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Pyrroformyl-containing 2,4-diaminopyrimidine derivatives as a new optimization strategy of ALK inhibitors combating mutations

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ABSTRACT

Aiming to identify new optimization strategy effective for ALK-mutations, two series of pyrroformyl-containing 2,4-diaminopyrimidine compounds (**11a-o**, **12a-o**) were designed, synthesized and evaluated for their anti-proliferative activities against three cancer cell lines *in vitro* by MTT assay. The biological evaluations on cellular assay resulted in discovery of compound **11k**, which performed considerable activity with IC_{50} value of 0.034 μM against H2228 cell. Meanwhile, **11k** exhibited outstanding enzymatic inhibitory potency with IC_{50} values of 1.9 nM and 3.1 nM against ALK^{WT} and ALK^{L1196M}, respectively, surpassing the reference ceritinib (IC_{50} = 2.4 nM and 7.6 nM). Ultimately, the binding mode of **11k** with ALK was established to explore the SARs. Overall, **11k** was considered as a promising ALK inhibitor for mutation treatment.

1. Introduction

In the receptor tyrosine kinase superfamily, anaplastic lymphoma kinase (ALK) as a membrane-bound receptor is part of insulin receptor (IR) protein.[1,2] There are nearly 30 fusion proteins of ALK have been identified as oncogenic drives in many types of cancer,[3–5] among which nucleophosmin (NPM)-ALK in anaplastic large cell lymphoma (ALCL) and echinoderm microtubule-associated protein-like-4 (EML4)-ALK in nonsmall-cell lung cancer (NSCLC) are the two common formalizations.[6–8] Blockading of different signaling cascades of ALK has been considered as an effective way to inhibit the amplification of ALK-positive cells.[9] Recently, a number of small-molecule inhibitors have been identified with the capacity to inhibit growth of the tumor associated to ALK,[10] including crizotinib,[11] ceritinib,[12] lorlatinib,[13] and so forth[14–16] (Fig. 1). Unfortunately, the success of crizotinib was overshadowed by the rapid development of drug resistance mutation arising from ALK targeted point mutations including G1269A, G1202R, L1196M, etc.[17–20] To combating mutations, urgent obligation compeled us to find a new optimization strategy of ALK inhibitors.

In light of the advantages of fragment optimization in discovering promising compounds, ceritinib was selected as lead owning to its potency for ALK.[21] The structure of ceritinib was divided into four parts, including **A** (isopropyl sulfonyl moiety), **B** (2,4-diaminopyrimidine core moiety), **C** (isopropoxy moiety), and **D** (hydrophilic 'tail'). The co-crystal structure of ceritinib with ALK protein revealed 2,4-diarylaminopyrimidine (DAAP) skeleton was the critical functional group, which was responsible for the key interactions with ALK residues (Fig. 2).[22] In the new design strategy, DAAP framework was reserved to prevent the off-target effect and retain intention of combating most mutations.

In previous study, we found that the substitute of isopropoxy with methoxy group on **C** moiety could give rise to a decent inhibitory for mutational ALK,[23–25] and thus methoxy was retained in this paper. Meanwhile, optimization toward isopropyl sulfonyl of **A** moiety have been investigated extensively, indicating that modifications on 'head' were tolerant.[24] In this situation, methyl sulfonamide and methylamine acyl instead of the isopropyl sulfonyl moiety of **A** were incorporated. Moreover, we believe that the introduction of motif ('linker') between **C** and **D** moiety could enhance the target protein affinity. Pyrroformyl group was regarded as a favorite linker for that it could generate additional interactions indicated in docking simulation, and thus exhibit better inhibitory potency. In addition, hydrophilic moiety of **D** ('tail') extended to solvent region, tolerating to further modification with various aliphatic amines.[24,25]

Herein, a variety of novel 2,4-diaminopyrimidine derivatives (**11a-o** and **12a-o**) bearing pyrroformyl were rationally designed and synthesized as depicted in Figs. 2 and 3. All compounds were evaluated for the anti-proliferative activity *in vitro* against three cell lines (H2228, Karpas299 and A549). Subsequently, four compounds were picked into further enzymatic assays on ALK^{WT} and ALK^{L1196M}. Finally, the possible binding mode of **11k** bearing hydroxyethylamine motif with ALK protein were established according to the corresponding co-crystal structure.

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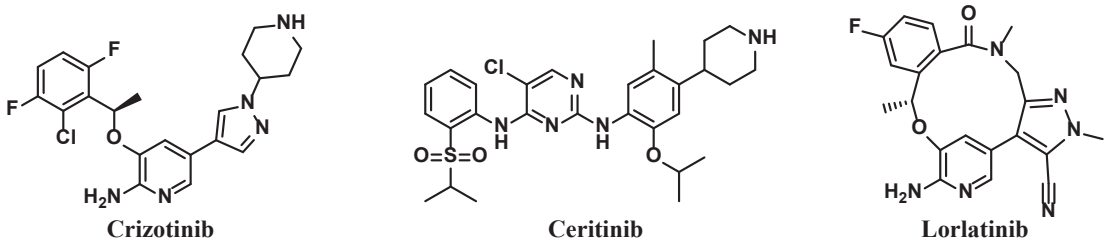


Fig. 1. Structures of marketed representative ALK inhibitors.

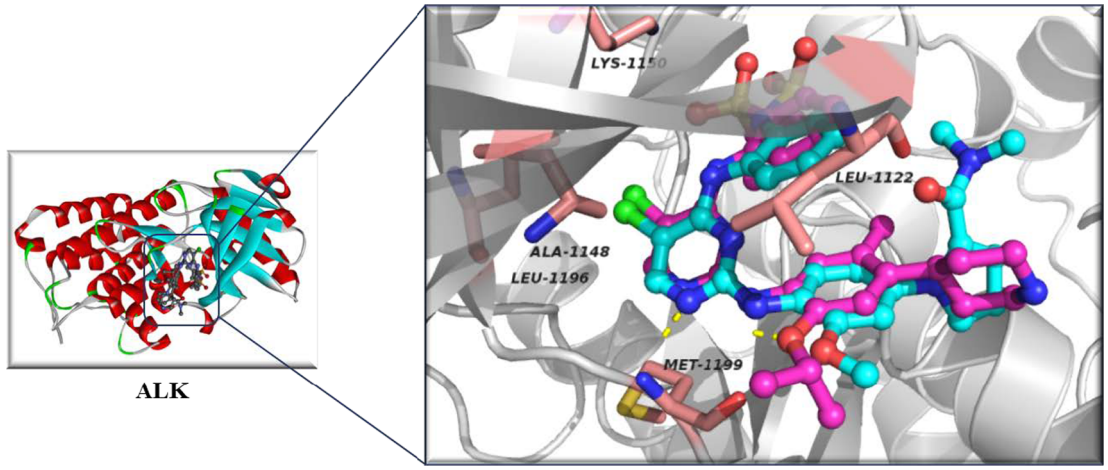


Fig. 2. Composite modes of ceritinib (pink sticks) and target compound (blue sticks) with ALK.

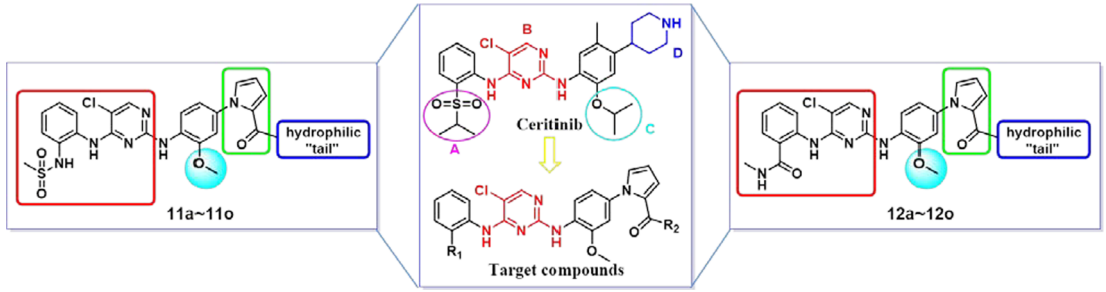


Fig. 3. Structure design strategies of **11a-o** and **12a-o**.

2. Results and discussion

2.1. Chemistry

The general methods for synthesizing compounds **11a-o** and **12a-o** are shown in Scheme 1. The intermediate **3** was readily prepared from commercially available 2,4,5-trichloropyrimidine (**1**) by treatment with benzene-1,2-diamine (**2**) in a 84.5% yield.[25] Acylation of **3** with methanesulfonyl chloride in the presence of pyridine at THF gave birth to *N*-(2-((2,5-dichloro-pyrimidin-4-yl)amino)phenyl)methanesulfonamide **4** in high yield. Isatin anhydride (**5**) reacted with methylamine in ethanol to provide intermediate **6**, which was alkylated with 2,4,5-trichloropyrimidine (**1**) in the company of *N*,*N*-diisopropylethylamine (DIPEA) at isopropanol to afford the key intermediate **7**.

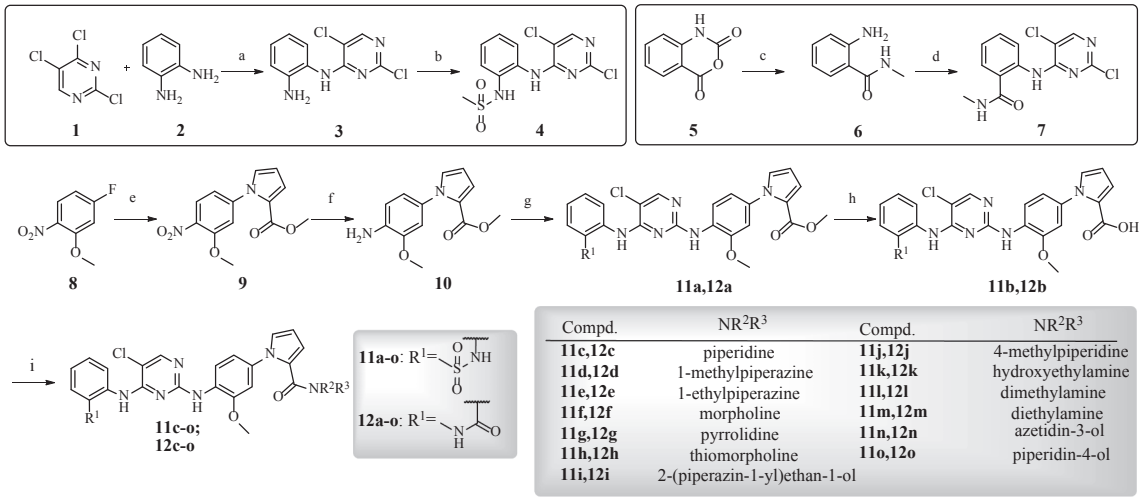
Treatment of 1*H*-pyrrole-2-carboxylate with K_2CO_3 followed by 4-fluoro-2-methoxy-1-nitrobenzene **8** gave intermediate **9** in 75.6% yield. Subsequently, the nitro group of **9** was reduced under activated iron powder in ethanol/water (9:1) to generate pivotal amide **10**. Nextly, *N*-alkylation of **10** with compound **4** or **7** provided **11a** and **12a**,[26] which converted to carboxylic derivatives **11b** and **12b** upon condition of hydrolysis in high yield. Both **11b** and **12b** were reacted with *N*,*N*-diisopropylethylamine (DIPEA) and 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) followed by addition of aliphatic amines to afford target compounds **11c-o** and **12c-o** in satisfactory yields.

2.2. Biological evaluation

2.2.1. Physical properties, in vitro anti-proliferative activity and SARs study

In this paper, a preliminary *in vitro* druggability evaluation[27] of synthesized compounds (**11a-o, 12a-o**) were carried out. Physical properties such as cLogP and topological polar surface area (TPSA) were calculated for each compounds using Molsoft (http://molsoft.com/mprop/), and data was presented in Table 1. As shown, the values of cLogP and TPSA were detected below 5 and 120, respectively, indicating majority of compounds possessing better druggability, which was beneficial for further pharmacological evaluation.

Subsequently, all target compounds (**11a-o**, **12a-o**) were screened for their anti-proliferative activity by the MTT-based assay using ceritinib as positive control. Two cancer cells including ALK-addicted H2228 (Human NSCL cell), Karpas299 (Human ALCL cell) expressing NPM-ALK were selected to tested cytotoxicity. Meanwhile, EGFR-



Compd.	NR^2R^3	Compd.	NR^2R^3
11c,12c	piperidine	**11j,12j**	4-methylpiperidine
11d,12d	1-methylpiperazine	**11k,12k**	hydroxyethylamine
11e,12e	1-ethylpiperazine	**11l,12l**	dimethylamine
11f,12f	morpholine	**11m,12m**	diethylamine
11g,12g	pyrrolidine	**11n,12n**	azetidin-3-ol
11h,12h	thiomorpholine	**11o,12o**	piperidin-4-ol
11i,12i	2-(piperazin-1-yl)ethan-1-ol		

Scheme 1. Reagents and conditions: (a) DIPEA, isopropanol, 80 °C; (b) methanesulfonyl chloride, pyridine, THF, rt.; (c) methylamine, ethanol, rt.; (d) 2,4,5-trichloropyrimidine, DIPEA, isopropanol, 80 °C; (e) methyl 1*H*-pyrrole-2-carboxylate, K_2CO_3, DMF, 110 °C; (f) iron powder, hydrochloric acid, ethanol/water (9:1), 80 °C; (g) **4**/**7**, isopropanol, 80 °C; (h) NaOH, methanol, 65 °C; (i) aliphatic amines, DIPEA, HATU, HOAT, DMF, rt.

positive A549 (Human ASCL cell) was employed to evaluated the potential off-target effects. The results were expressed as IC_{50} values outlined in Table 1.

As deposited in Table 1, all the synthesized compounds containing pyrroformyl group exhibited acceptable cytotoxic activities against ALK-addicted H2228 cell line with IC_{50} values below 1 μM. To our surprise, two compounds (**11k**, **11n**) displayed significant activities against H2228 with IC_{50} values of 0.034 μM and 0.044 μM, respectively. In the meantime, all compounds showed reduced activities against Karpas299 more and less than positive control. As expected, majority of compounds has poor performances in A549 cell line, demonstrating the on-target effects of these derivatives.

To determine influences of the **A** moiety, two different 'head' were introduced. As summarized in Table 1, the set of derivatives bearing methansulfonyl amino (**11a-o**) exhibited 1- to 5-fold improved potency against ALK-addicted H2228 than molecules bearing methylamino acyl (**12a-o**), except for the case that **D** moiety ('tail') is 1-methylpiperazine (**11d**). For Karpas299 cell line, better activities of most methansulfonyl amino compounds also were detected, in addition to chemical entities **11d**, **11e**, **11g** and **11i**. Compared to **12b**, **11b** bearing methansulfonyl amino with IC_{50} values of 0.064 μM and 0.072 μM against H2228 and Karpas299 cell lines, as was 1.3-fold and 2.4-fold ameliorated anti-proliferative activity. On the whole, introduction of methansulfonyl amino moiety would result in satisfactory activities on cytotoxicity.

Due to an occupation by **D** moiety, the solvent region was tolerated to further modification with groups possessing excellent hydrophilia property. Based on this perspective, introduction of versatile secondary amines, such as piperidine, 1-methylpiperazine and 1-ethylpiperazine were envisioned for the enhanced activity. Nevertheless, descents on the anti-proliferation (**11c-e**) were detected along with magnification in size of secondary amines. As an example, compound **11e** bearing 1-ethylpiperazine showed comparative low activity (beyond 1 μM), indicated that the negative effects of overlarge hydrophilic tails on cytotoxicity. Likewise, the derivatives bearing 2-(piperazin-1-yl)ethan-1-ol in **11i** and 4-methylpiperidine in **11j** displayed comparable activities (IC_{50} ~ 1 μM) against H2228 and Karpas299 cell lines. Subsequently, morpholine and thiomorpholine were utilized to explore the impact of 4-position atom. Delightly, compared with **11c**, **11f** and **11h** showed improved anti-proliferative effects with IC_{50} values of 0.61 μM and 0.70 μM toward ALK-addicted H2228, 0.64 μM and 0.93 μM toward Karpas299, respectively, demonstrated oxygen and sulfur atom of 4-position exert positive effect on inhibited activity.

As consequence, a set of tiny secondary amino groups were introduced, such as hydroxyethylamine, dimethylamine and diethylamine, which obtained **11k-m**. An obvious improvement in the anti-proliferative activities was detected, especially **11k** with IC_{50} values of 0.034 μM, 0.045 μM and beyond 10 μM against H2228, Karpas299 and A549, respectively, which were comparable to or better than the positive control. Meanwhile, **11l** and **11m** exhibited mild descend on activities for tested cell lines than chemistry entity **11k**, might due to the absence of electro negativity of terminal fragment. As for **11n**, embedment of azetidin-3-ol gave rise to a 1.1-fold increase in cytotoxicity against H2228 cell line. Consistent with the assumption, the derivative **11o** exerted almost the same activity with **11f** and **11h** for their similarities.

With **12k** as an exception, the series of compounds bearing methylamino acyl (**12a-o**) showed depressed potency, while **12k** containing hydroxyethylamine displayed exceptional promissing cytotoxicity with IC_{50} values of 0.043 μM, 0.79 μM and beyond 10 μM against tested three cancer cell lines, respectively. Apart from individual compounds, such as **11d**, **12d** and **12i**, the pharmacological data of all target compounds supported the view that the size of hydrophilia group located in D moiety was a crucial part in anti-proliferative activity against ALK-addicted H2228 and Karpas299 cell lines.

2.2.2. *In vitro enzymatic assays*

To further confirm inhibitions of preferential compounds for wild-type ALK and secondary mutation (ALK^{L1196M}), four representative compounds (**11e**, **11h**, **11k** and **12k**) were evaluated on enzymatic assay *in vitro* with ceritinib as positive control. The results were outlined in Table 2.

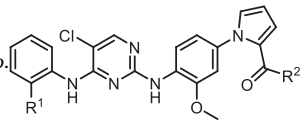
The results of enzymatic assay are consistent with the data in cellular assay mostly. As listed in Table 2, notably, compound **11k** exhibited outstanding potency on the tested two kinases with IC_{50} value of 1.9 nM and 3.1 nM, which was almost 1.3- to 2.5-fold more active relative to ceritinib. Given the fact that derivative **12k** showed limited inhibitions on ALK^{WT} (IC_{50} = 9.6 nM) and ALK^{L1196M} (IC_{50} = 13.4 nM), methylamino acyl was identified as an unfavorable motif to enzymatic inhibitory. In addition, derivative **11e** bearing bulky 1-ethylpiperazine was observed a loss in activity against ALK^{WT} (IC_{50} = 8.5 nM) and ALK^{L1196M} (IC_{50} = 11.7 nM), indicated appropriate size of **D** moiety play a vital role in activity.

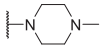
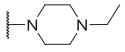
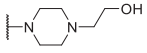
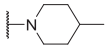
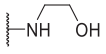
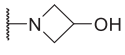
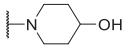
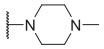
2.2.3. *Molecular docking studies*

To elucidate the binding mode, **11e** and **11k** were selected to perform molecular docking with co-crystal structure of ALK^{WT} and

Table 1

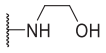
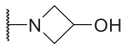
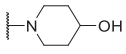
Physical properties, cytotoxicities of **11a-o** and **12a-o** against H2228, Karpas299 and A549 cell lines *in vitro*.



Compd.	R^1	R^2	IC_{50} [a] (μM) ± SD [b]			physical properties	
			H2228	Karpas299	A549	cLogP [c]	TPSA(A^2) [d]
11a			0.95 ± 0.03	0.79 ± 0.41	2.51 ± 0.03	4.39	109.56
11b			0.064 ± 0.07	0.072 ± 0.09	1.06 ± 0.19	4.09	116.79
11c			0.63 ± 0.17	1.04 ± 0.13	> 10	5.07	105.03
11d			1.05 ± 0.09	0.97 ± 0.08	> 10	3.87	108.51
11e			1.13 ± 0.30	1.41 ± 0.07	> 10	4.12	108.57
11f			0.61 ± 0.09	0.64 ± 0.021	1.77 ± 0.30	3.90	112.94
11g			0.60 ± 0.10	0.87 ± 0.013	4.58 ± 0.004	4.75	105.40
11h			0.70 ± 0.09	0.93 ± 0.30	1.47 ± 0.31	4.50	105.03
11i			1.08 ± 0.21	1.25 ± 0.011	> 10	3.38	120.48
11j			1.05 ± 0.05	1.37 ± 0.07	2.20 ± 0.014	5.05	105.03
11k			0.034 ± 0.013	0.045 ± 0.01	> 10	3.50	120.56
11l			0.054 ± 0.011	0.078 ± 0.09	> 10	3.65	104.38
11m			0.087 ± 0.21	0.105 ± 0.05	0.97 ± 0.09	4.67	104.31
11n			0.044 ± 0.04	0.081 ± 0.02	2.08 ± 0.16	3.45	120.95
11o			0.66 ± 0.41	0.97 ± 0.17	> 10	3.68	120.75
12a			0.97 ± 0.21	0.89 ± 0.09	8.51 ± 0.21	4.49	93.22
12b			0.087 ± 0.41	0.17 ± 0.13	2.49 ± 0.08	4.19	100.46
12c			0.99 ± 0.05	1.61 ± 0.07	> 10	5.17	88.70
12d			0.98 ± 0.03	0.09 ± 0.04	> 10	3.97	92.17
12e			1.27 ± 0.19	0.94 ± 0.01	1.57 ± 0.30	4.22	92.23
12f			0.84 ± 0.04	1.03 ± 0.09	2.21 ± 0.05	3.99	96.61
12g			0.77 ± 0.13	0.84 ± 0.21	> 10	4.85	89.06
12h			1.04 ± 0.17	1.14 ± 0.21	2.18 ± 0.13	4.60	88.70
12i			1.41 ± 0.09	0.43 ± 0.03	> 10	3.48	109.14
12j			1.07 ± 0.0.01	2.31 ± 0.06	> 10	5.05	88.70

(*continued on next page*)

Table 1 (*continued*)

Compd.	R^1	R^2	IC_{50} [a] (μM) ± SD [b]			physical properties	
			H2228	Karpas299	A549	cLogP [c]	TPSA(A^2) [d]
12k			0.043 ± 0.01	0.79 ± 0.21	> 10	3.59	113.23
12l			0.089 ± 0.03	1.07 ± 0.17	4.12 ± 0.31	3.75	88.05
12m			0.54 ± 0.05	0.27 ± 0.09	3.49 ± 0.05	4.77	87.98
12n			0.044 ± 0.04	0.74 ± 0.09	4.13 ± 0.01	3.54	104.62
12o			1.07 ± 0.02	0.97 ± 0.011	> 10	3.78	104.42
ceritinib			0.039 ± 0.02	0.041 ± 0.30	> 10	3.84	86.08

IC_{50} [a]: Values are the means of at least three independent experiments.
SD [b]: Standard deviation.
cLogP [c]: Measured between 1-octanol/water phosphate buffered.
TPSA[d]: Defined the surface sum over all polar atoms.

Table 2
Enzymatic inhibition of several compounds against ALK^{WT} and ALK^{L1196M} *in vitro*.

Compd.	IC_{50} [a] (nM)	
	ALK^{WT}	ALK^{L1196M}
11e	8.5	11.7
11h	4.5	8.3
11k	1.9	3.1
12k	9.6	13.4
ceritinib	2.4	7.6

IC_{50} [a]: Values are the means of at least two independent experiments.

ALK^{L1196M}, which was obtained from the Protein Date Bank (PDB code: 4MKC). Meanwhile, the binding configurations were analyzed using Discovery Studio 3.0. The predicted binding mode was shown in Fig. 4.

Obviously, **11k** occupied the same kinase domain with ceritinib, and exerted numerous interactions contribute to the on-target activity (Fig. 4A). Both **11k** and ceritinib formed two hydrogen bonds with Met1199 whereby amine and pyrimidine nitrogen atom. Simultaneously, the chlorine atom of 2,4-diaminopyrimidine skeleton is also crucial for the exertion of activity, as can be proven by the formation of two hydropholic interactions with Ala1148 and Leu1196. The above interactions seemed to be the reason for that DAAP is vital for the activity. Additionally, a halogen interaction was present between chlorine

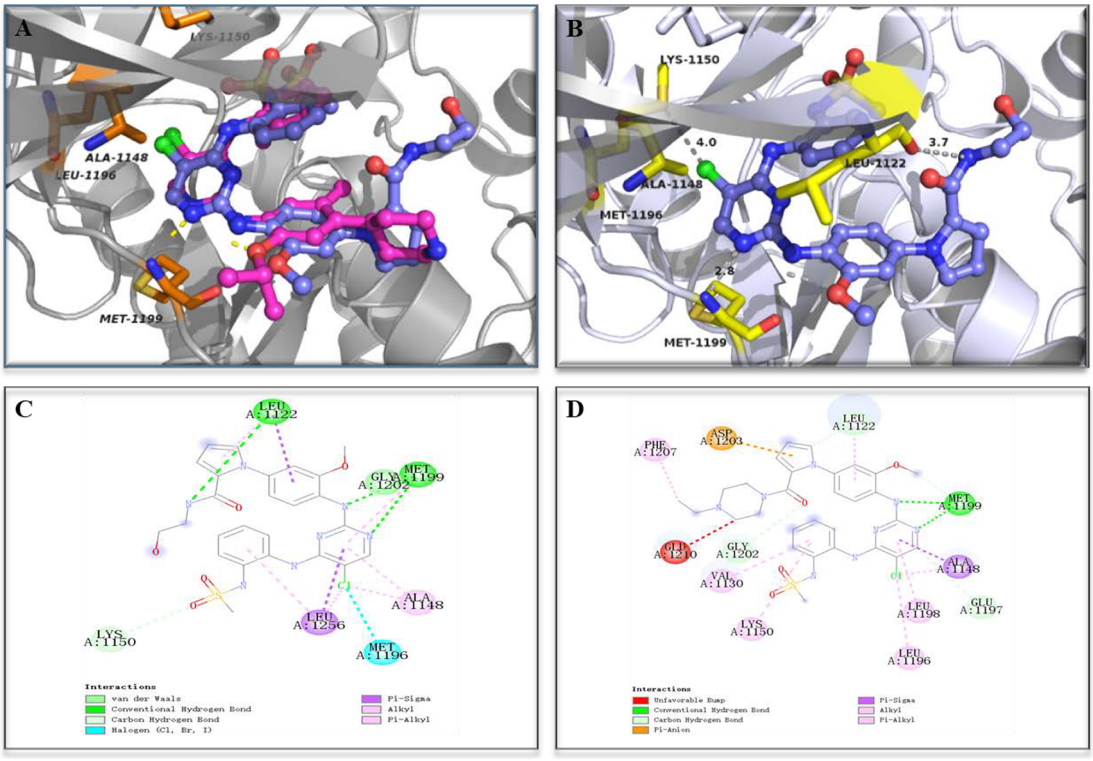


Fig. 4. The binding modes of ceritinib, **11e** and **11k** with ALK^{WT} and ALK^{L1196M}. (A) Binding mode of **11k** (blue sticks) with adjacent residues in the ALK^{WT} model and overlapping with ceritinib (pink sticks). (B) Predicted binding conformation for **11k** in the binding site cavity of ALK^{L1196M}. (C) 2D diagram of the interaction between **11k** and ALK^{L1196M}. (D) 2D diagram of the interaction between **11e** and ALK^{WT}.

atom in **11k** and L1196M, which may account for the combating effect of ALK^{L1196M} (Fig. 4B, C).

Also, oxygen atom of the 'head' was found to form carbon hydrogen bond with Lys1150, as illustrated the reasonability of the methyl sulfonamide. Furthermore, it is observed that the 'linker' form pi-alkyl interaction with Leu1122, which suggested the pyrroformyl group could inspire a new optimization idea of ALK inhibitor. Interestingly, the third hydrogen bond was detected between Leu1122 and the N—H of the water-soluable 'tail' belongs to **11k**. It can be boldly speculated that the third hydrogen bond was responsible for the improvement of activity. However, as shown in Fig. 4D, oversized hydrophilic tails forms an unfavorable bump with Glu1210, which were consistent with results of MTT assay.

3. Conclusions

In this investigation, we described the design, synthesis and biological evaluation of thirty novel 2,4-diaminopyrimidine compounds bearing pyrroformyl motif. Most of the tested compounds exhibited acceptable inhibitory activities against tested cancer cell lines (H2228, Karpas299 and A549), which suggested that pyrroformyl might produce a new structural optimization inspiration of ALK inhibitor. Interestingly, the MTT assay discovered **11k** as a potential lead which exhibited significant cytotoxicity with IC_{50} values of 0.034 μM, 0.045 μM and beyond 10 μM, respectively, superior to the positive control. Especially, representative compounds were examined for their enzymatic inhibitory, in which **11k** turned out to be effective against ALK^{WT} and ALK^{L1196M} kinases with IC_{50} values of 1.9 nM and 3.1 nM, surpassing the reference ceritinib (2.4 nM and 7.6 nM). Finally, the exploration of SARs was rationalized as was accordance with the docking simulation analysis of **11k** with ALK protein. As a whole, all results indicated that **11k** was expected to be a promising ALK inhibitor for combating L1196M mutation.

4. Experimental section

4.1. Chemistry

All materials of this paper were obtained from commercially available sources and were put in use without further purification. Melting points of all compounds were got on a Büchi Melting Point B-540 apparatus (BüchiLabortechnik, Switzerland), which were not underwent modification. Mass spectra (MS) were performed in ESI mode on Agilent 1100 LC-MS (Agilent, palo Alto, CA, USA). ^{1}H NMR and ^{13}C NMR spectra were accomplished using Bruker spectrometers (Bruker Bioscience, respectively, 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).

4.1.1. Preparation of N^1-(2,5-dichloropyrimidin-4-yl)benzene-1,2-diamine (3)

To the solution of 2, 4, 5-trichloropyrimidine **1** (30.0 g, 0.16 mol) in isopropanol (0.1 l) was added benzene-1,2-diamine **2** (17.7 g, 0.16 mol) and DIPEA (41.3 g, 0.32 mol). Then the mixture was heated to 80 °C. After the reaction mixture reacted for about 6 h, filtered the suspension at 80 °C and the filtrate was adjusted the pH to 7 with hydrochloric acid, then filtered and washed with hot isopropanol to afforded white solid **3** in a 84.5% yield. MS (ESI) *m/z*: 255.2 $[M+H]^+$, 253.1 $[M-H]^-$.

4.1.2. Preparation of N-(2-((2,5-dichloropyrimidin-4-yl)amino)phenyl) methanesulfonamide (4)

To a mixture of **3** (10.0 g, 0.02 mol) in tetrahydrofuran (THF, 50.0 mL) was added pyridine (9.5 mL, 0.06 mol), then methanesulfonyl chloride was dropwise added on the conditions of ice-salt baths. The reaction mixture was stirred for 5 h at room tempereture. Solvent was evaporated when the reaction was completed, and the residue was adjusted the pH to 6 with hydrochloric acid. The white solid was collected to give **4** in a satisfactory yield underwent filtration. MS (ESI) *m/z*: 333.1 $[M+H]^+$, 331.0 $[M-H]^-$.

4.1.3. Preparation of 2-amino-N-ethylbenzamide (6)

To a solution of isatin anhydride **5** (20.0 g, 0.12 mol) in ethyl alcohol (EtOH, 100.0 mL) was added methylamine (40% in water, 19.5 mL, 0.13 mol). The reaction mixture was stirred for 2 h at room tempereture before the reaction over. The mixture was added brine solution (100.0 mL) and was extracted with ethyl acetate (50.0 mL) for 3 times. The crude product **6** was obtained after evaporated the solvent. MS (ESI) *m/z*: 151.2 $[M+H]^+$, 149.1 $[M-H]^-$.

4.1.4. Preparation of 2-((2,5-dichloropyrimidin-4-yl)amino)-N-methylbenzamide (7)

2-amino-*N*-ethylbenzamide **6** (15.0 g, 0.10 mol) together with 2, 4, 5-trichloropyrimidine **1** (115 g, 0.10 mol) and DIPEA (33.0 mL, 0.20 mol) was added in isopropanol (100.0 mL). The reaction mixture was heated to refluxed for 6 h at 80 °C, which was filtered at 80 °C to get filter cake. The pale yellow solid was collected to give **7** in a 67.1% yield. MS (ESI) *m/z*: 297.1 $[M+H]^+$, 295.0 $[M-H]^-$.

4.1.5. Preparation of methyl 1-(3-methoxy-4-nitrophenyl)-1H-pyrrole-2-carboxylate (9)

To a solution of 4-fluoro-2-methoxy-1-nitrobenzene **8** (41.0 g, 0.24 mol) in dimethyl formamide (DMF, 100.0 mL) was added potassium carbonate (44.1 g, 0.32 mol) and methyl 1*H*-pyrrole-2-carboxylate (20.0 g, 0.16 mol). The mixture was heated and stirred for 5 h at refluxing state, which was poured into water (500.0 mL) and keep stirred for 0.5 h. The light brown solid **9** was collected by filtered and purified by diethyl ether in a good yield. MS (ESI) *m/z*: 277.1 $[M+H]^+$, 275.0 $[M-H]^-$.

4.1.6. Preparation of methyl 1-(4-amino-3-methoxyphenyl)-1H-pyrrole-2-carboxylate (10)

Iron powder reduction (18.0 g, 0.286 mol) was added in the solution of hydrochloric acid (HCl, 4.0 mL) in the ethyl alcohol (9:1, 200.0 mL). After activated for 0.5 h at 70 °C, the resulting solution was added 1-(3-methoxy-4-nitrophenyl)-1*H*-pyrrole-2-carboxylate **9** to maintained reaction for 3 h. The reaction mixture was filtered through celite at 70 °C, the solvent was whereafter evaporated to remove ethyl alcohol. The aqueous solution was adjusted pH to 8 and extracted with ethyl acetate (50.0 mL) for 3 times. The combined organic layers were concentrated under reduced pressure to afforded offwhite solid **10** in a better yield. MS (ESI) *m/z*: 247.1 $[M+H]^+$, 245.1 $[M-H]^-$.

4.1.7. General procedure for preparation of compounds (11a, 12a)

To a solution of methyl 1-(4-amino-3-methoxyphenyl)-1*H*-pyrrole-2-carboxylate **10** (0.012 mol) in isopropanol (30.0 mL) was added *N*-(2-((2,5-dichloropyrimidin-4-yl)amino)phenyl)methanesulfonamide **4** or 2-((2,5-dichloropyrimidin-4-yl)amino)-*N*-methylbenzamide **7** and *p*-toluenesulfonic acid (0.012 mol). The reaction mixture was filtered after maintained reaction for 48 h at 80 °C, the filtered cake was dissolved in water (20.0 mL) and was adjusted pH to 8. Ethyl acetate (20.0 mL) was employed to extracted for 3 times, and was combined to concentrated. The target compounds were obtained in a satisfactory yield.

4.1.7.1. Methyl 1-(4-((5-chloro-4-((2-(methylsulfonamido)phenyl)amino) pyrimidin-2-yl)amino)-3-methoxyphenyl)-1H-pyrrole-2-carboxylate (11a).

Yield: 69.2%; m. p.:172.4–173.1 °C. MS (ESI) *m/z*: 544.1 $[M+H]^+$, 541.3 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.31 (s, 1H), 8.62 (s, 1H), 8.20 (s, 1H), 8.00 (s, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 7.5 Hz, 1H), 7.34 (t, J = 7.4 Hz, 1H), 7.25 (d, J = 7.4 Hz, 1H), 7.21 (s, 1H), 7.02 (d, J = 2.2 Hz, 1H), 7.00 (d, J = 1.8 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.34–6.24 (m, 1H), 3.82 (s,

3H), 3.63 (s, 3H), 2.96 (s, 3H). Anal. Calcd for $C_{24}H_{23}ClN_6O_5S$, C, 53.09; H, 4.27; N, 15.48. Found: C, 53.10; H, 4.27; N, 15.48.

4.1.7.2. Methyl 1-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino)-3-methoxyphenyl)-1H-pyrrole-2-carboxylate ***(12a)***. Yield: 76.1%; m. p.:163.4–165.2 °C. MS (ESI) *m/z*: 505.8 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.65 (s, 1H), 8.77 (d, J = 4.4 Hz, 1H), 8.66 (d, J = 8.4 Hz, 1H), 8.33 (s, 1H), 8.22 (s, 1H), 7.90 (d, J = 8.5 Hz, 1H), 7.74 (d, J = 7.9 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.26 (s, 1H), 7.11 (t, J = 7.4 Hz, 1H), 7.06 (d, J = 2.0 Hz, 1H), 7.04 (dd, J = 3.8, 1.7 Hz, 1H), 6.88 (dd, J = 8.4, 2.0 Hz, 1H), 6.34–6.31 (m, 1H), 3.83 (s, 3H), 3.65 (s, 3H), 2.81 (d, J = 4.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C: 169.29, 160.36, 158.30, 155.46, 155.03, 150.40, 139.62, 135.86, 132.02, 131.00, 128.31, 128.08, 122.91, 122.32, 122.15, 121.69, 121.00, 118.99, 118.01, 109.95, 109.45, 105.70, 63.46, 56.39, 26.69. Anal. Calcd for $C_{25}H_{23}ClN_6O_4$, C, 59.25; H, 4.57; N, 16.59. Found: C, 59.23; H, 4.57; N, 16.58.

4.1.8. General procedure for preparation of compounds ***(11b, 12b)***

Sodium hydroxide (1.2 g, 0.032 mol) in water (4.0 mL) was poured into mixture of **11a** or **12a** (0.004 mol) in methyl alcohol (20.0 mL). The reaction mixture was heated to refluxed for 6 h, and was subsequently cooled to room temperature. The pH was adjusted to 6, and the solid was collected by filtration and air-dried in good yield.

4.1.8.1. 1-(4-((5-chloro-4-((2-(methylsulfonamido)phenyl)amino) pyrimidin-2-yl)amino)-3-methoxyphenyl)-1H-pyrrole-2-carboxylic acid ***(11b)***. Yield: 46.7%; m. p.:164.5–167.8 °C. MS (ESI) *m/z*: 529.3 $[M+H]^+$, 527.1 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.87 (s, 1H), 9.32 (m, 1H), 8.62 (s, 1H), 8.18 (s, 1H), 7.96 (s, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.32 (t, J = 7.3 Hz, 1H), 7.23 (t, J = 7.3 Hz, 1H), 7.12 (s, 1H), 6.98 (s, 1H), 6.95 (s, 1H), 6.70 (d, J = 8.1 Hz, 1H), 6.26 (s, 1H), 3.81 (s, 3H), 2.95 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C: 164.66, 160.92, 157.51, 155.95, 154.43, 148.59, 133.82, 129.94, 129.82, 129.48, 127.50, 126.67, 126.44, 125.77, 125.30, 119.72, 118.33, 117.32, 109.28, 108.56, 104.62, 55.91, 28.90. Anal. Calcd for $C_{23}H_{21}ClN_6O_5S$, C, 52.22; H, 4.00; N, 15.89. Found: C, 52.22; H, 4.02; N, 15.88.

4.1.8.2. 1-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino) pyrimidin-2-yl)amino)-3-methoxyphenyl)-1H-pyrrole-2-carboxylic acid ***(12b)***. Yield: 96.4%; m. p.:161.2–163.1 °C. MS (ESI) *m/z*: 491.8 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.61 (s, 1H), 8.79 (s, 1H), 8.64 (d, J = 8.4 Hz, 1H), 8.25 (s, 1H), 8.20 (s, 1H), 7.83 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 7.2 Hz, 1H), 7.50–7.45 (m, 1H), 7.14–7.07 (m, 1H), 7.01 (s, 2H), 6.84 (d, J = 7.8 Hz, 1H), 6.76 (s, 1H), 6.17 (s, 1H), 3.81 (s, 3H), 2.80 (s, 3H), 1.23 (s, 3H). Anal. Calcd for $C_{24}H_{21}ClN_6O_4$, C, 58.48; H, 4.29; N, 17.05. Found: C, 58.49; H, 4.29; N, 17.04.

4.1.9. General procedure for preparation of compounds ***(11c-o,12c-o)***

11b or **12b** (0.3 mmol) was added in the solvent of dimethyl formamide (DMF, 4.0 mL), then aliphatic amines (0.36 mmol), DIPEA (0.21 mL, 1.2 mmol), HATU (0.285 g, 0.75 mmol) and HOAT (0.014 g, 0.1 mmol) were added into the reaction mixture, which was added water (20.0 mL) after stirred for 3 h at room tempereture. The suspension was filtered to give target compounds (**11c-o**, **12c-o**).

4.1.9.1. N-(2-((5-chloro-2-((2-methoxy-4-(2-(piperidine-1-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl) methanesulfonamide ***(11c)***. Yield: 75.4%; m. p.:167.5–170.3 °C. MS (ESI) *m/z*: 596.2 $[M+H]^+$, 594.1 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.31 (s, 1H), 8.62 (s, 1H), 8.18 (s, 1H), 7.97 (s, 1H), 7.90 (d, J = 4.9 Hz, 1H), 7.82 (d, J = 7.2 Hz, 1H), 7.42 (d, J = 4.8 Hz, 1H), 7.33 (s, 1H), 7.25 (d, J = 6.4 Hz, 1H), 7.14 (s, 1H), 6.91 (s, 1H), 6.63 (d, J = 7.7 Hz, 1H), 6.39 (s, 1H), 6.25 (s, 1H), 3.83 (s, 3H), 3.40 (s, 4H), 2.94 (s, 3H), 1.53 (s, 2H), 1.34 (s, 4H). Anal. Calcd for $C_{28}H_{30}ClN_7O_4S$, C, 56.41; H, 5.07; N, 16.45. Found: C, 56.42; H, 5.07; N, 16.45.

4.1.9.2. N-(2-((5-chloro-2-((2-methoxy-4-(2-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl) methanesulfonamide ***(11d)***. Yield: 72.7%; m. p.:182.4–184.3 °C. MS (ESI) *m/z*: 611.6 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.56 (s, 1H), 8.66 (s, 1H), 8.18 (s, 1H), 7.99 (s, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.31 (t, J = 7.4 Hz, 1H), 7.23 (t, J = 7.3 Hz, 1H), 7.16 (s, 1H), 6.90 (d, J = 1.6 Hz, 1H), 6.65 (d, J = 8.9 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 6.27–6.24 (m, 1H), 3.83 (s, 3H), 3.44 (s, 4H), 2.94 (s, 3H), 2.14 (s, 4H), 1.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 162.40, 158.08, 156.53, 155.01, 149.82, 134.91, 134.36, 130.61, 127.60, 126.98, 126.86, 126.60, 126.26, 125.75, 124.63, 121.11, 115.37, 112.78, 109.03, 107.30, 105.19, 56.46(2C), 54.84, 48.00, 46.02 (2C), 29.46. Anal. Calcd for $C_{28}H_{31}ClN_8O_4S$, C, 55.03; H, 5.12; N, 18.34. Found: C, 55.03; H, 5.11; N, 18.34.

4.1.9.3. N-(2-((5-chloro-2-((4-(2-(4-ethylpiperazine-1-carbonyl)-1H-pyrrol-1-yl)-2-methoxyphenyl)amino)pyrimidin-4-yl)amino)phenyl) methanesulfonamide ***(11e)***. Yield: 67.5%; m. p.:173.2–174.9 °C. MS (ESI) *m/z*: 625.6 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 8.68 (d, J = 4.1 Hz, 1H), 8.62 (s, 1H), 8.47 (d, J = 8.4 Hz, 1H), 8.18 (s, 1H), 8.00 (s, 1H), 7.88 (dd, J = 32.6, 8.3 Hz, 2H), 7.42 (d, J = 7.9 Hz, 1H), 7.30 (dt, J = 33.9, 7.5 Hz, 2H), 7.20 (s, 1H), 6.64 (d, J = 8.3 Hz, 1H), 6.50 (d, J = 2.2 Hz, 1H), 6.29–6.26 (t, 1H), 3.84 (s, 3H), 3.56 (s, 4H), 2.96 (s, 3H), 2.64–2.60 (t, 2H), 2.58 (s, 4H), 2.50 (s, 3H). Anal. Calcd for $C_{29}H_{33}ClN_8O_4S$, C, 55.73; H, 5.32; N, 17.92. Found: C, 55.72; H, 5.32; N, 17.92.

4.1.9.4. N-(2-((5-chloro-2-((2-methoxy-4-(2-(morpholine-4-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl) methanesulfonamide ***(11f)***. Yield: 77.9%; m. p.:184.7–186.1 °C. MS (ESI) *m/z*: 596.6 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.29 (s, 1H), 8.65 (s, 1H), 8.18 (s, 1H), 7.98 (s, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 7.2 Hz, 1H), 7.30 (t, J = 7.3 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.18 (s, 1H), 6.93 (d, J = 2.0 Hz, 1H), 6.64 (d, J = 8.5 Hz, 1H), 6.46 (d, J = 2.1 Hz, 1H), 6.30–6.24 (m, 1H), 3.84 (s, 3H), 3.44 (s, 3H), 2.94 (s, 3H), 1.23 (s, 3H). Anal. Calcd for $C_{27}H_{28}ClN_7O_5S$, C, 54.23; H, 4.72; N, 16.39. Found: C, 54.22; H, 4.72; N, 16.39.

4.1.9.5. N-(2-((5-chloro-2-((2-methoxy-4-(2-(pyrrolidine-1-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl) methanesulfonamide ***(11g)***. Yield: 79.4%; m. p.:180.0–181.4 °C. MS (ESI) *m/z*: 580.7 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.32 (s, 1H), 8.61 (s, 1H), 8.17 (s, 1H), 7.98 (s, 1H), 7.91 (s, 1H), 7.79 (s, 1H), 7.40 (s, 1H), 7.33 (s, 1H), 7.23 (s, 1H), 7.12 (s, 1H), 6.90 (s, 1H), 6.62 (s, 1H), 6.56 (s, 1H), 6.23 (s, 1H), 3.81 (s, 3H), 3.48 (s,4H), 2.95 (s, 3H), 1.81 (s, 4H). Anal. Calcd for $C_{27}H_{28}ClN_7O_4S$, C, 55.71; H, 4.85; N, 16.85. Found: C, 55.71; H, 4.85; N, 16.84.

4.1.9.6. N-(2-((5-chloro-2-((2-methoxy-4-(2-(thiomorpholine-4-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl) methanesulfonamide ***(11h)***. Yield: 69.5%; m. p.:172.3–175.8 °C. MS (ESI) *m/z*: 612.9 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.31 (s, 1H), 8.63 (s, 1H), 8.18 (s, 1H), 7.97 (s, 1H), 7.86 (dd, J = 22.3, 7.9 Hz, 2H), 7.43 (d, J = 7.6 Hz, 1H), 7.30 (dt, J = 28.3, 7.1 Hz, 2H), 7.18 (s, 1H), 6.93 (s, 1H), 6.61 (d, J = 7.9 Hz, 1H), 6.46 (s, 1H), 6.26 (s, 1H), 3.84 (s, 3H), 3.71 (s, 4H), 2.94 (s, 3H), 2.48–2.46 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C: 162.44, 158.11, 156.57, 155.04, 149.86, 134.94, 134.39, 130.65, 127.63, 127.01, 126.89, 126.63, 126.29, 125.78, 124.66, 121.14, 115.40, 112.81, 109.07, 107.34, 105.23,

56.49, 54.87, 46.06, 29.49. Anal. Calcd for $C_{27}H_{28}ClN7O_4S_2$, C, 52.82; H, 4.60; N, 15.97. Found: C, 52.81; H, 4.60; N, 15.97.

*4.1.9.7. N-(2-((5-chloro-2-((4-(2-(4-(2-hydroxyethyl)piperazine-1-carbonyl)-1H-pyrrol-1-yl)-2-methoxyphenyl)amino)pyrimidin-4-yl)amino) phenyl)methanesulfonamide (**11i**)*. Yield: 68.4%; m. p.:192.4–194.1 °C. MS (ESI) *m/z*: 639.2 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.34 (s, 1H), 8.63 (s, 1H), 8.18 (s, 1H), 7.98 (s, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 8.6 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.33 (t, J = 7.4 Hz, 1H), 7.25 (t, J = 7.1 Hz, 1H), 7.16 (s, 1H), 6.89 (d, J = 2.1 Hz, 1H), 6.63 (s, 1H), 6.43 (d, J = 2.2 Hz, 1H), 6.27–6.23 (m, 1H), 4.42 (s, 1H), 3.83 (s, 3H), 3.45 (d, J = 4.6 Hz, 6H), 2.95 (s, 3H), 2.33 (t, J = 6.0 Hz, 4H), 2.27 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C: 161.73, 157.48, 155.98, 154.47, 149.24, 134.32, 133.85, 129.79, 128.76, 126.97, 126.69, 126.48, 125.95, 125.28, 124.08, 120.53, 114.73, 112.25, 108.46, 106.66, 104.57, 59.87, 58.19, 55.86, 55.33, 52.74, 28.90. Anal. Calcd for $C_{29}H_{33}ClN_8O_5S$, C, 54.33; H, 5.18; N, 17.48. Found: C, 54.33; H, 5.19; N, 17.48.

*4.1.9.8. N-(2-((5-chloro-2-((2-methoxy-4-(2-(4-methylpiperidine-1-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)phenyl) methanesulfonamide (**11j**)*. Yield: 80.4%; m. p.:177.7–178.1 °C. MS (ESI) *m/z*: 608.9 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.31 (s, 1H), 8.64 (s, 1H), 8.19 (s, 1H), 8.01 (s, 1H), 7.91 (s, 1H), 7.83 (s, 1H), 7.43 (s, 1H), 7.33 (s, 1H), 7.25 (s, 1H), 7.15 (s, 1H), 6.90 (s, 1H), 6.65 (s, 1H), 6.40 (s, 1H), 6.25 (s, 3H), 3.83 (s, 4H), 3.39 (s, 3H), 2.95 (s, 4H), 1.53 (s, 3H), 0.82 (s, 1H). Anal. Calcd for $C_{29}H_{32}ClN_7O_4S$, C, 57.08; H, 5.29; N, 16.07. Found: C, 57.09; H, 5.29; N, 16.07.

*4.1.9.9. 1-(4-((5-chloro-4-((2-(methylsulfonamido)phenyl)amino) pyrimidin-2-yl)amino)-3-methoxyphenyl)-N-(2-hydroxyethyl)-1H-pyrrole-2-carboxamide (**11k**)*. Yield: 68.1%; m. p.:166.6–168.4 °C. MS (ESI) *m/z*: 570.8 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.30 (s, 1H), 8.60 (s, 1H), 8.18 (s, 1H), 8.03 (t, J = 5.7 Hz, 1H), 7.98 (s, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 7.9 Hz, 1H), 7.35 (t, J = 7.7 Hz, 1H), 7.24 (t, J = 7.6 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H), 6.90 (d, J = 2.1 Hz, 1H), 6.80 (dd, J = 3.6, 1.5 Hz, 1H), 6.64 (dd, J = 8.5, 1.9 Hz, 1H), 6.22–6.19 (t, 1H), 4.69 (t, J = 5.6 Hz, 1H), 3.80 (s, 3H), 3.44 (q, J = 6.1 Hz, 2H), 3.20 (q, J = 6.0 Hz, 2H), 2.96 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C: 161.19, 158.07, 156.41, 154.95, 149.31, 135.80, 134.47, 129.98, 127.79, 127.49, 127.44, 127.40, 127.13, 126.28, 125.70, 120.60, 116.90, 113.69, 108.86, 108.41, 104.99, 60.37, 56.26, 41.91, 28.82. Anal. Calcd for $C_{25}H_{26}ClN_7O_5S$, C, 52.49; H, 4.59; N, 17.14. Found: C, 52.49; H, 4.58; N, 17.14.

*4.1.9.10. 1-(4-((5-chloro-4-((2-(methylsulfonamido)phenyl)amino) pyrimidin-2-yl)amino)-3-methoxyphenyl)-N,N-dimethyl-1H-pyrrole-2-carboxamide (**11l**)*. Yield: 66.7%; m. p.:169.2–171.4 °C. MS (ESI) *m/z*: 554.7 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.31 (s, 1H), 8.61 (s, 1H), 8.18 (s, 1H), 7.98 (s, 1H), 7.90 (s, 1H), 7.80 (s, 0H), 7.42 (s, 1H), 7.32 (s, 0H), 7.26 (s, 1H), 7.15 (s, 1H), 6.91 (s, 1H), 6.60 (s, 1H), 6.45 (s, 1H), 6.25 (s, 1H), 3.82 (s, 3H), 2.94 (s, 6H), 2.91 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C: 162.60, 158.27, 156.73, 155.20, 150.02, 135.10, 134.55, 130.81, 127.80, 127.17, 127.05, 126.79, 126.45, 125.95, 124.82, 121.30, 115.56, 112.97, 109.23, 107.50, 105.39, 56.65, 55.03, 29.66. Anal. Calcd for $C_{25}H_{26}ClN_7O_4S$, C, 54.01; H, 4.71; N, 17.62. Found: C, 54.00; H, 4.71; N, 17.63.

*4.1.9.11. 1-(4-((5-chloro-4-((2-(methylsulfonamido)phenyl)amino) pyrimidin-2-yl)amino)-3-methoxyphenyl)-N,N-diethyl-1H-pyrrole-2-carboxamide (**11m**)*. Yield: 75.1%; m. p.:165.5–167.9 °C. MS (ESI) *m/z*: 582.8 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.31 (s, 1H), 8.62 (s, 1H), 8.18 (s, 1H), 7.95 (s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.42 (d, J = 7.5 Hz, 1H), 7.32 (t, J = 7.4 Hz, 1H), 7.25 (t, J = 7.4 Hz, 1H), 7.12 (s, 1H), 6.90 (s, 1H), 6.61 (d, J = 8.3 Hz, 1H), 3.82 (s, 3H), 3.31 (d, J = 6.6 Hz, 4H), 2.94 (s, 4H), 1.01 (t, J = 5.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C: 162.84, 158.51, 156.97, 155.45, 150.26, 135.34, 134.80, 131.05, 128.04, 127.42, 127.29, 127.04, 126.70, 126.19, 125.06, 121.55, 115.80, 113.21, 109.47, 107.74, 105.63, 56.90, 55.27, 46.46, 29.90. Anal. Calcd for $C_{27}H_{30}ClN_7O_4S$, C, 55.52; H, 5.18; N, 16.79. Found: C, 55.52; H, 5.18; N, 16.79.

*4.1.9.12. N-(2-((5-chloro-2-((4-(2-(3-hydroxyazetidine-1-carbonyl)-1H-pyrrol-1-yl)-2-methoxyphenyl)amino)pyrimidin-4-yl)amino)phenyl) methanesulfonamide (**11n**)*. Yield: 63.4%; m. p.:173.8–174.9 °C. MS (ESI) *m/z*: 582.8 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.31 (s, 1H), 8.66 (s, 1H), 8.19 (s, 1H), 8.06 (s, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.42 (d, J = 7.7 Hz, 1H), 7.36 (t, J = 7.4 Hz, 1H), 7.26 (t, J = 7.5 Hz, 1H), 7.11 (s, 1H), 6.93 (d, J = 1.9 Hz, 1H), 6.63 (d, J = 2.5 Hz, 1H), 6.61 (s, 1H), 4.49 (s, 1H), 3.83 (s, 3H), 3.38 (s, 4H), 2.96 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C: 159.54, 156.42, 154.76, 153.30, 147.66, 134.16, 132.82, 128.34, 126.14, 125.84, 125.79, 125.75, 125.48, 124.63, 124.06, 118.96, 115.25, 112.05, 107.21, 106.76, 103.34, 58.72, 54.61, 40.26, 27.17. Anal. Calcd for $C_{26}H_{26}ClN_7O_5S$, C, 53.47; H, 4.49; N, 16.78. Found: C, 53.47; H, 4.49; N, 16.79.

*4.1.9.13. N-(2-((5-chloro-2-((4-(2-(4-hydroxypiperidine-1-carbonyl)-1H-pyrrol-1-yl)-2-methoxyphenyl)amino)pyrimidin-4-yl)amino)phenyl) methanesulfonamide (**11o**)*. Yield: 78.1%; m. p.:193.4–195.7 °C. MS (ESI) *m/z*: 610.8 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 9.36 (s, 1H), 8.70 (s, 1H), 8.17 (s, 1H), 7.95 (s, 2H), 7.85 (s, 1H), 7.38 (s, 1H), 7.22 (s, 1H), 7.15 (s, 1H), 6.90 (s, 1H), 6.63 (s, 2H), 6.41 (s, 1H), 6.25 (s, 1H), 4.76 (s, 3H), 3.83 (s, 1H), 3.68 (s, 2H), 3.13 (s, 5H), 1.63 (s, 2H), 0.85 (s, 2H). Anal. Calcd for $C_{28}H_{30}ClN_7O_5S$, C, 54.95; H, 4.94; N, 16.02. Found: C, 54.94; H, 4.94; N, 16.02.

*4.1.9.14. 2-((5-chloro-2-((2-methoxy-4-(2-(piperidine-1-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide (**12c**)*. Yield: 76.1%; m. p.:188.4–190.7 °C. MS (ESI) *m/z*: 558.8 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.66 (s, 1H), 8.79 (d, J = 4.4 Hz, 1H), 8.65 (d, J = 8.3 Hz, 1H), 8.30 (s, 1H), 8.20 (s, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.20 (s, 1H), 7.11 (t, J = 7.5 Hz, 1H), 6.96 (d, J = 2.0 Hz, 1H), 6.83 (dd, J = 8.5, 2.0 Hz, 1H), 6.41 (d, J = 2.6 Hz, 1H), 6.27 (t, J = 3.0 Hz, 1H), 3.84 (s, 3H), 3.43 (s, 4H), 2.81 (d, J = 4.4 Hz, 3H), 1.53 (s, 2H), 1.36 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 169.37, 162.40, 158.41, 155.54, 155.13, 151.06, 139.73, 135.83, 132.01, 128.43, 127.32, 127.05, 124.34, 123.05, 122.37, 121.76, 121.10, 115.27, 112.26, 109.04, 107.36, 105.72, 63.55, 56.31(2C), 26.77 (2C), 26.09, 24.45. Anal. Calcd for $C_{29}H_{30}ClN_7O_3$, C, 62.18; H, 5.40; N, 17.51. Found: C, 62.19; H, 5.40; N, 17.51.

*4.1.9.15. 2-((5-chloro-2-((2-methoxy-4-(2-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide (**12d**)*. Yield: 67.5%; m. p.:199.1–201.3 °C. MS (ESI) *m/z*: 573.8 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.65 (s, 1H), 8.77 (s, 1H), 8.65 (s, 1H), 8.32 (s, 1H), 8.20 (s, 1H), 7.88 (s, 1H), 7.74 (s, 1H), 7.48 (s, 1H), 7.22 (s, 1H), 7.12 (s, 1H), 6.95 (s, 1H), 6.83 (s, 1H), 6.45 (s, 1H), 6.28 (s, 1H), 3.85 (s, 3H), 3.46 (s, 4H), 2.81 (s, 3H), 2.09 (s, 4H), 1.04 (d, J = 154.1 Hz, 3H). Anal. Calcd for $C_{29}H_{31}ClN_8O_3$, C, 60.57; H, 5.42; N, 19.50. Found: C, 60.57; H, 5.43; N, 19.49.

*4.1.9.16. 2-((5-chloro-2-((4-(2-(4-ethylpiperazine-1-carbonyl)-1H-pyrrol-1-yl)-2-methoxyphenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide (**12e**)*. Yield: 71.2%;m. p.:187.6–189.4 °C. MS (ESI) *m/z*: 587.8 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.65 (s, 1H), 8.76 (d, J = 4.5 Hz, 1H), 8.65 (d, J = 8.2 Hz, 1H), 8.30 (s, 1H), 8.20 (s, 1H), 7.89 (d, J = 8.5 Hz, 1H), 7.75 (dd, J = 7.8, 1.0 Hz, 1H), 7.48 (t, J = 7.4 Hz, 1H), 7.22–7.20 (m, 1H), 7.11 (t, J = 7.6 Hz, 1H), 6.94 (d, J = 2.2 Hz, 1H), 6.84 (dd, J = 8.5, 2.3 Hz, 1H), 6.45 (dd, J = 3.6,

1.5 Hz, 1H), 6.29–6.27 (m, 1H), 3.84 (s, 3H), 3.45 (s, 4H), 2.81 (d, J = 4.5 Hz, 3H), 2.23 (dd, J = 14.3, 7.2 Hz, 2H), 2.17 (s, 4H), 0.92 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{30}H_{33}ClN_8O_3$, C, 61.18; H, 5.65; N, 19.02. Found: C, 61.17; H, 5.65; N, 19.02.

4.1.9.17. 2-((5-chloro-2-((2-methoxy-4-(2-(morpholine-4-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide ***(12f)***. Yield: 78.9%; m. p.:174.6–178.9 °C. MS (ESI) m/z: 560.8 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.66 (s, 1H), 8.80 (s, 1H), 8.65 (d, J = 7.1 Hz, 1H), 8.31 (s, 1H), 8.20 (s, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.23 (s, 1H), 7.11 (t, J = 7.1 Hz, 1H), 6.98 (s, 1H), 6.83 (d, J = 8.1 Hz, 1H), 6.49 (s, 1H), 6.29 (s, 1H), 3.85 (s, 3H), 3.45 (d, J = 20.8 Hz, 8H), 2.81 (d, J = 3.9 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 169.35, 162.55, 158.39, 155.59, 155.09, 151.03, 139.73, 135.67, 131.98, 128.45, 127.52, 126.27, 124.98, 122.98, 122.36, 121.81, 121.18, 115.52, 113.17, 109.13, 107.58, 105.78, 66.52 (2C), 56.39, 36.25 (2C), 26.75. Anal. Calcd for $C_{28}H_{28}ClN_7O_4$, C, 59.84; H, 5.02; N, 17.46. Found: C, 59.84; H, 5.02; N, 17.45.

4.1.9.18. 2-((5-chloro-2-((2-methoxy-4-(2-(pyrrolidine-1-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide ***(12g)***. Yield: 69.4%; m. p.:164.7–168.1 °C. MS (ESI) m/z: 544.7 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.64 (s, 1H), 8.76 (d, J = 4.6 Hz, 1H), 8.65 (d, J = 8.4 Hz, 1H), 8.30 (s, 1H), 8.20 (s, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.74 (d, J = 6.8 Hz, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.17 (dd, J = 2.5, 1.7 Hz, 1H), 7.10 (t, J = 7.5 Hz, 1H), 6.96 (d, J = 2.2 Hz, 1H), 6.81 (dd, J = 8.5, 2.3 Hz, 1H), 6.58 (dt, J = 17.3, 8.7 Hz, 1H), 6.27–6.25 (m, 1H), 3.83 (s, 3H), 3.44 (d, J = 58.5 Hz, 4H), 2.80 (d, J = 4.5 Hz, 3H), 1.80 (d, J = 6.4 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C: 169.25, 162.28, 158.29, 155.43, 155.01, 150.95, 139.62, 135.72, 131.89, 128.31, 127.21, 126.94, 124.23, 122.93, 122.25, 121.65, 120.98, 115.15, 112.15, 108.93, 107.25, 105.60, 63.43, 56.99, 26.65, 25.97. Anal. Calcd for $C_{28}H_{28}ClN_7O_3$, C, 61.58; H, 5.17; N, 17.96. Found: C, 61.59; H, 5.17; N, 17.96.

4.1.9.19. 2-((5-chloro-2-((2-methoxy-4-(2-(thiomorpholine-4-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide ***(12h)***. Yield: 74.1%; m. p.:161.7–163.4 °C. MS (ESI) m/z: 576.7 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.64 (s, 1H), 8.76 (s, 1H), 8.65 (d, J = 7.7 Hz, 1H), 8.32 (s, 1H), 8.21 (s, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.48 (s, 1H), 7.23 (s, 1H), 7.13 (d, J = 7.4 Hz, 1H), 6.98 (s, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.49 (s, 1H), 6.29 (s, 1H), 3.85 (s, 3H), 3.74 (s, 4H), 2.81 (s, 3H), 2.50 (s, 4H). Anal. Calcd for $C_{28}H_{28}ClN_7O_3S$, C, 58.18; H, 4.89; N, 16.96. Found: C, 58.18; H, 4.88; N, 16.96.

4.1.9.20. 2-((5-chloro-2-((4-(2-(4-(2-hydroxyethyl)piperazine-1-carbonyl)-1H-pyrrol-1-yl)-2-methoxyphenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide ***(12i)***. Yield: 77.2%; m. p.:184.6–185.9 °C. MS (ESI) m/z: 603.6 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.64 (s, 1H), 8.76 (s, 1H), 8.65 (s, 1H), 8.31 (s, 1H), 8.20 (s, 1H), 7.89 (s, 1H), 7.74 (s, 1H), 7.48 (s, 1H), 7.21 (s, 1H), 7.12 (s, 1H), 6.94 (s, 1H), 6.84 (s, 1H), 6.45 (s, 1H), 6.27 (s, 1H), 4.40 (s, 1H), 3.84 (s, 3H), 3.45 (s, 6H), 2.80 (s, 3H), 2.30 (s, 4H), 2.25 (s, 2H). Anal. Calcd for $C_{30}H_{33}ClN_8O_4$, C, 59.55; H, 5.50; N, 18.52. Found: C, 59.55; H, 5.50; N, 18.52.

4.1.9.21. 2-((5-chloro-2-((2-methoxy-4-(2-(4-methylpiperidine-1-carbonyl)-1H-pyrrol-1-yl)phenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide ***(12j)***. Yield: 77.2%; m. p.:189.0–191.3 °C. MS (ESI) m/z: 603.6 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.65 (s, 1H), 8.75 (d, J = 3.8 Hz, 1H), 8.65 (d, J = 8.4 Hz, 1H), 8.30 (s, 1H), 8.20 (s, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.74 (d, J = 7.7 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.20 (s, 1H), 7.11 (t, J = 7.5 Hz, 1H), 6.95 (d, J = 1.9 Hz, 1H), 6.84 (dd, J = 8.5, 1.9 Hz, 1H), 6.41 (d, J = 2.1 Hz, 1H), 6.27 (t, J = 3.0 Hz, 1H), 3.84 (s, 3H), 2.81 (d, J = 4.4 Hz, 4H), 1.53 (s, 3H), 0.80 (d, J = 6.0 Hz, 4H), 0.74 (s, 1H). Anal. Calcd for $C_{30}H_{32}ClN_7O_3$, C, 62.78; H, 5.62; N, 17.08. Found: C, 62.77; H, 5.62; N, 17.08.

4.1.9.22. 1-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin-2-yl)amino)-3-methoxyphenyl)-N-(2-hydroxyethyl)-1H-pyrrole-2-carboxamide ***(12k)***. Yield: 78.4%; m. p.:189.0–192. °C. MS (ESI) m/z: 534.5 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.64 (s, 1H), 8.76 (s, 1H), 8.66 (d, J = 7.7 Hz, 1H), 8.30 (s, 1H), 8.20 (s, 1H), 8.07 (s, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.50 (t, J = 7.7 Hz, 1H), 7.13–7.07 (m, 2H), 6.96 (s, 1H), 6.84–6.78 (m, 2H), 6.22 (s, 1H), 4.71 (s, 1H), 3.82 (s, 3H), 3.45 (s, 2H), 3.21 (d, J = 1.8 Hz, 2H), 2.81 (d, J = 2.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C: 169.11, 162.32, 158.16, 155.35, 154.89, 154.85, 150.79, 139.50, 135.44, 131.74, 128.22, 127.28, 126.04, 124.74, 122.75, 122.13, 121.57, 120.94, 115.28, 112.94, 108.89, 107.34, 105.55, 66.28, 54.56, 36.01, 26.51. Anal. Calcd for $C_{26}H_{26}ClN_7O_4$, C, 58.26; H, 4.88; N, 18.29. Found: C, 58.26; H, 4.89; N, 18.29.

4.1.9.23. 1-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin-2-yl)amino)-3-methoxyphenyl)-N,N-dimethyl-1H-pyrrole-2-carboxamide ***(12l)***. Yield: 81.0%; m. p.:194.2–196.0 °C. MS (ESI) m/z: 518.7 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.64 (s, 1H), 8.76 (s, 1H), 8.64 (s, 1H), 8.31 (s, 1H), 8.20 (s, 1H), 7.85 (s, 1H), 7.74 (s, 1H), 7.47 (s, 1H), 7.20 (s, 1H), 7.11 (s, 1H), 6.97 (s, 1H), 6.80 (s, 1H), 6.47 (s, 1H), 6.27 (s, 1H), 3.83 (s, 3H), 2.93 (s, 6H), 2.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C: 170.43, 163.46, 159.47, 156.60, 156.19, 152.12, 140.79, 136.89, 133.07, 129.49, 128.38, 128.11, 125.40, 124.11, 123.43, 122.82, 122.16, 116.33, 113.32, 110.10, 108.42, 106.77, 64.61, 55.96, 27.82. Anal. Calcd for $C_{26}H_{26}ClN_7O_3$, C, 60.06; H, 5.03; N, 18.86. Found: C, 60.06; H, 5.04; N, 18.86.

4.1.9.24. 1-(4-((5-chloro-4-((2-(methylcarbamoyl)phenyl)amino)pyrimidin-2-yl)amino)-3-methoxyphenyl)-N,N-diethyl-1H-pyrrole-2-carboxamide ***(12m)***. Yield: 69.9%; m. p.:195.3–196.9 °C. MS (ESI) m/z: 546.8 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.63 (s, 1H), 8.76 (d, J = 4.6 Hz, 1H), 8.63 (d, J = 8.4 Hz, 1H), 8.29 (s, 1H), 8.20 (s, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.74 (d, J = 6.9 Hz, 1H), 7.46 (t, J = 7.8 Hz, 1H), 7.21–7.17 (m, 1H), 7.12 (t, J = 7.5 Hz, 1H), 6.96 (d, J = 2.2 Hz, 1H), 6.82 (dd, J = 8.5, 2.2 Hz, 1H), 6.42 (dd, J = 3.5, 1.5 Hz, 1H), 6.27–6.24 (m, 1H), 3.83 (s, 3H), 3.36 (d, J = 7.0 Hz, 4H), 2.80 (d, J = 4.5 Hz, 3H), 1.02 (t, J = 6.7 Hz, 6H). Anal. Calcd for $C_{28}H_{30}ClN_7O_3$, C, 61.37; H, 5.53; N, 17.89. Found: C, 61.37; H, 5.52; N, 17.89.

4.1.9.25. 2-((5-chloro-2-((4-(2-(3-hydroxyazetidine-1-carbonyl)-1H-pyrrol-1-yl)-2-methoxyphenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide ***(12n)***. Yield: 67.8%; m. p.:169.5–171.8 °C. MS (ESI) m/z: 546.8 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.68 (s, 1H), 8.76 (s, 1H), 8.67 (s, 1H), 8.34 (s, 1H), 8.21 (s, 1H), 7.83 (s, 1H), 7.75 (s, 1H), 7.53 (s, 1H), 7.14 (d, J = 12.4 Hz, 2H), 7.00 (s, 1H), 6.81 (s, 1H), 6.64 (s, 1H), 6.27 (s, 1H), 5.74 (s, 1H), 4.49 (s, 1H), 3.84 (s, 3H), 2.85 (d, J = 33.8 Hz, 4H), 2.74 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C: 169.29, 162.69, 162.25, 158.31, 155.44, 155.05, 150.95, 139.62, 135.75, 131.97, 128.31, 127.22, 126.76, 124.50, 122.95, 122.32, 121.69, 121.00, 115.17, 112.41, 108.95, 107.27, 66.43, 56.22, 36.16, 31.15, 26.69. Anal. Anal. Calcd for $C_{27}H_{26}ClN_7O_4$, C, 59.18; H, 4.78; N, 17.88. Found: C, 59.18; H, 4.78; N, 17.89.

4.1.9.26. 2-((5-chloro-2-((4-(2-(4-hydroxypiperidine-1-carbonyl)-1H-pyrrol-1-yl)-2-methoxyphenyl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide ***(12o)***. Yield: 71.4%; m. p.:188.4–189.9 °C. MS (ESI) m/z: 574.8 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 11.64 (s, 1H), 8.76 (d, J = 4.2 Hz, 1H), 8.65 (d, J = 8.1 Hz, 1H), 8.29 (s, 2H), 8.20 (s, 1H), 7.89 (d, J = 8.5 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.20 (s, 1H), 7.12 (t, J = 7.4 Hz, 1H), 6.96 (s, 1H),

6.82 (d, J = 8.3 Hz, 1H), 6.43 (s, 1H), 6.27 (s, 1H), 4.76 (d, J = 3.2 Hz, 1H), 3.84 (s, 3H), 3.68 (s, 1H), 3.16 (s, 2H), 2.89 (s, 1H), 2.81 (d, J = 4.2 Hz, 3H), 2.73 (s, 1H), 1.64 (s, 2H), 1.24 (d, J = 4.0 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ_C: 169.50, 162.90, 162.47, 158.53, 155.66, 155.27, 151.17, 139.84, 135.97, 132.18, 128.53, 127.43, 126.98, 124.72, 123.17, 122.54, 121.91, 121.22, 115.39, 112.63, 109.17, 107.49, 66.01, 56.43, 36.38, 31.36, 26.91. Anal. Calcd for $C_{29}H_{30}ClN_7O_4$, C, 60.47; H, 5.26; N, 17.02. Found: C, 60.47; H, 5.25; N, 17.02.

4.2. MTT assay in vitro

The H2228, Karpas299, A549 cell lines were selected to evaluated the anti-proliferative activities of all the target compounds (**11a-o**, **12a-o**). Above three cancer cell lines were cultured in minimum essential medium supplement with 10% fetal bovine serum. Cells were seeded into 96-well plates in approximate 5 × 10^4 wells, incubated in 5% CO_2 at 37 ˚C for 24 h. Triplicate wells were treated with concentrations of compounds and media. Subsequently, tested compounds were added to the culture medium and incubated for 72 h. Fresh MTT were added to each well at the 5 μg/mL. After 4 h of incubation, the MTT mediun was removed, and 100 μL DMSO was added to each wells. The result was determined with microplate reader (MK3, Themo, Germony). All of the compounds were tested three times, and the IC_{50} values were defined as the concentration that reduced the absorbance of the negative wells by 50% of vehicle in the MTT assay.

4.3. Enzymatic assay in vitro

In vitro enzymatic assay, four compounds (**11e**, **11h**, **12k** and **11k**) were selected to examined enzymatic activities against ALK^{WT} and ALK^{L1196M}. The solution of peptide substrates, ATP, approprite kinase, and diluted compound wasmixed 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 started with the addition of tyrosine kinase proteins diluted in 39 μL of kinase reaction buffer solution and incubated at 28 ˚C for 1 h. Subsequently, 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} was presented from the inhibition curves.

4.4. Molecular docking

The molecular docking was performed within Discovery Studio 3.0. The protein coordidates (PDB code: 4MKC, 2YFX) were downloaded from the Protein Data Bank (http://www.rcsb.org/). In the docking process, firstly, the protein was prepared by insertion of missing atoms in residues, addition of formal charges, and so forth. Then, CHARMm force field was used to type the protein model and binding sphere within 12 Å around ceritinib was defined as binding site. The ceritinib, **11e** and **11k** were drawn with Chemsketch 3D and thoroughly minimazed by the CHARMm force field. Finally, possible conformations were searching in the binding site with the Glide protocol in default settings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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