-Br	1.40 ± 0.10	1.2 ± 0.15	1.09 ± 0.12	>10	1.03 ± 0.20
6u	2-Cl	5.62 ± 0.49	6.30 ± 0.28	7.90 ± 0.53	7.12 ± 0.69	>10
6v	3-CF_3	0.065 ± 0.0032	0.080 ± 0.0069	3.34 ± 0.28	>10	5.50 ± 0.45
6w	2,4-di-F	0.10 ± 0.014	0.11 ± 0.0093	2.28 ± 0.31	3.36 ± 0.34	>10
6x	3,4,5- tri-OCH_3	0.020 ± 0.0018	0.019 ± 0.0012	1.15 ± 0.21	1.23 ± 0.14	2.15 ± 0.19
7a	4-OCH_3	0.034 ± 0.0028	0.031 ± 0.0019	2.12 ± 0.17	2.50 ± 0.28	>10
7b	4-F	0.020 ± 0.0017	0.028 ± 0.0023	>10	>10	>10
7c	2,4-di-F	0.44 ± 0.029	0.67 ± 0.042	1.65 ± 0.18	>10	2.12 ± 0.22
7d	2-F	0.54 ± 0.038	0.60 ± 0.079	>10	7.15 ± 0.92	>10
7e	4-CH_3	2.23 ± 0.14	2.84 ± 0.14	>10	>10	>10
7f	4-Br	2.50 ± 0.34	2.38 ± 0.10	4.84 ± 0.53	>10	>10
7g	4-SCH_3	0.49 ± 0.039	0.85 ± 0.074	>10	>10	5.43 ± 0.48
7h	4-CN	0.27 ± 0.017	0.31 ± 0.048	>10	6.84 ± 0.37	>10
Ceritinib[b]		0.027 ± 0.0016	0.018 ± 0.0026	>10	>10	>10
Crizotinib[b]		0.068 ± 0.0039	0.34 ± 0.0018	>10	>10	>10

[a] Values are the means of at least three independent experiments.
[b] Used as positive control.

were summarized in Table 1. Compound **6a** exhibited good activity against KARPAS299 and HCC78, with IC_{50} values of 0.25 and 0.47 μM, respectively. Additionally, 6a demonstrated diminished potency against A549, H460 and HT-29 cells with IC_{50} values of >1 μM. These results indicate that compound **6a** could possibly act as a selective inhibitor. Compared with compound **6a** (Ar = Ph), incorporation of five or six-membered heterocyclic groups at the Ar position such as compounds **6b-e**, led to approximately 0.7- to 4.3-fold loss in potency against KARPAS299 and HCC78. Compound **6d** showed moderate activity against five cell lines, indicating that a general cytotoxicity might exist. The presence of an aryl ring at the Ar position plays a critical role in determining the potency and selectivity of these inhibitors. Accordingly, **6a** derivatives were synthesized in order to improve potency and selectivity.

6a derivatives with diverse R_1 groups were synthesized and examined for potency (Table 2). The SAR based on the IC_{50} values of **6f-x** showed that variations of R_1 groups have a marked impact on activity against cancer cells. The introduction of fluorine (**6f**, IC_{50} = 16 nM against KARPAS299, IC_{50} = 19 nM against HCC78) in the *para*-position of the aryl ring led to a 14.6-fold increase in potency against KARPAS299 and a 23.7-fold increase against HCC78 relative to **6a**. However, the replacement of fluorine with other halogens such as chlorine (**6g**) and bromine (**6h**) resulted in a decreased activity. The *para*-methyl analog (**6j**) turned out to be much less potent than **6a**, while the replacement of methyl group with trifluoromethyl moiety (**6k**) led to a 6.1-fold increase in cytotoxic activity against KARPAS299 and a 4.7-fold increase against HCC78 relative to **6a**. Interestingly, shifting the methoxy group (**6l**) to the trifluoromethoxy moiety (**6m**) did not substantially affect inhibitory activity. These results indicate that the variation instead of the electronic property of substitutions on the benzene ring is critical for cytotoxic activities, and the methoxy moiety and groups containing fluorine are favorable substitutions.

It can be noted from Table 2 that the *para*-methoxy derivative (**6l**) and the corresponding *meta*-substituted analog (**6r**) turned out to show approximately the potency against KARPAS299 and HCC78 cells. The same trend in potency was observed between the trifluoromethyl-substituted compounds **6k** and **6v**. However, moving the methoxy group to the *ortho*-position seen in **6q** led to an obvious decrease in cytotoxicity against KARPAS299 and HCC78 cells. The *ortho*-fluoro derivative **6s** provided moderate potency against KARPAS299 and HCC78 cancer cells relative to **6a**, but much less than the corresponding *para*-substituted analog **6f**. The loss of activity might be due to steric hindrance resulting in a steric clash within the hydrophobic pocket. These proved evidence that *para*- and *meta*-position are more favorable than *ortho*-position.

The compound **6x**, which possesses three favorable methoxys maintained significant activity against KARPAS299 and HCC78 cells but not significantly more so than the related monosubstituted analogs 6**l** and **6r**. This indicates that incorporating simultaneously favorable substitutions does not provide additive effects.

As a continuation of the SAR study, we prepared a series of derivatives (**7a**-**h**) by introducing a methyl moiety on the hydrazone moiety (Table 2). Although compounds **7a**-**h** exhibited less potent activities than the related analogs without methyl group, the 4-methoxy derivative, **7a**, and 4-fluorine derivative, **7b**, maintained significant activity against KARPAS299 and HCC78 cancer cells. It is noteworthy that the introduction of methyl group did lead to a loss in potency against A549, H460 and HT-29. By Comparing compound **6f** with **7b**, an obvious drop in potency against A549, H460 and HT-29 was observed when incorporating a methyl moiety on the hydrazone moiety. These results indicate that the introduction of a methyl group increases the selectivity for KARPAS299 and HCC78 over A549, H460 and HT-29.

3.2. In vitro enzymatic assays

Based on the cellular assays, eight compounds were selected for further *in vitro* ALK, L1196M ALK, ROS1, c-Met and EGFR inhibitory studies. The results summarized in Table 3 were expressed as IC_{50} values, which were derived from two independent experiments.

In parallel with the cellular results, all of the selected compounds displayed good to significant potency against ALK with IC_{50} values ranging from 2.3 to 6.2 nM. Likewise, the selected compounds showed significant potency against ROS1 with IC_{50} values of 1.8–5.8 nM. Among the compounds tested, the fluorine-substituted compounds **6f** and **7b** exhibited the most potent ALK inhibitory activity with an IC_{50} below 3 nM, which was 9-fold higher than crizotinib and was as potent as ceritinib. Meanwhile, compound **6f** is the most potent ROS1 inhibitor with a IC_{50} of 1.8 nM, which is even more potent than crizotinib and ceritinib. Notably, all of the selected compounds showed good inhibitory activity against the L1196M ALK with IC_{50} values ranging from 45 to 86 nM, which turned out to be 20-fold more potent than crizotinib (IC_{50} = 980 nM).

Comparing compound **7b** and **6f**, a loss of activity against c-Met and EGFR was observed, which indicated that the introduction of a methyl moiety on the hydrazone moiety did diminish inhibitory activity against c-Met and EGFR significantly.

This data revealed that 4-arylaminopyrimidine derivatives possessing hydrazone moiety are novel L1196M ALK and ROS1 dual inhibitors and the methyl group on the hydrazone might have afforded high selectivity over off-target kinases.

3.3. Binding mode analysis

To further elucidate the binding mode, molecular docking of the most potent compound **7b** was performed in the binding site cavity of ALK. Crystal structure of ALK (PDB ID: 4MKC) and **7b** were pretreated by Discovery Studio 3.5. All docking runs were utilizing LigandFit Dock protocol of Discovery Studio 3.5 systems. The image files were generated by the Accelrys DS visualizer 4.5 systems.

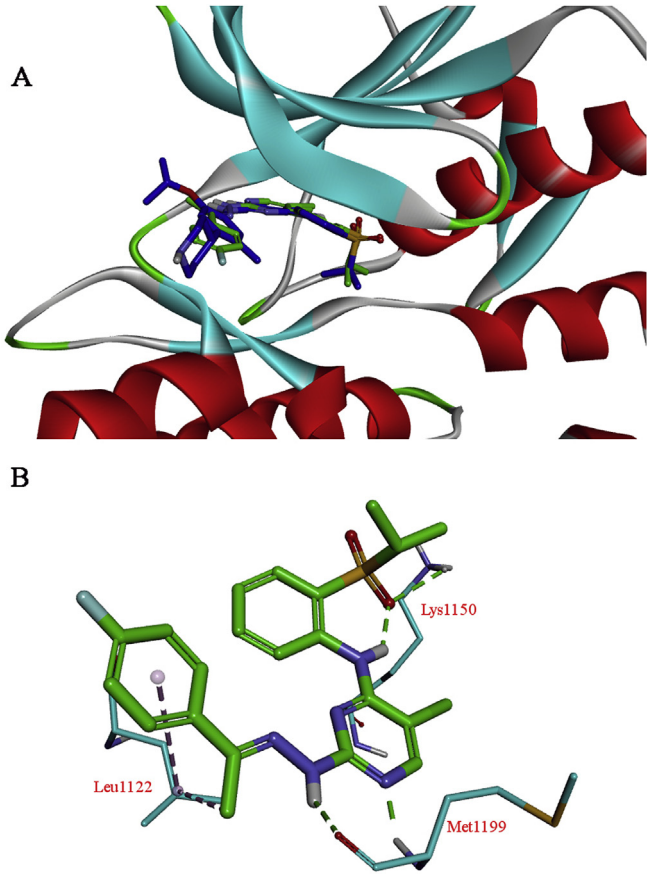


Fig. 3. Docking model of compound **7b** with ALK (PDB ID: 4MKC). A: a representation of the overlap of docking model of **7b** (green) with ceritinib (blue) in the binding site cavity of ALK. B: The binding mode of compound **7b** and ALK. The amino acid residues were displayed by sticks and the compound **7b** was displayed by green sticks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3(A) shows the binding model overlay of the binding model of compound **7b** with ceritinib. It suggests that they act in a very similar way with matching occupancy of the hydrophobic pocket. As shown in Fig. 3(B), compound **7b** binds well to ALK domain *via* three hydrogen bonds. The isopropylsulfonyl group of compound **7b** formed hydrogen-bonding interactions with residues Lys1150 in the catalytic region and the aminopyrimidine nuclei interacts with Met1199 *via* two hydrogen bonds. One π-alkyl interaction is formed between the benzene ring of **7b** and protein residues Leu1122 which may be helpful to improve its activity. One alkyl interaction is observed between the methyl on the hydrazone moiety of compound **7b** and Leu1122, which is a probable explanation for its selectivity.

Table 3
Enzymatic inhibition of **6f**, **6k**, **6l**, **6m**, **6r**, **6v**, **7a** and **7b** against ALK, L1196M ALK, ROS1, c-Met and EGFR *in vitro*.

Compd.	IC_{50}[a] (nmol/L)				
	ALK	L1196M ALK	ROS1	c-Met	EGFR
6f	2.3	45	1.8	220	570
6k	3.6	73	3.4	380	593
6l	4.8	82	2.3	157	587
6m	5.5	79	5.5	393	898
6r	6.2	86	5.8	376	601
6v	5.5	69	5.0	479	460
7a	4.9	80	4.7	>1000	>1000
7b	2.5	67	2.7	>1000	>1000
Ceritinib[b]	2.3	64	2.0	>1000	>1000
Crizotinib[b]	25	980	6.5	2.4	>1000

[a] Values are the means of at least two independent experiments.
[b] Used as positive control.

4. Conclusions

In summary, a series of 4-arylaminopyrimidine derivatives possessing a hydrazone moiety were designed, synthesized and evaluated for their biological activity. Most compounds exhibited moderate to excellent cytotoxic activity against ALK-addicted KARPAS299 and ROS1-addicted HCC78 cell lines. Additionally, they showed drastically less potent activity against A549, H460 and HT-29, whose growth was not dependent on ALK and/or ROS1, as

compared with crizotinib and ceritinib. An extensive, structured activity study was conducted. The presence of the phenyl ring in the aryl position is of significance for inhibition potency and selectivity. The variation instead of the electronic property of substitutions on the phenyl ring was critical for cytotoxic activities and *para*- and *meta*-positions of the aryl ring were more favorable than the *ortho*-position. The introduction of a methyl moiety on the hydrazone increased the selectivity for KARPAS299 and HCC78 over A549, H460 and HT-29. Moreover, the enzymatic assays identified these derivatives as L1196M ALK and ROS1 dual inhibitors. The methyl group on the hydrazine moiety made contributions to selectivity over off-target kinases.

Compound **7b** was identified as a potent and selective ALK and ROS1 dual inhibitor. It also exhibited good inhibitory activity against the L1196M ALK with an IC_{50} value of 67 nM. It showed high antiproliferative effects on ALK-addicted KARPAS299 and ROS1-addicted HCC78. Further pharmacological profile is on-going.

5. Experimental

5.1. Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC–MS (Agilent, Palo Alto, CA, USA). The ^{1}H (1D and 2D) and ^{13}C NMR were performed using Bruker ARX-400 or ARX-600 spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Column chromatography was run on silica gel (200–300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). Unless otherwise noted, all materials were obtained from commercially available sources and were used without further purification. The IR spectra were recorded by means of the KBr pellet technique on a Bruker FTS 135 spectrometer.

5.1.1. Isopropyl(2-nitrophenyl)sulfane (*1*)

To a solution of isopropyl mercaptan (83.9 g, 1.1 mol) in dry DMF (1000 mL) was added 2-fluoronitrobenzene (141.1 g, 1.0 mol) and K_2CO_3 (276.4 g, 2.0 mol). The reaction mixture was heated and stirred for 10 h at 110 °C. After cooling, the reaction mixture was poured into water and extracted with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield **1** as yellow oil in an 85% yield. MS (ESI) *m*/*z*(%): 198.1 $[M+H]^+$.

5.1.2. 1-(Isopropylsulfonyl)-2-nitrobenzene (*2*)

To a solution of **1** (49.3 g, 0.5 mol) in CH_3COOH (500 mL), 30% H_2O_2 (61 mL, 2.0 mol) was added dropwise while maintaining the temperature below 50 °C. After the addition was completed, the mixture was heated to 80 °C for another 8 h. After cooling, the reaction mixture was poured into water to give a light yellow precipitate. This was collected by filtration and air-dried to give **2** as a light yellow solid in an 89% yield. MS (ESI) *m*/*z*(%): 230.0 $[M+H]^+$.

5.1.3. 2-(Isopropylsulfonyl)aniline (*3*)

To a mixture of **1** (23.0 g, 0.1 mol), activated carbon (0.12 g, 0.01 mol) and $FeCl_3$ (4.9 g, 0.02 mol) in EtOH (230 mL) was added dropwise 80% $N_2H_4\cdot H_2O$ (36 mL, 0.6 mol) while maintaining the temperature below 60 °C. After the addition was completed, the mixture was heated to reflux for another 7 h. After completion of the reaction as indicated by TLC, the mixture was filtered immediately. The filtrate was concentrated under reduced pressure, and the residue was poured into water to give a white solid. The solid was collected by filtration and air-dried to give **3** in an 89% yield. MS (ESI) *m*/*z*(%): 200.0 $[M+H]^+$.

5.1.4. 2,5-Dichloro-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (*4*)

To the suspension of NaH (4.5 g, 0.12 mol) in DMF (160 mL), 2-(isopropylsulfonyl)aniline (16.0 g, 0.08 mol) was added at 0 °C. The mixture was stirred for 30 min at 0 °C, and then 2,4-dichloro-5-methylpyrimidine (29.3 g, 0.16 mol) diluted in DMF (6 mL)was added slowly. The mixture was warmed to room temperature and stirred overnight. The reaction mixture was poured into water to give a dark red precipitate which was collected by filtration. The crude product was directly crystallized from cold CH_3CN to afford **4** as a light yellow solid in a 59% yield. M.p.:152–154 °C; MS (ESI) *m*/*z*(%): 346.0 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.63 (d, J = 8.4 Hz, 1H), 8.30 (s, 1H), 7.92 (dd, J = 8.0, 1.5 Hz, 1H), 7.77–7.68 (m, 1H), 7.35–7.29 (m, 1H), 3.21 (hept, J = 6.9 Hz, 1H), 1.31 (d, J = 6.9 Hz, 6H).

5.1.5. 2,5-Dichloro-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (*5*)

To a solution of **4** (10.4 g, 0.03 mol) in EtOH (160 mL), 80% $N_2H_4\cdot H_2O$ (3.6 mL, 0.06 mol) was added dropwise. The mixture was stirred for 10 h at 25 °C, whereby a white solid was formed. This was collected by filtration and air-dried in an 86% yield. M.p.:144–145 °C; MS (ESI) *m*/*z* (%): 342.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 9.54 (s, 1H), 8.91 (d, J = 7.6 Hz, 1H), 8.30 (s, 1H), 8.16 (s, 1H), 7.82 (dd, J = 7.9, 1.4 Hz, 1H), 7.77–7.71 (m, 1H), 7.32 (t, J = 7.6 Hz, 1H), 4.23 (s, 2H), 3. 44 (hept, J = 6.8 Hz, 1H), 1.18 (d, J = 6.8 Hz, 6H).

5.1.6. General procedure for preparation of the target compounds (*6a-x, 7a-h*)

To a mixture of **5** (0.34 g, 1.0 mmol) in EtOH (5 mL) was added an appropriate aromatic aldehyde or ketone (1.2 mmol). The mixture was stirred for 5h at 60 °C. The resulting solid was collected by filtration and washed with EtOH to afford the target compounds **6a-x**, **7a-h**.

*5.1.6.1. (E)-2-(2-benzylidenehydrazinyl)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**6a**).* White solid; yield: 81%; M.p.: 237–239 °C; MS (ESI) *m*/*z* (%): 430.1 $[M+H]^+$; IR (KBr) cm^{-1}: 3427.5, 2919.7, 2850.6, 1623.8, 1572.5, 1544.2, 1456.4, 1384.3, 1320.0, 1263.0, 1124.6, 878.7, 619.1, 586.2; ^{1}H NMR (600 MHz, DMSO) δ 11.38 (s, 1H), 9.78 (s, 1H), 9.30 (s, 1H), 8.31 (s, 1H), 8.14 (s, 1H), 7.85–7.43 (m, 2H), 7.72 (d, J = 7.5 Hz, 2H), 7.48 (t, J = 7.5 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 3.51 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 158.04, 155.97, 155.40, 142.05, 139.02, 135.64, 135.40, 131.49, 129.53, 129.29 (2C), 126.71 (2C), 123.57 (2C), 122.97, 105.68, 55.48, 15.36 (2C). Anal. calcd. for $C_{20}H_{20}ClN_5O_2S$ (%): C, 55.88; H, 4.69; N, 16.29. Found (%): C, 55.85; H, 4.67; N, 16.25.

*5.1.6.2. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(pyridin-4-ylmethylene)hydrazinyl)pyrimidin-4-amine (**6b**).* White solid; yield: 85%; M.p.: 236–238 °C; MS (ESI) *m*/*z* (%): 431.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.69 (s, 1H), 9.80 (s, 1H), 9.21 (s, 1H), 8.65 (d, J = 5.6 Hz, 2H), 8.36 (s, 1H), 8.10 (s, 1H), 7.98–7.86 (m, 2H), 7.64 (d, J = 5.8 Hz, 2H), 7.40 (t, J = 7.6 Hz, 1H), 3.52 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 7H). Anal. calcd. for $C_{19}H19ClN_6O_2S$ (%):C, 52.96; H, 4.44; N, 19.50. Found (%): C, 52.90; H, 4.48; N, 19.53.

*5.1.6.3. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(pyridin-3-ylmethylene)hydrazinyl)pyrimidin-4-amine(**6c**).* White solid; yield: 84%; M.p.: 234–236 °C; MS (ESI) *m*/*z* (%): 431.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.55 (s, 1H), 9.78 (s, 1H), 9.24 (s, 1H), 8.87 (s, 1H), 8.57 (dd, J = 4.7, 1.4 Hz, 1H), 8.33 (s, 1H), 8.17 (s, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.88–7.82 (m, 2H), 7.50–7.53 (m, 1H), 7.40 (t,

J = 7.5 Hz, 1H), 3.51 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{19}H_{19}ClN_6O_2S$ (%): C, 52.96; H, 4.44; N, 19.50. Found (%): C, 52.98; H, 4.45; N, 19.54.

*5.1.6.4. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(thiophen-2-ylmethylene)hydrazinyl)pyrimidin-4-amine (**6d**).* White solid; yield: 57%; M.p.: 234–236 °C; MS (ESI) *m/z*(%): 436.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.37 (s, 1H), 9.82 (s, 1H), 9.37 (s, 1H), 8.32 (s, 1H), 8.30 (s, 1H), 7.88–7.82 (m, 2H), 7.61 (d, J = 5.0 Hz, 1H), 7.39–7.34 (m, 2H), 7.12–7.10 (m, 1H), 3.52 (hept, J = 6.8 Hz, 1H), 1.21 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{18}H_{18}ClN_5O_2S_2$ (%): C, 49.59; H, 4.16; N, 16.07. Found (%): C, 49.56; H, 4.18; N, 16.10.

*5.1.6.5. (E)-5-chloro-2-(2-(furan-2-ylmethylene)hydrazinyl)-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**6e**).* Grey solid; yield: 57%; M.p.: 217–218 °C; MS (ESI) *m/z* (%): 450.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.27 (s, 1H), 9.71 (s, 1H), 9.09 (s, 1H), 8.29 (s, 1H), 8.04 (s, 1H), 7.86–7.80 (m, 3H), 7.37 (t, J = 7.6 Hz, 1H), 6.79 (d, J = 3.1 Hz, 1H), 6.62 (s, 1H), 3.50 (hept, J = 6.7 Hz, 1H), 1.20 (d, J = 6.7 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 157.89, 155.93, 155.38, 150.74, 144.71, 138.86, 135.58, 132.65, 131.45, 123.70, 123.61, 123.17, 112.52, 111.27, 105.79, 55.44, 15.35 (2C). Anal. calcd. for $C_{18}H_{18}ClN_5O_3S$ (%): C, 51.49; H, 4.32; N, 16.68. Found (%): C, 51.51; H, 4.36; N, 16.65.

*5.1.6.6. (E)-5-chloro-2-(2-(4-fluorobenzylidene)hydrazinyl)-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**6f**).* White solid; yield: 74%; M.p.: 227–228 °C; MS (ESI) *m/z* (%): 448.1 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO) δ 11.38 (s, 1H), 9.78 (s, 1H), 9.26 (s, 1H), 8.31 (s, 1H), 8.14 (s, 1H), 7.87–7.86 (m, 2H), 7.77 (dd, J = 8.2, 5.8 Hz, 2H), 7.38 (t, J = 7.4 Hz, 1H), 7.33 (t, J = 8.6 Hz, 2H), 3.51 (hept, J = 6.8 Hz, 1H), 1.21 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 164.22, 161.78, 158.03, 155.95, 155.40, 140.90, 138.99, 135.50, 132.26, 132.24, 131.48, 128.70, 128.62, 123.58, 122.95, 116.44, 116.22, 105.69, 55.47, 15.35 (2C). Anal. calcd. for $C_{20}H_{19}ClFN_5O_2S$ (%): C, 53.63; H, 4.28; N, 15.64. Found (%): C, 53.65; H, 4.24; N, 15.67.

*5.1.6.7. (E)-5-chloro-2-(2-(4-chlorobenzylidene)hydrazinyl)-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**6g**).* White solid; yield: 77%; M.p.: 237–238 °C; MS (ESI) *m/z*(%): 464.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.43 (s, 1H), 9.78 (s, 1H), 9.24 (s, 1H), 8.31 (s, 1H), 8.12 (s, 1H), 7.89–7.85 (m, 2H), 7.73 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.38 (t, J = 7.6 Hz, 1H), 3.50 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{20}H_{19}Cl_2N_5O_2S$ (%): C, 51.73; H, 4.12; N, 15.08. Found (%): C, 51.74; H, 4.10; N, 15.10.

*5.1.6.8. (E)-2-(2-(4-bromobenzylidene)hydrazinyl)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**6h**).* White solid; yield: 70%; M.p.: 254–256 °C; MS (ESI) *m/z* (%): 508.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.46 (s, 1H), 9.78 (s, 1H), 9.25 (s, 1H), 8.32 (s, 1H), 8.10 (s, 1H), 7.90–7.86 (m, 2H), 7.71–7.65 (m, 4H), 7.39 (t, J = 7.3 Hz, 1H), 3.49 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 157.94, 155.95, 155.49, 140.71, 138.95, 135.58, 134.95, 132.28 (2C), 131.49, 128.48 (2C), 123.64, 123.55, 122.94, 122.56, 105.89, 55.47, 15.36 (2C). Anal. calcd. for $C_{20}H_{19}BrClN_5O_2S$ (%): C, 47.21; H, 3.76; N, 13.76. Found (%): C, 47.25; H, 3.73; N, 13.73.

*5.1.6.9. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(4-nitrobenzylidene)hydrazinyl)pyrimidin-4-amine (**6i**).* Yellow solid; yield: 69%; M.p.: 234–236 °C; MS (ESI) *m/z* (%): 475.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.74 (s, 1H), 9.80 (s, 1H), 9.21 (s, 1H), 8.36–8.34 m, 3H), 8.22 (s, 1H), 7.95 (d, J = 8.6 Hz, 3H), 7.87 (d, J = 7.8 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 3.52 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{20}H_{19}ClN_6O_4S$ (%):C, 50.58; H, 4.00; N, 17.70. Found (%): C, 50.55; H, 4.03; N, 17.68.

*5.1.6.10. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(4-methylbenzylidene))hydrazinyl)pyrimidin-4-amine (**6j**).* White solid; yield: 79%; M.p.: 238–239 °C; MS (ESI) *m/z* (%): 444.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.31 (s, 1H), 9.77 (s, 1H), 9.31 (s, 1H), 8.30 (s, 1H), 8.10 (s, 1H), 7.90–7.80 (m, 2H), 7.61 (d, J = 8.0 Hz, 2H), 7.39 (t, J = 7.6 Hz, 1H), 7.29 (d, J = 7.9 Hz, 2H), 3.51 (hept, J = 6.8 Hz, 1H), 2.35 (s, 3H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 158.06, 155.96, 155.38, 142.16, 139.19, 139.06, 135.40, 132.94, 131.48, 129.89 (2C), 126.68 (2C), 123.54 (2C), 122.94, 105.49, 55.49, 21.44, 15.36 (2C). Anal. calcd. for $C_{21}H_{22}ClN_5O_2S$ (%): C, 56.82; H, 5.00; N, 15.78. Found (%): C, 56.80; H, 5.03; N, 15.80.

*5.1.6.11. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(4-(trifluoromethyl)benzylidene)hydrazinyl)pyrimidin -4-amine (**6K**).* White solid; yield: 75%; M.p.: 249–251 °C; MS (ESI) *m/z* (%): 498.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 9.92 (s, 1H), 9.04 (d, J = 8.1 Hz, 1H), 8.66 (s, 1H), 8.23 (s, 1H), 7.96–7.92 (m, 2H), 7.86 (d, J = 8.1 Hz, 2H), 7.76–7.69 (m, 1H), 7.67 (d, J = 8.2 Hz, 2H), 7.30 (t, J = 7.8 Hz, 1H), 3.26 (hept, J = 6.9 Hz, 1H), 1.34 (d, J = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 157.77, 155.42 (2C), 140.21, 140.20, 139.24, 138.96, 135.08, 131.21, 129.90, 129.58, 126.80 (2C), 125.64, 125.60, 123.41, 123.01, 122.96, 122.66, 106.29, 55.52, 15.32 (2C). Anal. calcd. for $C_{21}H_{19}ClF_3N_5O_2S$ (%): C, 50.66; H, 3.85; N, 14.07. Found (%): C, 50.68; H, 3.84; N, 14.09.

*5.1.6.12. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(4-methoxybenzylidene)hydrazinyl)pyrimidin-4-amine (**6l**).* White solid; yield: 75%; M.p.: 225–227 °C; MS (ESI) *m/z* (%): 460.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.21 (s, 1H), 9.76 (s, 1H), 9.29 (s, 1H), 8.28 (s, 1H), 8.09 (s, 1H), 7.87–7.83 (m, 2H), 7.66 (d, J = 8.7 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 7.05 (d, J = 8.7 Hz, 2H), 3.82 (s, 3H), 3.50 (hept, J = 6.8 Hz, 1H), 1.21 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{21}H_{22}ClN_5O_3S$ (%): C, 54.84; H, 4.82; N, 15.23. Found (%): C, 54.86; H, 4.80; N, 15.25.

*5.1.6.13. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(4-(trifluoromethoxy)benzylidene)hydrazinyl)pyrimidi n-4-amine (**6m**).* White solid; yield: 85%; M.p.: 219–221 °C; MS (ESI) *m/z*(%): 514.1 $[M+H]^+$; IR (KBr) cm^{-1}: 3428.1, 2919.9, 2850.8, 1623.7, 1573.2, 1455.6, 1384.3, 1313.3, 1127.6, 773.3, 619.0, 587.3; ^{1}H NMR (400 MHz, DMSO) δ 11.48 (s, 1H), 9.78 (s, 1H), 9.25 (s, 1H), 8.32 (s, 1H), 8.16 (s, 1H), 7.93–7.80 (m, 4H), 7.49 (d, J = 8.2 Hz, 2H), 7.39 (t, J = 7.6 Hz, 1H), 3.51 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 157.95, 155.96, 155.43, 148.99, 140.40, 138.94, 135.60, 134.97, 131.49, 128.35 (2C), 123.64, 123.56, 122.94, 121.93 (2C), 105.95, 55.46, 15.35 (2C). Anal. calcd. for $C_{21}H_{19}ClF_3N_5O_3S$ (%): C, 49.08; H, 3.73; N, 13.63. Found (%): C, 49.10; H, 3.77; N, 13.65.

*5.1.6.14. (E)-4-((2-(5-chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)hydrazono)methyl)benzonitrile (**6n**).* White solid; yield: 85%; M.p.: 230–232 °C; MS (ESI) *m/z* (%): 455.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.66 (s, 1H), 9.80 (s, 1H), 9.22 (s, 1H), 8.34 (s, 1H), 8.16 (s, 1H), 7.94 (d, J = 8.2 Hz, 2H), 7.92–7.84 (m, 4H), 7.39 (t, J = 7.7 Hz, 1H), 3.50 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{21}H_{19}ClN_6O_2S$ (%): C, 55.44; H, 4.21; N, 18.47. Found (%):C, 55.43; H, 4.24; N, 18.44.

*5.1.6.15. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(4-(methylthio)benzylidene)hydrazinyl)pyrimidin-4- amine (**6o**).* White solid; yield: 84%; M.p.: 243–244 °C; MS (ESI) *m/z* (%): 476.1

$[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.33 (s, 1H), 9.77 (s, 1H), 9.29 (s, 1H), 8.30 (s, 1H), 8.09 (s, 1H), 7.86 (d, J = 7.7 Hz, 2H), 7.65 (d, J = 8.1 Hz, 2H), 7.40–7.35 (m, 3H), 3.51 (hept, J = 6.7 Hz, 1H), 2.53 (s, 3H), 1.20 (d, J = 6.7 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 158.01, 155.95, 155.38, 141.70, 140.04, 139.03, 135.52, 132.18, 131.48, 127.12 (2C), 126.33 (2C), 123.56, 123.48, 122.92, 105.55, 55.48, 15.36 (2C), 14.89. Anal. calcd. for $C_{21}H_{22}ClN_5O_2S_2$ (%): C, 52.99; H, 4.66; N, 14.71. Found (%): C, 53.00; H, 4.63; N, 14.74.

*5.1.6.16. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(4-(methylsulfonyl)benzylidene)hydrazinyl)pyrimidin- 4-amine (**6p**).* White solid; yield: 80%; M.p.: 275–277 °C; MS (ESI) m/z(%): 508.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.60 (s, 1H), 9.75 (s, 1H), 9.18 (s, 1H), 8.29 (s, 1H), 8.14 (s, 1H), 7.96–7.82 (m, 6H), 7.35 (s, 1H), 3.45 (s, 1H), 3.20 (s, 3H), 1.15 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 157.82, 155.96, 155.49, 140.83, 140.50, 139.95, 138.88, 135.59, 131.51, 128.07 (2C), 127.10 (2C), 123.73, 122.99, 106.39, 55.47, 44.02, 15.35 (2C). Anal. calcd. for $C_{21}H_{22}ClN_5O_4S_2$ (%): C, 49.65; H, 4.37; N, 13.79. Found (%):C, 49.63; H, 4.35; N, 13.76.

*5.1.6.17. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(2-methoxybenzylidene)hydrazinyl)pyrimidin-4-amine (**6q**).* White solid; yield: 78%; M.p.: 294–296 °C; MS (ESI) m/z (%): 460.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.35 (s, 1H), 9.77 (s, 1H), 9.30 (s, 1H), 8.48 (s, 1H), 8.29 (s, 1H), 7.95 (d, J = 7.1 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.81 (t, J = 7.9 Hz, 1H), 7.38 (t, J = 7.7 Hz, 2H), 7.14–7.04 (m, 2H), 3.86 (s, 3H), 3.51 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{21}H_{22}ClN_5O_3S$ (%): C, 54.84; H, 4.82; N, 15.23. Found (%): C, 54.82; H, 4.87; N, 15.25.

*5.1.6.18. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(3-methoxybenzylidene)hydrazinyl)pyrimidin-4-amine (**6r**).* White solid; yield: 84%; M.p.: 203–204 °C; MS (ESI) m/z (%): 460.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.39 (s, 1H), 9.76 (s, 1H), 9.24 (s, 1H), 8.31 (s, 1H), 8.10 (s, 1H), 7.93–7.76 (m, 2H), 7.39 (t, J = 7.0 Hz, 2H), 7.28 (d, J = 7.7 Hz, 2H), 6.97 (d, J = 8.0 Hz, 1H), 3.82 (s, 3H), 3.51 (hept, J = 6.6 Hz, 1H), 1.20 (d, J = 6.6 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 160.04, 158.01, 155.96, 155.43, 141.94, 138.99, 137.06, 135.31, 131.49, 130.37, 123.64, 123.56, 122.97, 119.49, 115.31, 111.54, 105.73, 55.65, 55.44, 15.36 (2C). Anal. calcd. for $C_{21}H_{22}ClN_5O_3S$ (%): C, 54.84; H, 4.82; N, 15.23. Found (%): C, 54.85; H, 4.84; N, 15.26.

*5.1.6.19. (E)-5-chloro-2-(2-(2-fluorobenzylidene)hydrazinyl)-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**6s**).* White solid; yield: 90%; M.p.: 245–247 °C; MS (ESI) m/z (%): 448.3 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.52 (s, 1H), 9.79 (s, 1H), 9.24 (s, 1H), 8.35 (s, 1H), 8.33 (s, 1H), 7.98 (t, J = 7.2 Hz, 1H), 7.87–7.82 (m, 2H), 7.48–7.36 (m, 3H), 7.31–7.26 (m, 1H), 3.51 (hept, J = 6.6 Hz, 1H), 1.20 (d, J = 6.6 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 161.94, 159.47, 157.88, 155.96, 155.44, 138.94, 135.41, 134.64, 131.49, 131.28, 131.20, 126.11, 125.37, 123.63, 123.21, 123.12, 123.00, 116.56, 116.36, 106.04, 55.47, 15.35 (2C). Anal. calcd. for $C_{20}H_{19}ClFN_5O_2S$ (%): C, 53.63; H, 4.28; N, 15.64. Found (%): C, 53.65; H, 4.26; N, 15.60.

*5.1.6.20. (E)-2-(2-(3-bromobenzylidene)hydrazinyl)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**6t**).* White solid; yield: 76%; M.p.: 217–218 °C; MS (ESI) m/z (%): 508.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.53 (s, 1H), 9.79 (s, 1H), 9.26 (s, 1H), 8.32 (s, 1H), 8.09 (s, 1H), 7.98 (s, 1H), 7.93–7.84 (m, 2H), 7.64 (d, J = 7.7 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.44–7.38 (m, 2H), 3.50 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 157.91, 155.97, 155.44, 140.01, 138.97, 138.16, 135.30, 131.95, 131.53, 131.47, 128.23, 126.31, 123.69, 123.59, 122.98, 122.72, 106.01, 55.47, 15.36 (2C). Anal. calcd. for $C_{20}H_{19}BrClN_5O_2S$ (%): C, 47.21; H, 3.76; N, 13.76. Found (%): C, 47.23; H, 3.74; N, 13.74.

*5.1.6.21. (E)-5-chloro-2-(2-(2-chlorobenzylidene)hydrazinyl)-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine (**6u**).* White solid; yield: 76%; M.p.: 244–246 °C; MS (ESI) m/z (%): 464.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.60 (s, 1H), 9.78 (s, 1H), 9.22 (s, 1H), 8.52 (s, 1H), 8.34 (s, 1H), 8.08 (d, J = 7.9 Hz, 1H), 7.91–7.78 (m, 2H), 7.52–7.48 (m, 2H), 7.43–7.37 (m, 2H), 3.50 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 157.84, 155.99, 155.45, 138.90, 137.84, 135.41, 132.83, 132.75, 131.51, 130.87, 130.38, 128.03, 126.53, 123.69 (2C), 123.03, 106.21, 55.46, 15.35 (2C). Anal. calcd. for $C_{20}H_{19}Cl_2N_5O_2S$ (%): C, 51.73; H, 4.12; N, 15.08. Found (%): C, 51.75; H, 4.10; N, 15.10.

*5.1.6.22. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(3-(trifluoromethyl)benzylidene)hydrazinyl)pyrimidin -4-amine (**6v**).* White solid; yield: 73%; M.p.: 217–219 °C; MS (ESI) m/z (%): 498.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.57 (s, 1H), 9.78 (s, 1H), 9.21 (s, 1H), 8.34 (s, 1H), 8.21 (s, 1H), 8.07 (s, 1H), 7.96 (d, J = 5.5 Hz, 1H), 7.87 (d, J = 7.4 Hz, 1H), 7.80–7.69 (m, 3H), 7.39 (t, J = 7.4 Hz, 1H), 3.50 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 157.90, 155.97, 155.49, 140.29, 138.91, 136.79, 135.06, 131.52, 130.91, 130.45, 130.22, 129.90, 125.97, 124.00, 123.57, 123.27, 122.98, 106.13, 55.42, 15.35 (2C). Anal. calcd. for $C_{21}H_{19}ClF_3N_5O_2S$ (%): C, 50.66; H, 3.85; N, 14.07. Found (%):C, 50.64; H, 3.87; N, 14.09.

*5.1.6.23. (E)-5-chloro-2-(2-(2,4-difluorobenzylidene)hydrazinyl)-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4- amine (**6w**).* White solid; yield: 77%; M.p.: 229–231 °C; MS (ESI) m/z (%): 466.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.51 (s, 1H), 9.78 (s, 1H), 9.22 (s, 1H), 8.32 (s, 1H), 8.28 (s, 1H), 8.02–7.96 (m, 1H), 7.87–7.83 (m, 2H), 7.43–7.25 (m, 3H), 3.50 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{20}H_{18}ClF_2N_5O_2S$ (%): C, 51.56; H, 3.89; N, 15.03. Found (%): C, 51.53; H, 3.86; N, 15.00.

*5.1.6.24. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)pyrimidin-4- amine (**6x**).* White solid; yield: 86%; M.p.: 227–229 °C; MS (ESI) m/z (%): 520.0 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 11.33 (s, 1H), 9.68 (s, 1H), 9.06 (s, 1H), 8.31 (s, 1H), 8.07 (s, 1H), 7.86 (dd, J = 7.9, 1.3 Hz, 1H), 7.76 (t, J = 7.9 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 6.98 (s, 2H), 3.84 (s, 6H), 3.70 (s, 3H), 3.51 (hept, J = 6.8 Hz, 1H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 157.99, 155.94, 155.49, 153.65 (2C), 142.48, 139.03, 138.86, 135.24, 131.48, 131.06, 123.93, 123.58, 123.07, 105.61, 104.11 (2C), 60.59, 56.40 (2C), 55.30, 15.35 (2C). Anal. calcd. for $C_{23}H_{26}ClN_5O_5S$ (%): C, 53.13; H, 5.04; N, 13.47. Found (%): C, 53.10; H, 5.07; N, 13.45.

*5.1.6.25. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)pyrimidin -4-amine (**7a**).* White solid; yield: 83%; M.p.: 80–83 °C; MS (ESI) m/z (%): 474.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 10.22 (s, 1H), 9.78 (s, 1H), 9.39 (d, J = 7.3 Hz, 1H), 8.30 (s, 1H), 7.86–7.82 (m, 3H), 7.79 (t, J = 7.9 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.02 (d, J = 8.8 Hz, 2H), 3.80 (s, 3H), 3.49 (hept, J = 6.8 Hz, 1H), 2.27 (s, 3H), 1.18 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 160.25, 158.89, 155.79, 155.35, 147.25, 139.14, 135.41, 131.66, 131.45, 127.53 (2C), 123.47, 123.23, 122.89, 114.19 (2C), 105.41, 55.73, 55.52, 15.36 (2C), 13.84. Anal. calcd. for $C_{22}H_{24}ClN_5O_3S$ (%): C, 55.75; H, 5.10; N, 14.78. Found (%): C, 55.77; H, 5.13; N, 14.75.

*5.1.6.26. (E)-5-chloro-2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4- amine (**7b**).* White solid; yield: 83%; M.p.: 196–198 °C; MS (ESI) m/z (%): 461.8 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 10.36 (s, 1H), 9.81 (s, 1H), 9.36 (d, J = 8.1 Hz, 1H), 8.34 (s, 1H), 7.96–7.93 (m, 2H), 7.86 (dd,

J = 7.9, 1.0 Hz, 1H), 7.82 (t, J = 7.9 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.33 (t, J = 8.8 Hz, 2H), 3.51 (hept, J = 6.8 Hz, 1H), 2.32 (s, 3H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 164.15, 161.71, 158.82, 155.82, 155.40, 146.19, 139.05, 135.70, 135.45, 131.46, 128.20, 128.12, 123.58, 123.36, 122.93, 115.79, 115.58, 105.78, 55.51, 15.36 (2C), 13.93. Anal. calcd. for $C_{21}H_{21}ClFN_5O_2S$ (%): C, 54.60; H, 4.58; N, 15.16. Found (%): C, 54.63; H, 4.55; N, 15.19.

*5.1.6.27. (E)-5-chloro-2-(2-(1-(2,4-difluorophenyl)ethylidene)hydrazinyl)-N-(2-(isopropylsulfonyl)phenyl)pyrimidi n-4-amine (**7c**).* White solid; yield: 86%; M.p.: 216–218 °C; MS (ESI) m/z (%): 480.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 10.41 (s, 1H), 9.81 (s, 1H), 9.28 (d, J = 8.4 Hz, 1H), 8.34 (s, 1H), 7.86–7.82 (m, 1H), 7.81–7.76 (m, 1H), 7.73 (t, J = 7.4 Hz, 1H), 7.39–7.31 (m, 2H), 7.27 (td, J = 8.5, 2.3 Hz, 1H), 3.49 (hept, J = 6.8 Hz, 1H), 2.33 (s, 3H), 1.19 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{21}H_{20}ClF_2N_5O_2S$ (%): C, 52.56; H, 4.20; N, 14.59. Found (%):C, 52.53; H, 4.23; N, 14.57.

*5.1.6.28. (E)-5-chloro-2-(2-(1-(2-fluorophenyl)ethylidene)hydrazinyl)-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4- amine (**7d**).* White solid; yield: 80%; M.p.: 187–189 °C; MS (ESI) m/z (%): 462.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 10.40 (s, 1H), 9.81 (s, 1H), 9.32 (d, J = 8.4 Hz, 1H), 8.34 (s, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.77 (t, J = 7.4 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 7.46 (dd, J = 13.0, 5.9 Hz, 1H), 7.39–7.25 (m, 3H), 3.50 (hept, J = 6.8 Hz, 1H), 2.34 (s, 3H), 1.20 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{21}H_{21}ClFN_5O_2S$ (%): C, 54.60; H, 4.58; N, 15.16. Found (%): C, 54.63; H, 4.55; N, 15.14.

*5.1.6.29. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(1-(p-tolyl)ethylidene)hydrazinyl)pyrimidin-4-amine (**7e**).* White solid; yield: 89%; M.p.: 172–173 °C; MS (ESI) m/z (%): 458.1 $[M+H]^+$; IR (KBr) cm^{-1}: 3429.5, 3363.5, 3314.7, 2919.9, 2850.8, 1611.9, 1570.4, 1526.2, 1505.2, 1450.0, 1410.9, 1384.6, 1318.8, 1286.2, 1259.4, 1125.1, 773.6, 741.3; ^{1}H NMR (400 MHz, DMSO) δ 10.26 (s, 1H), 9.79 (s, 1H), 9.40 (d, J = 7.9 Hz, 1H), 8.30 (s, 1H), 7.84–7.74 (m, 4H), 7.36 (t, J = 7.5 Hz, 1H), 7.26 (d, J = 8.1 Hz, 2H), 3.47 (hept, J = 6.8 Hz, 1H), 2.33 (s, 3H), 2.27 (s, 3H), 1.17 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 158.85, 155.79, 155.37, 147.14, 139.14, 138.52, 136.37, 135.29, 131.46, 129.39 (2C), 126.05 (2C), 123.45, 123.27, 122.90, 105.59, 55.51, 21.29, 15.36 (2C), 13.80. Anal. calcd. for $C_{22}H_{24}ClN_5O_2S$ (%): C, 57.70; H, 5.28; N, 15.29. Found (%): C, 57.72; H, 5.25; N, 15.27.

*5.1.6.30. (E)-2-(2-(1-(4-bromophenyl)ethylidene)hydrazinyl)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)pyrimidin- 4-amine (**7f**).* Grey solid; yield: 83%; M.p.: 214–215 °C; MS (ESI) m/z (%): 522.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 10.40 (s, 1H), 9.81 (s, 1H), 9.34 (d, J = 8.0 Hz, 1H), 8.34 (s, 1H), 7.88–7.80 (m, 4H), 7.68 (d, J = 8.6 Hz, 2H), 7.40 (t, J = 7.6 Hz, 1H), 3.50 (hept, J = 6.8 Hz, 1H), 2.31 (s, 6H), 1.20 (d, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 158.71, 155.81, 155.42, 145.86, 139.00, 138.34, 135.48, 131.72 (2C), 131.45, 128.04 (2C), 123.63, 123.37, 122.92, 122.40, 105.98, 55.50, 15.36 (2C), 13.71. Anal. calcd. for $C_{21}H_{21}BrClN_5O_2S$ (%): C, 48.24; H, 4.05; N, 13.39. Found (%):C, 48.27; H, 4.03; N, 13.37.

*5.1.6.31. (E)-5-chloro-N-(2-(isopropylsulfonyl)phenyl)-2-(2-(1-(4-(methylthio)phenyl)ethylidene)hydrazinyl) pyrimidin-4-amine (**7g**).* White solid; yield: 78%; M.p.: 212–214 °C; MS (ESI) m/z (%): 490.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 10.31 (s, 1H), 9.82 (s, 1H), 9.39 (d, J = 6.7 Hz, 1H), 8.33 (s, 1H), 7.87–7.80 (m, 4H), 7.41–7.34 (m, 3H), 3.49 (hept, J = 6.7 Hz, 1H), 2.54 (s, 3H), 2.30 (s, 3H), 1.20 (d, J = 6.7 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 158.78, 155.78, 155.38, 146.72, 139.38, 139.08, 135.63, 135.44, 131.45, 126.54 (2C), 125.97 (2C), 123.54, 123.27, 122.90, 105.69, 55.51, 15.36 (2C), 15.00, 13.73. Anal. calcd. for $C_{22}H_{24}ClN_5O_2S_2$ (%): C, 53.92; H, 4.94; N, 14.29. Found (%): C, 53.90; H, 4.93; N, 14.30.

*5.1.6.32. (E)-4-(1-(2-(5-chloro-4-((2-(isopropylsulfonyl)phenyl) amino)pyrimidin-2-yl)hydrazono)ethyl)benzonitrile (**7h**).* Grey solid; yield: 70%; M.p.: 285–287 °C; MS (ESI) m/z (%): 469.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ 10.62 (s, 1H), 9.85 (s, 1H), 9.33 (d, J = 8.5 Hz, 1H), 8.39 (s, 1H), 8.09 (d, J = 8.4 Hz, 2H), 7.90–7.87 (m, 2H), 7.88 (t, J = 6.7 Hz, 2H), 7.43 (t, J = 7.6 Hz, 1H), 3.52 (hept, J = 6.7 Hz, 1H), 2.37 (s, 3H), 1.22 (d, J = 6.8 Hz, 6H). Anal. calcd. for $C_{22}H_{21}ClN_6O_2S$ (%): C, 56.35; H, 4.51; N, 17.92. Found (%): C, 56.33; H, 4.54; N, 17.95.

5.2. Pharmacology

5.2.1. MTT assay in vitro

The antiproliferative activities of compounds **6a**-**x** and **7a**-**h** were evaluated against KARPAS299, HCC78, A549, H460 and HT-29 cell lines by the standard MTT assay *in vitro*, with crizotinib and ceritinib as the positive controls. 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 into each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The tested compounds at the indicated final concentrations were added to the culture medium and incubated for 72 h. Fresh MTT was added to each well at the terminal concentration of 5 μg/mL, and incubated with cells at 37 °C for 4 h. The formazan crystals in each well were dissolved in 100 μL DMSO, 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 average of three determinations and calculated relative to the vehicle (DMSO) control by the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

5.2.2. In vitro enzymatic assays

The *in vitro* enzymatic assays versus ALK, L1196M ALK, ROS1, c-Met and EGFR were evaluated by homogeneous time-resolved fluorescence (HTRF) assay.

In enzymatic assay, the solution of peptide substrates, ATP, appropriate kinase, and diluted compound was mixed with the kinase reaction buffer (50 mM HEPES, pH 7.5, 0.0015% Brij-35, 10 mM $MgCl_2$, 2 mM DTT), with blank DMSO solution as the negative control. The kinase reaction was initiated by the addition of tyrosine kinase proteins diluted in 39 μL of kinase reaction buffer solution and incubated at 28 °C for 60 min. And then add 25 μL of stop buffer (100 mM HEPES, pH = 7.5, 0.015% Brij-35, 0.2% Coating Reagent #3, 50 mM EDTA) to stop reaction. The plate was read by Caliper at 320 nm and 615 nm. IC_{50} values were calculated from the inhibition curves.

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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.2016.06.056.

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