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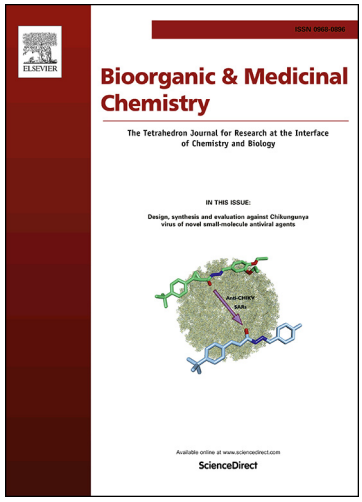

Discoveryof selectiveEGFRmodulatortoinhibitL858R/T790M doublemutants-bearinga*N*-9-Diphenyl-9*H*-purin-2-aminescaffold

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Discovery of selective EGFR modulator to inhibit L858R/T790M double mutants bearing a *N*-9-Diphenyl-9*H*-purin-2-amine scaffold

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Abstract

Based upon the modeling binding mode of marketed **AZD9291** with T790M, a series of *N*-9-Diphenyl-9*H*-purin-2-amine derivatives were designed and synthesized with the purpose to overcome the drug resistance resulted from T790M/L858R double mutations. The most potent compound **23a** showed excellent enzyme inhibitory activities and selectivity with nanomolar IC_{50} values for both the single T790M and double T790M/L858R mutant EGFRs, and was more than 8-fold selective for wild type EGFR. Compound **23a** displayed strong antiproliferative activity against the H1975 non-small cell lung cancer (NSCLC) cells bearing T790M/L858R. And it was less potent against A549 (WT EGFR and k-Ras mutation) and HT-29 (non-special gene type) cells, showing a high safety index.

Keywords: Non-small cell lung cancer; *N*-9-Diphenyl-9*H*-purin-2-amine derivatives; L858R/T790M double mutants

The epidermal growth factor receptor (EGFR) tyrosine kinase played a crucial role in cellular signal-transduction pathways and was closely related to cell proliferation, survival, adhesion, migration, and differentiation.[1,2] Thus, EGFR tyrosine kinase has become an attractive therapeutic target for cancer treatment, especially for nonsmall-cell lung cancer (NSCLC).[3-5]

The first generation of EGFR drugs, **gefitinib** (1, Fig.1) [6,7] and **erlotinib** (2, Fig.1) [8] relied on reversible affinity to achieve potency. More recently, a covalent inhibition strategy has been used because it delivered distinct pharmacological properties. Irreversible kinase inhibitors can achieve advantages over reversible compounds by delivering complete and sustained target engagement in the presence of high intracellular concentrations of ATP in the cells and by requiring the physical turnover of kinase proteins to restore inhibited signaling pathways.[9-11] Indeed, second generation, irreversible inhibitors of WT EGFR (**afatinib**) (3, Fig 1)[12] which covalently modify EGF Cys797 demonstrate increased cellular potency against T790 mutants relative to their reversible counterparts. However because these compounds maintained the aniline moiety that clashed with Met790 side chain, their T790M activity was less than their activity against the primary activating EGFR mutants. As a result, their clinical efficacy to treat T790M

patients was limited due to dose-limiting toxicity associated with inhibition of WT EGFR.

In response to this unmet need and because irreversible inhibitors demonstrated better potency against EGFR T790M mutants than reversible inhibitors, an effort was initiated to discover highly potent irreversible inhibitors of the drug resistant T790M mutants that spare EGFR WT activity to the greatest extent as a means to minimize mechanism-based toxicities. Some irreversible inhibitors of EGFR T790M have been pursued, resulting in the identification of **WZ4002** (4, Fig 1) [13], **CO-1686** (6, Fig 1) [14] and **AZD9291** (5, Fig 1) [15, 16]. In addition, there are several recent publications discussing the design and SAR for novel third generation irreversible EGFR inhibitors.[15,17-19] Among them, **AZD9291** showed 200-fold selectivity for T790M/L858R double mutants over WT EGFR [20] and was approved for the treatment of NSCLC patients with EGFR T790M mutation by FDA in November 2015.

AZD9291 had been reported to have a few common adverse events in clinical trials, such as diarrhea, rash and decreased appetite. And the morbidity of diarrhea, rash and cardiotoxicity increased with escalating doses of **AZD9291** [21]. To overcome the drawbacks of **AZD9291**, in this manuscript, we described the design, synthesis, in vitro biological evaluation of a series of novel selective T790M inhibitors.

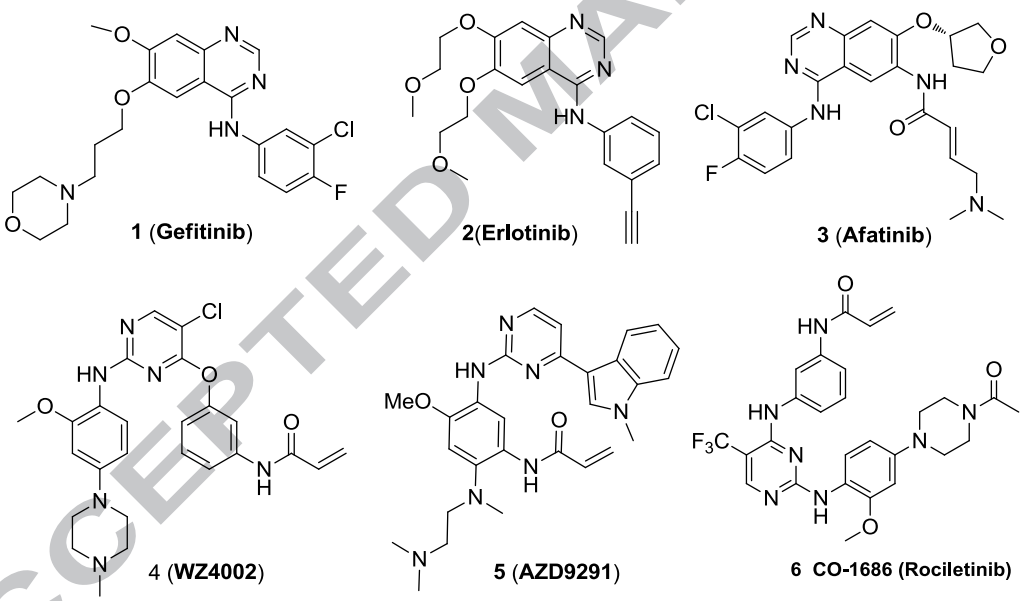


Fig. 1. Structures of different generation EGFR inhibitors

2. **Results and discussion**

2.1. Design strategy of the new compounds

Without the crystal complex of **AZD9291** binding to the T790M EGFR kinase domain, we employed a published T790M structure (PDB code: 3IKA) to model the binding mode with **AZD9291** (**Fig. 2**).The docking mode indicated that **AZD9291** bound to the outer edge of the ATP-binding pocket. The pyrimidine core of the molecule formed two hydrogen bonds with Met793 residue in the kinase hinge: one with the indole group adjacent to the gatekeeper residue, and the other with the dimethylamine moiety positioned in the solvent channel, while Cys797 covalently bonded to the acrylamide group [15]. These interactions were envisioned to be

important for improving the activity and selectivity against mutated EGFR kinase [22].

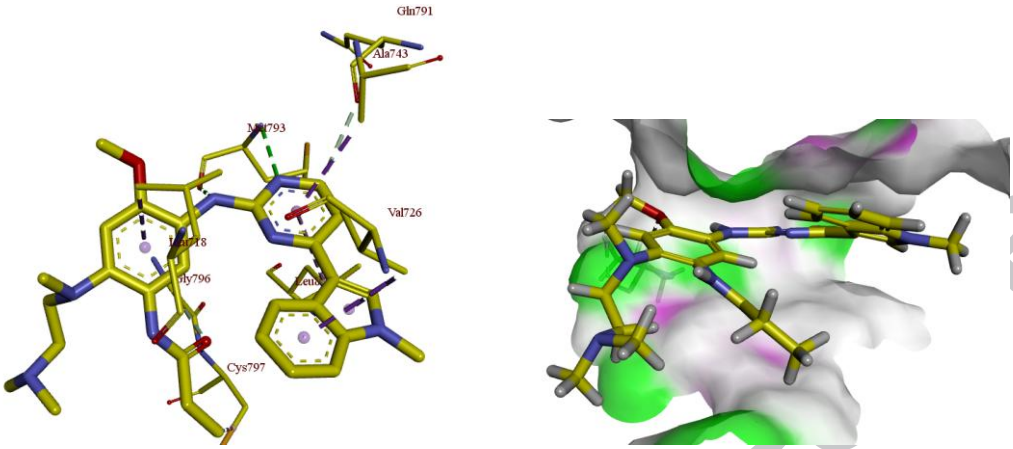


Fig. 2. Interaction map of AZD9291 with the EGFR T790M mutant and the overview in the binding site.

To our knowledge, the purin moiety have been widely applied as building blocks in drug design, and they could be found in several therapeutic agents, such as seliciclib and GS-4059 [23,24]. Based upon the binding mode of **AZD9291** with T790M active domain, we designed and synthesized a series of *N*-9-diphenyl-9*H*-purin-2-amine analogues (**Fig 3**) by applying the conformational constraint strategy, which is an effective method to improve ligand selectivity for a molecular target. Moreover, additional hydrogen bond donors and acceptors were expected to contribute to the interaction with EGFR. More importantly, the aminopyrimidines and a Michael receptor group were expected to contribute to the interaction with EGFR. And a number of side chain amines (R_1) were also modified with the purpose to improve the potency and physical properties of the inhibitors.

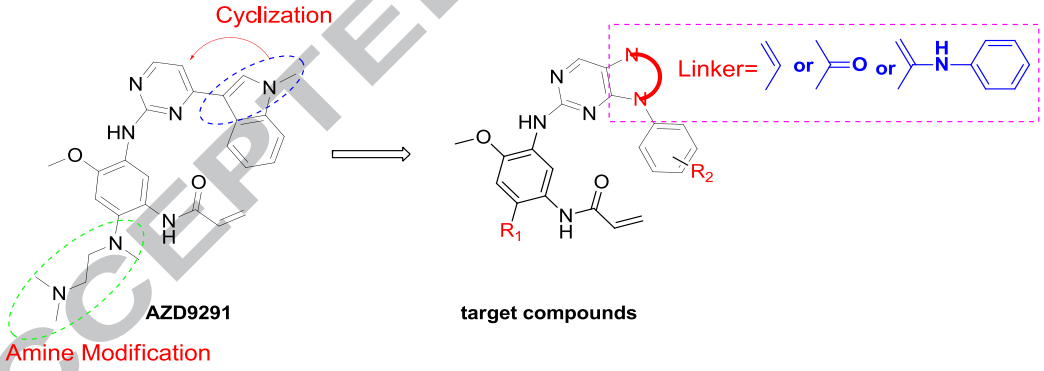


Fig. 3. Design strategy of target compounds.

The novel target scaffold was docked to the EGFR binding pocket (PDB: 3IKA) and overlay results showed that the conformation of this novel scaffold is well consistent with **AZD9291**'s binding conformation, which suggested that a *N*-9-Diphenyl-9*H*-purin-2-amine Scaffold moiety could serve as a scaffold from which to build a novel series of EGFR inhibitors (**Fig. 4**).

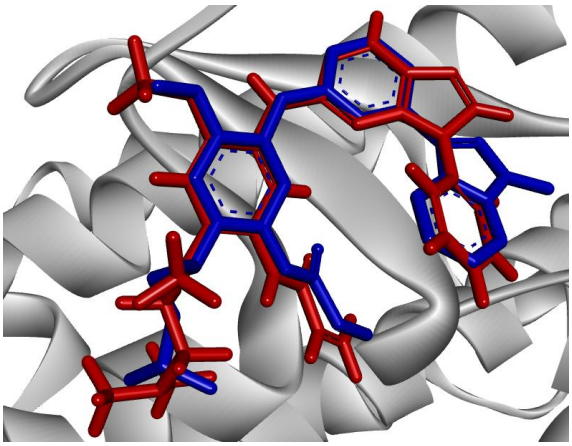


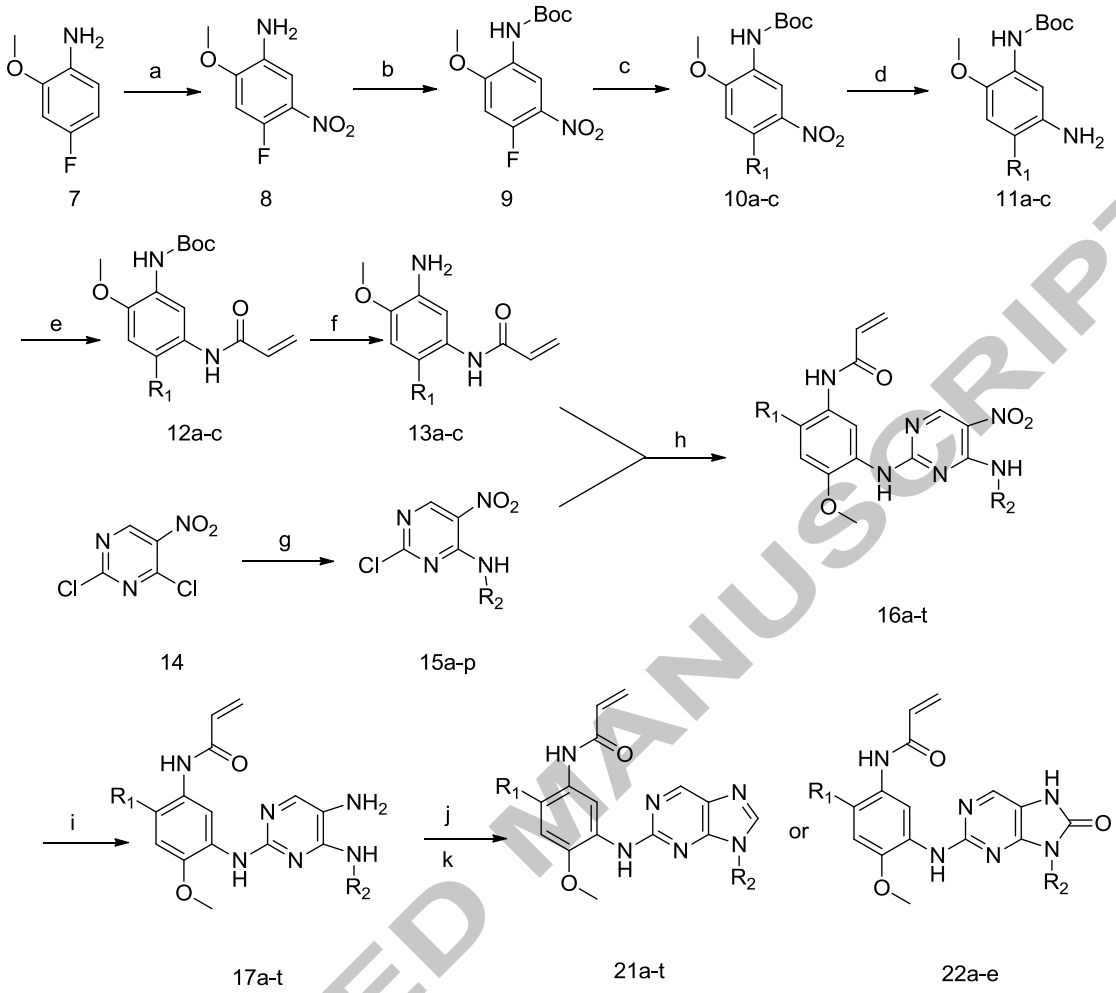
Fig.4 Docking structure of target scaffold (red) overlayed with **AZD9291** (blue) in the complex of $EGFR^{T790M}$ mutant. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. *CHEMISTRY*

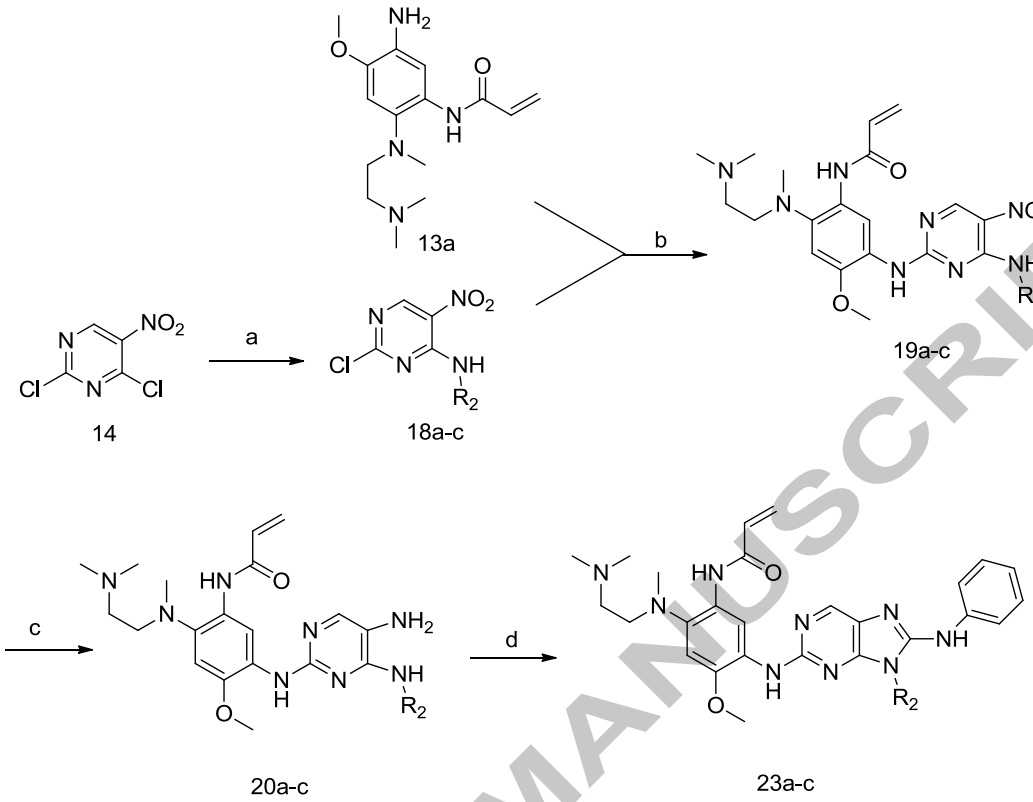
The synthesis of target compounds **21a−t** and **22a-c** were described in **Scheme 1.** The intermediate **8** was synthesized by nitration of commercially available 4-fluoro-2-methoxyaniline 7. Substituted aniline **8** was protected with $(Boc)_2O$ generated the intermediate **9**, which was reacted with various aliphatic amines under basic conditions to formed intermediates **10**. Subsequent reduction of the nitro groups of **10** with hydrogen gave amines **11**, which were not purified and used in the next reaction directly. Acylation of **11a-c** with acryloyl chloride conveniently produced compounds **12a-c**. The key intermediates **13** was obtained by the deprotection of **12** in the presence of trifluoroacetic acid.

Anilines regioselectively reacted with the 4-chloro atom of the 2,4-dichloro-5-nitropyrimidine **14** in the presence of *N*,*N*-diisopropylethylamine (DIPEA) to produce the key pyrimidine intermediates **15**, which coupled with anilines **13** to obtain pyrimidine template compounds **16a-t**. Subsequent reduction of the nitro groups of **16a-t** with stannous chloride gave amines **17a-t** in excellent yield (68−85%). Finally, compounds **17a-t** were treated with formic acid or carbonyldiimidazole to yield the desired 2,9-dianilinopurine derivatives **21a-t** or **22a-e**.

The general steps for preparing the final compounds **23a-c** are outlined in **Scheme 2**. Intermediate **20a-c** was obtained from intermediate **14** via a three-step reaction using similar conditions as described for **16** and **17**. And the resultant intermediates (**20a-c**) were treated with isothiocyanatobenzene to yield the final 2,9-dianilinopurine derivatives **23a-c**.



Scheme 1. Reagents and conditions: (a) KNO_3/H_2SO_4, -15°C, 3h; (b)Boc_2O, DMAP, DCM, 25°C, 6h; (c) appropriate alkyl amine, K_2CO_3, DMF, 65°C, 3-5h; (d) Pd/C, H_2, MeOH, 25°C, 12h; (e) acryloyl chloride, DIPEA, DCM, 25°C, 2h; (f) TFA, DCM, 25°C, 5-7 h; (g) $ArNH_2$, K_2CO_3, DCM, -5-0°C, 2 h; (h) TFA, 1,4-dioxane/IPA, 25°C, 25h; (i) $SnCl_2{\cdot}2H_2O$, MeOH, 65°C,3-4h; (j) formic acid/PPA, 3 h, 125 °C; (k) carbonyldiimidazole, CH_2Cl_2, 3 h, rt.



Scheme 2. Reagents and conditions: (a) appropriate alkyl amine, K_2CO_3, DCM, -5-0°C, 2h; (b) TFA, 1,4-dioxane /IPA, 25°C, 25h; (c) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 3-4h; (d) CH_2Cl_2, DIPEA, DCC, isothiocyanatobenzene, reflux, 12 h.

2.3. Biological evaluation

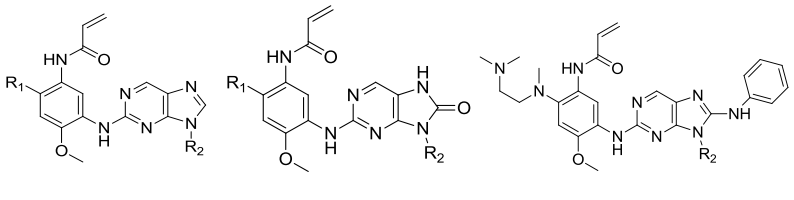
2.3.1. Inhibitory activity against A549, H1975, and HT-29 cells

NCI-H1975 adenocarcinoma cell lines bearing $EGFR^{L858R,T790M}$ point mutations were used for potency optimization while A549 epidermoid carcinoma cell lines bearing overexpressed $EGFR^{WT}$ were used as a counterscreen, allowing an early assessment of selectivity over $EGFR^{WT}$. In addition, these compounds were screened in HT-29 colon cancer cells, which expressed a non-special gene type, to test their toxic effects. The results were expressed as IC_{50} values and summarized in Table 1. The IC_{50} values were the average of at least three independent experiments.

As shown in Table 1, All the target compounds showed moderate-to-excellent cytotoxic activity against H1975 cell line and some exhibited more or similar potent activities against certain cancer

Table 1

Cell cytotoxicity of representative compounds [a].



Compd	R_1	R_2	$IC_{50}(\mu M)$[a]

			A549	H1975	HT-29	Selectivity Ratio A549/H1975
21a		-Ph	0.58±0.13	0.11±0.05	8.08±0.17	58
21b		4-CH_3O-Ph	3.33±0.04	0.16±0.07	1.74±0.05	42
21c		4-CH_3-Ph	0.36±0.07	0.14±0.07	9.23±0.02	3
21d		4-IPE-Ph	14.52±1.24	**0.074±0.024**	15.26±3.01	196
21e		4-C_2H_5-Ph	1.97±0.18	0.13±0.08	8.18±0,17	16
21f		4-F-Ph	0.42±0.08	**0.061±0.031**	11.96±1.22	7
21g		4-Cl-Ph	1.86±0.03	0.085±0.027	7.69±0.03	30
21h		4-Br-Ph	1.15±0.04	0.14±0.06	1.46±0.002	8
21i		4-I-Ph	8.71±0.23	**0.076±0.043**	9.52±0.18	115
21j		2-F-Ph	5.82±0.47	6.72±0.57	9.75±1.44	<1
21k		2-Cl-Ph	12.60±2.34	1.71±0.35	11.33±0.16	7
21l		2-Br-Ph	2.09±0.36	1.80±0.12	16.24±3.34	1
21m		2,4-$(Cl)_2$-Ph	11.2±1.87	1.61±0.23	20.55±2.67	7
21n		3-Br-4-F-Ph	3.02±.023	**0.035±0.012**	3.64±0.09	86
21o		3-Cl-4-F-Ph	5.19±1.54	0.10±0.08	1.61±0.04	35
21p		C_5H_9	2.90±0.21	0.33±0.07	20.85±3.16	9
21q		4-IPE-Ph	9.25±0.05	1.93±0.42	7.69±0.08	5
21r		4-I-Ph	6.31±1.57	7.32±0.16	20.70±0.15	<1
21s		4-IPE-Ph	4.23±0.13	5.10±0.47	23.02±1.63	<1
21t		4-I-Ph	1.52±0.02	29.84±1.69	35.14±0.94	<1
22a		4-I-Ph	1.23±0.05	0.83±0.07	4.82±0.07	15
22b		4-IPE-Ph	2.05±0.67	2.14±0.40	14.29±0.71	1
22c		4-I-Ph	0.49±0.03	10.41±0.21	39.30±3.47	<1
22d		4-IPE-Ph	1.17±0.08	4.04±0.35	1.54±0.16	<1
22e		4-I-Ph	18.52±3.19	4.31±0.43	19.58±0.02	4
23a		CH_3	6.08±1.39	**0.038±0.014**	8.70±.034	160
23b		$(CH_3)_2CH_2$	1.42±0.07	**0.046±0.022**	4.72±0.02	31
23c		$(CH_2)_4CH$	0.78±0.04	0.23±0.05	11.44±0.13	3
AZD9291			0.87±0.05	0.060±0.022	0.65±0.06	9

[a] The biological data are generated from at least three independent experiments.

lines in comparison with **AZD9291**, which indicated that the replacement of pyrimidine moiety in **AZD9291 with** *N*-9-Diphenyl-9*H*-purin-2-amine scaffold via cyclization strategy maintained potent cytotoxicity. In addition, the target compounds suppressed H1975 cells more effectively than they suppressed A549 cells, which suggests that the observed potent efficacy might be attributable to selective inhibition of the mutant form of EGFR. The most promising compounds **21n**, **23a** and **23b** exhibited more potent antitumour activity against H1975 cell line than the positive control **AZD9291** with IC_{50} values from 0.025 μM to 0.046 μM. These compounds are also evaluated against HT-29 colon cancer cells, which expressed a non-special gene type, to test their toxic effects. As shown in **Table 1** compounds **21n**, **23a** and **23b** were less potent against A549 (WT EGFR and k-Ras mutation) and HT-29 (non-special gene type) cells, showing a high safety index. Therefore, compounds **21n**, **23a** and **23b** might be promising candidates to overcome drug resistance mediated by the EGFR T790 Mutant[25].

Our initial effort was to explore the cytotoxic activity of different substituent compounds on the

phenyl ring in order to understand the structure-activity relationship. Primarily, the introduction of electron-withdrawing groups exhibited a positive effect on the cytotoxic activityon on H1975 cells, such as compound **21f** (R_2=4-fluorophenyl, IC_{50} =0.061 μM), **21i** (R_2 = 4-iodophenyl) and **21n** (R_2 =3-bromo-4-fluorophenyl, IC_{50} =0.035 μM), which are similar to or better than that of **AZD9291** (IC_{50} =0.060 μM). However, compound **21f** showed less selectivity between WT and TL EGFR (the ratio of WT/TL was 7). By contrast, monoelectron-donating groups (EDGs) such as 4-methoxy analogues (**21b**-**21c**, **21e**) exhibited a negative effect. Exceptively, compound **21d** exhibited a positive effect on the cytotoxic activity (IC_{50} =0.074 μM). The greatly influenced in cytotoxic potency was observed when halogen atom was introduced to the different position of phenyl ring. For example, shifting the Cl atom from the para position (**21g**, IC_{50}=0.085 μM against H1975) to the ortho position (**21k**, IC_{50}=1.71 μM against H1975) significant decreased inhibition of H1975 cells, while a slightly reduced the inhibition on A549 and HT-29 cells. Moreover, incorporation of another halogen atom (**21m**, R_2 = 2, 4-dichloro, IC_{50} = 1.61 μM against H1975) showed somewhat a decreased cellular activity compared with 4-chloro analogue (**21g**: R_2 = 4-chloro), which indicated monosubstitution of phenyl is more preferred. Interestingly, Isopropyl group at R_2 position increased the TL activities and selectivity in a certain extent. Compared with compound **21a**, isopropyl substituted compound **21d** was merely 1.5-fold potent in activity, but its selectivity was increased by more than 3 folds. Based on the activities and selectivity, compound **21d** and **21i** were selected as leads to optimize.

After the optimization of R_2 moiety, the modification of amine R_1 groups was carried out. As seen from the data on **Table 1**, replacing the ethylenediamine group with rigid N-methyl piperazine group (**21q**, **21r**) or morpholine group (**21s**, **21t**) was lower tolerated and somewhat decreased the biological activity. For example, compared with **21d** (IC_{50} =0.074 μM), compound **21q** (IC_{50} =1.93μM) decreased potency by approximately 26-fold in H1975 cancer, which demonstrated that the *N,N,N'*-trimethylethane-1,2-diamine group was essential for high activity. With regard to compounds **22a**-**22e**, due to the presence of a hydroxyl group on the pyrimidine core, they exhibited very weak against H1975 activity, with IC_{50} values of more than 0.23 μM. In addition, the selectivity between WT and TL EGFR is low (WT / TL <10). Satisfactorily, 2,9-dianilinopurine derivative **23a** (IC_{50} =0.038 μM**)** exhibited a significant enhancement of potency against H1975 cells, as well as it showed less potent against A549 (IC_{50}=6.08μM, WT EGFR and k-Ras mutation) and HT-29 (IC_{50}=8.70μM, non-special gene type) cells, showing a high safety index. Further studies about 2,9-dianilinopurine derivative are in progress in our laboratories and will be reported upon in the future.

2.3.2. Inhibitory activity against EGFR kinase

To rationalize their appealing cellular inhibition, the optimized compounds (**21d**, **21f**, **21g**, **21i**, **21n**, **23a** and **23b**) were evaluated using EGFR kinases with different status including EGFR T790M/L858R, EGFR T790M, and WT EGFR. The results obtained using a well-established mobility shift assay are summarized in **Table 2**, with **AZD9291** as the positive controls.

As shown in **Table 2**, all seven tested compounds exhibited excellent EGFR T790M/L858R enzymatic potency with IC_{50} values ranging from 2.1 to 5.1 nM, which were comparable to the enzymatic activity of **AZD9291**.

The inhibitory activities of compounds **21n** and **23a** were particularly more pronounced with IC_{50} values less than 3 nM. All the tested compounds showed low-level inhibition of WT EGFR (IC_{50}, 21.3-42nM) and among them, compounds **23a** and **23b** were up to 8-fold more selective

against EGFR T790M/L858R than they were against WT EGFR. Although **21n** showed high levels of inhibition of T790M/L858R activity, it was less selective between WT and TL EGFR.

Compound **23a** showed the more potent activity with an IC_{50} value of 2.7 nM, which was similar to that of the positive control, **AZD9291** (IC_{50} = 2.0 nM), indicating that this compound deserves further study with regard to its application in the treatment of cancer. So based on the selectivity and activity data against these three EGFRs, compound **23a** were selected to be further evaluated the drug availability. In addition, it was noteworthy that compounds **21n** and **23a** possessed distinct substructures in their skeletons, highlighting a potential for further investigation of this series.

Table 2

Inhibitory activity against EGFR kinase

Compd	EGFR IC_{50} (nM)[a]			
	T790M/L858R	T790M	WT	TL[b]/WT selectivity
21d	4.2	ND	25	6.0
21f	3.2	5.8	18.2	5.6
21g	3.9	6.4	15.6	7.0
21i	3.5	4.7	17	4.9
21n	2.3	ND	10	4.3
23a	**2.7**	**3.5**	**22.4**	**8.3**
23b	5.1	ND	41	8.0
AZD9291	2.0	3.1	16.7	8.4

ND: not determined.

[a] The biological data are means from at least two replicated experiments.

[b] TL represents EGFR T790M/L858R.

3. Molecular modeling analysis

To further elucidate the binding mode of compounds, a detail docking analysis was performed. In our study, the co-crystal structure of **WZ4002** with $EGFR^{T790M}$ was selected as the docking model (PDB ID code: 3IKA). The docking simulation was conducted using Glide XP (Schrodinger 2014), since Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. The image files were generated using Accelrys DS visualizer 4.0 system. The binding model was exemplified by the interaction of compound **23a** with $EGFR^{T790M}$. As shown in Fig.5, compound **23a** could tightly contact with $EGFR^{T790M}$ through several important binding forces, including: (1) The purin core of the molecule formed two hydrogen bonds with Met793 residue in the kinase hinge; (2) A covalent bond between the acryl amide and the amino acid Cys797; (3) Furthermore, several vander Waals interactions existed between **23a** and the residues of protein, such as the purin ring with Ala743、Met790, and phenyl ring with Met766 and ASP855, respectively; (4)The pep interaction is formed between the phenyl ring and Lys745. All these interactions contribute to the tight binding and greatly enhance the inhibitory potency of **23a**. Compared with **AZD9291**, van der Waals forces of purine core with Met790 and the pep interaction of aniline with Lys745 increased the effect with $EGFR^{T790}$. However, the aniline of compound **23a** lacks the hydrogen bond interaction with $EGFR^{T790M}$, so that further

structural modifications will be focused on the hydrogen bond donor / acceptor substituents in the aniline.

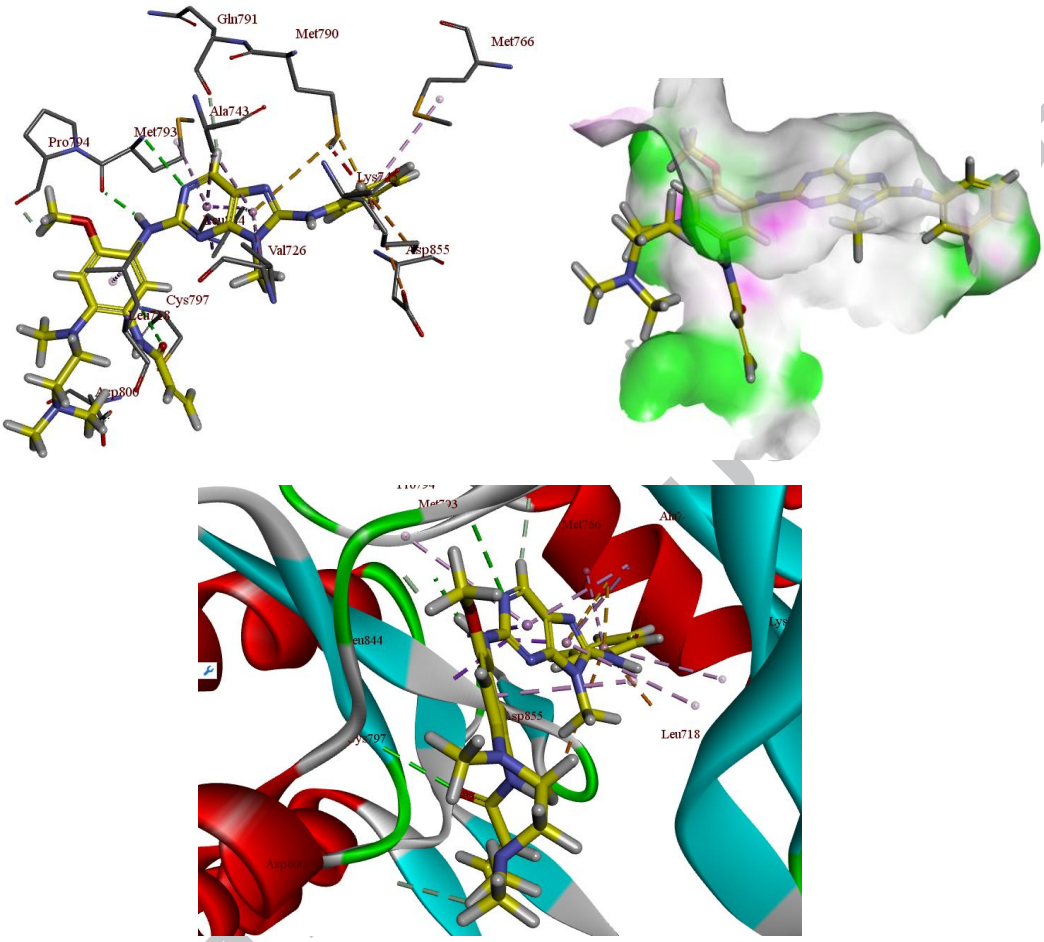


Fig. 5. The EGFR^{T790M} enzyme (PDB code: 3IKA) active site in complex with compound **23a**. Compound **23a** was shown in colored sticks (yellow:carbon atom, blue: nitrogen atom, red: oxygen atom). H-bonding interactions between the **23a** and EGFR^{T790M} were indicated with dotted lines in green. pep interactions were shown with dotted lines in pink. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In addition, we wanted the new compound to be "druglike" with respect to their predicted physicochemical properties. One of our success criteria was that the resulting compounds should be lead-like with respect to their physicochemical properties. As a metric to demonstrate lead-likeness, we chose to evaluate the lipophilic ligand efficiency (LIPE)[26-29] of our most potent analogues and compare them to **AZD9291**. It was gratifying to note that **23a** displayed very good predicted druglike characteristics (LIPE 4.44), comparing favorably to **AZD9291**, the results are shown in Table 3. Although the predicted water solubility was equivalent to that of **AZD9291** (solubility at pH 7.4 in water: <0.01 mg/mL)[30], further analysis showed **23a** to have a favorable topological polar surface area (TPSA)[31] value of 109 Å2.

Table 3

Calculated physicochemical parameters of **23a** and **AZD9291**

Compound	pIC_{50}	cLogP[a]	cLogD[a]	MWt	LIPE[b]
23a	8.57	4.13	3.01	515.6	4.44
AZD9291	8.70	4.49	2.65	499.6	4.21

[a]Calculated using instant JChem. [b]LIPE = pIC_{50} − cLogP.

4. Conclusion

A novel series of selective covalent EGFR T790M inhibitors were designed and synthesized based on the modeling binding mode of marketed drug **AZD9291** with EGFR T790M mutant. The structureactivity relationship (SAR) was discussed in detail and the results demonstrated that substitution with bearing a *N*,9-Diphenyl-9*H*-purin-2-amine scaffold was considered as a novel strategy for maintaining EGFR T790M inhibitory activity and selectivity. Among the most promising inhibitors, compound **23a** potently inhibited the enzymatic function of EGFR T790M/L858R with an IC_{50} value of 2.7 nM and NSCLC H1975 cell with an IC_{50} value of 38 nM. It was also less potent against A549 (WT EGFR and k-Ras mutation) and HT-29 (non-special gene type) cells, showing a high safety index. Subsequent satisfactory predictions were obtained by predicting the physicochemical properties of compound **23a**. Further studies on structural optimization and biological activities about these derivatives are still underway in our laboratory and will be reported in the future.

5. Experimental section

5.1. Chemistry

Reagents and solvents were obtained from commercial sources and used without further purification. The purity of the synthesized compounds was measured by high performance liquid chromatography (HPLC, Agilent, USA). Flash chromatography was performed using silica gel (200-300 mesh). All reactions were monitored by TLC on silica gel plates. Melting points were deter determined on Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ARX-400, 400MHz or Bruker ARX-600, 600 MHz spectrometers (Bruker Bioscience Billerica, MA, USA) with TMS as an internal standard. Mass spectra (MS) were measured in ESI mode on an Aglient 1100 LC-MS (Agilent, Palo Alto, CA, USA). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). The IR spectra were recorded by means of the KBr pellet technique on a Bruker FTS 135 spectrometer.

5.2 Preparation of 4-Fluoro-2-methoxy-5-nitro-phenylamine (**8**)

4-Fluoro-2-methoxyaniline(12.0g, 85.0 mmol) was added to cooled concentrated H_2SO_4 (80 mL). The mixture was stirred at 0-10°C for 15-30 min. KNO_3 (8.6g, 85.0 mmol) was added to the mixture. The resulting mixture was stirred at 0-5°C for 1-2 h and then poured into ice/water. The mixture was neutralised with concentrated K_2CO_3. The resulting solid was filtered off and dried under vacuum. The corresponding amine was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO) δ 7.35 (d, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 13.4 Hz, 1H), 5.28 (s, 1H), 3.91 (s, 1H).

5.3. Preparation of tert-butyl (4-fluoro-2-methoxy-5-nitrophenyl)carbamate (**9**)

A solution of **8** (8.0g, 43.0 mmol) in CH_2Cl_2 (100 mL) was cooled to 0-5°C in an ice/water bath. Boc_2O (9.4g, 43.0 mmol) in DCM (20 mL) was added to the mixture slowly. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated to dryness under reduced pressure. The crude product was purified by flash silica chromatography, with gradient 10−40% EtOAc in hexane to yield intermediate (**9**) (12.0 g, 42.1 mmol, 98%) as a yellow solid. m.p. 88-90 °C (EtOAc /hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (br, 1H), 6.99 (s, 1H), 6.72 (d, *J*=12.0, 1H), 4.0 (s, 3H), 1.567 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.5, 152.6, 152.5,152.2, 150.9, 124.7, 124.7, 114.7, 100.0, 99.7, 56.8, 28.3, 7.9; IR 3432, 2985, 1723, 1536, 1485, 1158. MS calcd for $C_{12}H_{15}FN_2O_5$[M-H]$^-$ 285.0887; found: 285.0865

*5.4 General procedure for preparation of compounds (**10a-c**)*

Appropriate alkyl amine (2.7 g, 27.0 mmol) was added to a solution of intermediate (**9**) (6.3g, 22.0 mmol) and DIPEA (*N*,*N*-Diisopropylethylamine) (3.82 mL, 22.0 mmol) in DMF (100 mL). The mixture was heated to 60 °C and stirred at this temperature for 2h. Water (200 mL) was added to the reaction mixture, which was then extracted with DCM (50 mL*3). The combined extracts were dried over anhydrous magnesium sulfate. The solvent was removed under vacuum to afford compounds **10a-c**, respectively, which were used without further purification.

*5.4.1. tert-butyl (4-((2-(dimethylamino)ethyl)(methyl)amino)- 2-methoxy-5 -nitrophenyl) carbamate(**10a**)*

^{1}H NMR (400MHz，DMSO-d_6) δ 8.12 (m, 2H), 6.74 (s, 1H), 3.90 (s, 3H), 3.21 (m, 2H), 2.81 (s, 3H), 2.44 (m, 2H), 2.14 (s, 6H), 1.45 (s, 9H); ^{13}C NMR (100MHz，DMSO-d_6) δ 153.5, 144.9, 132.1, 120.0, 102.4, 79.8, 56.8, 56.7, 53.1, 45.9, 40.8, 40.6, 40.4, 28.5, 27.9; IR (KBr) cm^{-1}: 3441, 2979, 2359, 1708, 1546, 1162; ESI-MS m/z: 369.2 [M+H]$^+$.

*5.4.2. tert-butyl (2-methoxy-4-(4-methylpiperazin-1-yl)-5-nitrophenyl)carbamate(**10b**)*

Yellow solid. Yield: 78%. ESI-MS m/z: 367.2 [M +H]$^+$.

*5.4.3. tert-butyl (2-methoxy-4-morpholino-5-nitrophenyl)carbamate(**10c**)*

Yellow solid. Yield: 75%. ESI-MS m/z: 354.3[M +H]$^+$.

*5.5. General procedure for preparation of compounds (**11a-c**)*

To a solution of **10** (22.6 mmol) in ethanol (150 mL) was added 10% Pd/C (0.5 g). The reaction mixture was stirred at room temperature under an atmosphere of H_2 for 9 h. After completion of the reaction, the resulting mixture was filtered through Celite, and the filtered catalyst was washed with ethanol. The filtrate was concentrated under high vacuum to afford compound **11a-c**, respectively.

*5.5.1 tert-butyl (5-amino-4-((2-(dimethylamino)ethyl)(methyl)amino)- 2-methoxyphenyl) carbamate(**11a**).*

Brown liquid. Yield: 78%. ^{1}H NMR (DMSO-d_6) δ 8.12 (m, 2H), 6.74 (s, 1H), 3.90 (s, 3H), 3.21 (m, 2H), 2.81 (s, 3H), 2.44 (m, 2H), 2.14 (s, 6H), 1.45 (s, 9H). ESI-MS m/z: 369.2 [M+H]$^+$.

*5.5.2. tert-butyl (5-amino-2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)carbamate(**11b**)*

Brown liquid. Yield: 69%. ESI-MS m/z: 337.2 $[M+H]^+$.

*5.5.3. tert-butyl (5-amino-2-methoxy-4-morpholinophenyl)carbamate(**11c**)*

Brown liquid. Yield: 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (s, 1H), 7.10 (s, 1H), 6.62 (s, 1H), 4.47 (s, 1H), 3.76 – 3.70 (m, 4H), 3.69 (s, 3H), 2.80 – 2.71 (m, 4H), 1.44 (s, 9H). ESI-MS m/z: 324.2 $[M+H]^+$.

*5.6. General procedure for preparation of compounds (**12a-c**)*

To a solution of **11** (2.0 g, 7.9 mmol) and DIPEA (1.38 mL, 7.9 mmol) in CH_2Cl_2 (50 mL) at 0 °C, acryloyl chloride (0.64 mL, 7.9 mmol) was added dropwise. The mixture was stirred for 1 h, then extracted with water (2-50 mL). The organic layerwas dried over anhydrous Na_2SO_4, and evaporated to give the crude residue. Compounds (**12a-c)** was obtained as a pale-yellow solid after purification by silica gel column chromatography ((CH_2Cl_2:MeOH, 30:1 to 15:1 v/v).

*5.6.1. tert-butyl 5-acrylamido-4-((2-(dimethylamino)ethyl)(methyl)amino) -2-methoxyphenyl) carbamate(**12a**)*

Brown solid. Yield: 54%. m.p. 92-94°C. ESI-MS m/z: 393.5$[M+ H]^+$.

*5.6.2. tert-butyl (5-acrylamido-2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)carbamate (**12b**).*

Brown solid. Yield: 57%. ESI-MS m/z: 393.5$[M+ H]^+$.

*5.6.3. tert-butyl (5-acrylamido-2-methoxy-4-morpholinophenyl)carbamate(**12c**)*

Brown solid. Yield: 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.17 (s, 1H), 7.88 (s, 1H), 6.80 (s, 1H), 6.63 (dd, J = 16.8, 10.6 Hz, 1H), 6.21 (d, J = 17.1 Hz, 1H), 5.72 (d, J = 10.1 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 4H), 2.80 (s, 4H), 1.44 (s, 9H).ESI-MS m/z: 378.2$[M+ H]^+$.

*5.7. General procedure for preparation of compounds (**13a-c**)*

TFA (20 mmol) was added to a solution of **12** (2 mmol) in CH_2Cl_2 (50 mL) at room temperature. The reaction mixture was stirred for 2-7 h, and was monitored by TLC. The mixture was extracted with water (2*100 mL), the combined aqueous layer was neutralized with aqueous K_2CO_3 to pH 7-8. The precipitate was filtered and dried to generate the compounds **13a-c**, respectively.

*5.7.1. N-(5-amino-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide (**13a**)*

Gray solid. Yield: 75.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H), 7.59 (s, 1H), 6.78 (s, 1H), 6.45 (s, 1H), 6.19 (dd, *J* = 16.9, 1.9 Hz, 1H), 5.69 (dd, *J* = 10.1, 1.9 Hz, 1H), 4.64 (s, 2H), 3.74 (s, 3H), 2.86 (s, 2H), 2.57 (s, 3H), 2.29 (s, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.3, 143.6, 133. 9, 132.4, 132.3, 129.9, 125.9, 107.1, 105.2, 57.2, 55.8, 45.15, 44.2; IR 3452, 3342, 2935, 2823, 1670, 1526, 1207; ESI-MS m/z: 293.2$[M+ H]^+$

*5.7.2. N-(5-amino-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide(**13b**)*

Gray solid. Yield: 73.1%. ESI-MS m/z: 291.2$[M+ H]^+$

*5.7.3. N-(5-amino-4-methoxy-2-morpholinophenyl)acrylamide(**13c**)*

Gray solid. Yield: 67.3%. ESI-MS m/z: 278.1$[M+ H]^+$

*5.8. General procedure for preparation of compounds (**16a-t**)*

A solution of **13** (3.0 g, 12.4 mmol) and **15** (2.2 g, 12.4 mmol) in 1,4-dioxane/IPA (20 mL) added 10% TFA (300 mg), and the resulting suspension was stirred at 25°C under an atmospheric pressure of N_2 for 12 h. The resulting precipitate was collected by filtration, rinsing with EtOH, to give compounds **16a-t** as its hydrochloride salt.

*5.8.1. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((5-nitro-4-(phenylamino)pyrimidin-2-yl)amino)phenyl)acrylamide(**16a**)*

Yellow solid; Yield: 58.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.75 (s, 1H), 7.75 (dd, *J* = 15.1, 3.1 Hz, 2H), 7.33 (t, *J* = 15.0 Hz, 2H), 7.12 (s, 1H), 7.05 (t, *J* = 3.0 Hz, 1H), 6.43 (s, 1H), 6.28 (dd, *J* = 34.1, 19.2 Hz, 2H), 6.05 (dd, *J* = 20.0, 4.6 Hz, 1H), 5.69 (dd, *J* = 33.3, 4.6 Hz, 1H), 3.86 (s, 3H), 3.57 – 3.47 (m, 2H), 2.75 (s, 3H), 2.50 (t, *J* = 10.4 Hz, 2H), 2.21 (s, 6H). MS (ESI) m/z(%): 492.4 $[M+H]^+$, 514.4 $[M+Na]^+$.

*5.8.2. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-((4-methoxyphenyl)amino)-5-nitropyrimidin-2-yl)amino)phenyl)acrylamide(**16b**)*

Yellow solid; Yield: 55.6%; MS (ESI) m/z(%): 537.2 $[M+H]^+$, 559.2 $[M+Na]^+$.

*5.8.3. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((5-nitro-4-(p-tolylamino)pyrimidin-2-yl)amino)phenyl)acrylamide(**16c**)*

Yellow solid; Yield: 50.1%; MS (ESI) m/z(%): 521.3 $[M+H]^+$, 543.3 $[M+Na]^+$.

*5.8.4. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((4-((4-isopropylphenyl)amino)-5-nitropyrimidin-2-yl)amino)-4-methoxyphenyl)acrylamide(**16d**)*

Yellow solid; Yield: 43.7%; MS (ESI) m/z(%): 549.3 $[M+H]^+$, 556.2 $[M+Na]^+$.

*5.8.5. N-(5-((4-((4-ethylphenyl)amino)-5-nitropyrimidin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide(**16e**)*

Yellow solid; Yield: 45.5%; MS (ESI) m/z(%): 520.2 $[M+H]^+$, 571.3 $[M+Na]^+$

*5.8.6. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((4-((4-fluorophenyl)amino)-5-nitropyrimidin-2-yl)amino)-4-methoxyphenyl)acrylamide(**16f**)*

Yellow solid; Yield: 67.5%; MS (ESI) m/z(%): 525.2 $[M+H]^+$, 547.2 $[M+Na]^+$

*5.8.7. N-(5-((4-((4-chlorophenyl)amino)-5-nitropyrimidin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**16g**)*

Light yellow solid; Yield: 72.1%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 9.07 (s, 1H), 8.19 (s, 1H), 7.74 (s, 1H), 7.19 (s, 2H), 7.00 (s, 2H), 6.22 (dd, *J* = 26.4, 9.0 Hz, 2H), 5.74 – 5.67 (m, 1H), 3.76 (s, 3H), 2.96 (s, 2H), 2.69 – 2.64 (m, 3H), 2.31 (s, 8H). MS (ESI) m/z(%): 541.2 $[M+H]^+$, 563.2 $[M+Na]^+$

*5.8.8. N-(5-((4-((4-bromophenyl)amino)-5-nitropyrimidin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**16h**)*

Yellow solid; Yield: 69.5%; MS (ESI) m/z(%): 585.1 $[M+H]^+$, 607.1 $[M+Na]^+$

*5.8.9. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((4-((4-iodophenyl)amino)-5-nitropyrimidin-2-yl)amino)-4-methoxyphenyl)acrylamide(**16i**)*

Yellow solid; Yield: 73.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (s, 1H), 9.04 (s, 1H), 8.16 (s, 1H), 7.71 (s, 2H), 7.53 (s, 2H), 7.23 (s, 1H), 7.07 (s, 1H), 6.82 (s, 1H), 6.52 (s, 1H), 6.24 (s, 1H), 5.74 (s, 1H), 3.78 (s, 3H), 2.93 (s, 2H), 2.70 (s, 3H), 2.24 (d, *J* = 36.8 Hz, 8H). MS (ESI) m/z(%): 633.1 $[M+H]^+$, 655.1 $[M+Na]^+$

*5.8.10. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((4-((2-fluorophenyl)amino)-5-nitropyrimidin-2-yl)amino)-4-methoxyphenyl)acrylamide(**16j**)*

Yellow solid; Yield: 73.5%; MS (ESI) m/z(%): 525.2 $[M+H]^+$, 563.2 $[M+K]^+$

*5.8.11. N-(5-((4-((2-chlorophenyl)amino)-5-nitropyrimidin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**16k**)*

Yellow solid; Yield: 75.5%; MS (ESI) m/z(%): 541.2$[M+H]^+$, 563.2 $[M+Na]^+$

*5.8.12. N-(5-((4-((2-bromophenyl)amino)-5-nitropyrimidin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**16l**)*

Yellow solid; Yield: 78.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 9.11 (s, 1H), 8.14 (d, *J* = 33.3 Hz, 2H), 7.60 (s, 1H), 7.01 (s, 2H), 6.73 (s, 1H), 6.21 (dd, *J* = 18.7, 13.5 Hz, 1H), 5.73 (dd, *J* = 12.8, 9.0 Hz, 1H), 3.79 (d, *J* = 19.8 Hz, 3H), 3.04 (s, 2H), 2.69 (s, 3H), 2.38 (s, 8H); MS (ESI) m/z(%): 585.1$[M+H]^+$, 607.1 $[M+Na]^+$

*5.8.13. N-(5-((4-((2,4-dichlorophenyl)amino)-5-nitropyrimidin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**16m**)*

Yellow solid; Yield: 62.5%; MS (ESI) m/z(%): 575.2$[M+H]^+$, 597.2 $[M+Na]^+$

*5.8.14. N-(5-((4-((4-bromo-3-fluorophenyl)amino)-5-nitropyrimidin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**16n**)*

Yellow solid; Yield: 67.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 9.04 (s, 1H), 8.18 (dd, *J* = 6.3, 2.5 Hz, 1H), 8.01 (s, 1H), 7.69 – 7.66 (m, 1H), 7.39 (s, 1H), 7.27 (s, 1H), 6.96 (d, *J* = 1.9 Hz, 1H), 6.21 (d, *J* = 16.4 Hz, 1H), 5.71 (dd, *J* = 9.6, 1.0 Hz, 1H), 3.80 (s, 3H), 3.25 (s, 2H), 3.12 (s, 3H), 2.76 (d, *J* = 4.8 Hz, 6H), 2.60 (s, 2H); MS (ESI) m/z(%): 603.1$[M+H]^+$, 625.1 $[M+Na]^+$

*5.8.15. N-(5-((4-((3-chloro-4-fluorophenyl)amino)-5-nitropyrimidin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**16o**)*

Yellow solid; Yield: 56.3%; MS (ESI) m/z(%): 559.2$[M+H]^+$, 582.2 $[M+Na]^+$

*5.8.16. N-(5-((4-(cyclopentylamino)-5-nitropyrimidin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**16p**)*

Yellow solid; Yield: 58.6%; MS (ESI) m/z(%): 499.3$[M+H]^+$, 521.3$[M+Na]^+$

*5.8.17. N-(5-((4-((4-isopropylphenyl)amino)-5-nitropyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide(**16q**)*

Yellow solid; Yield: 77.6%; MS (ESI) m/z(%): 547.3$[M+H]^+$, 569.3$[M+Na]^+$

*5.8.18. N-(5-((4-((4-iodophenyl)amino)-5-nitropyrimidin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide(**16r**)*

Yellow solid; Yield: 73.4%; MS (ESI) m/z(%): 631.1$[M+H]^+$, 653.1$[M+Na]^+$

*5.8.19. N-(5-((4-((4-isopropylphenyl)amino)-5-nitropyrimidin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide(**16s**)*

Yellow solid; Yield: 54.5%; MS (ESI) m/z(%): 534.2$[M+H]^+$, 556.2$[M+Na]^+$

*5.8.20. N-(5-((4-((4-iodophenyl)amino)-5-nitropyrimidin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide(**16t**)*

Yellow solid; Yield: 59.5%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.21 (s, 1H), 9.10 (d, *J* = 45.0 Hz, 2H), 7.93 (s, 1H), 7.38 (d, *J* = 70.3 Hz, 3H), 6.93 (s, 1H), 6.76 – 6.60 (m, 1H), 6.20 (d, *J* = 16.9 Hz, 1H), 5.73 (d, *J* = 10.0 Hz, 1H), 3.85 (s, 3H), 3.75 (s, 4H), 2.92 (s, 4H); MS (ESI) m/z(%):618.1$[M+H]^+$, 640.1$[M+Na]^+$

*5.9. General procedure for preparation of compounds (**21a-t**)*

Compound **16** was dissolved in a mixture of EtOH and water ((v/v) 3.6/1). Iron powder and NH_4Cl were added to it, and the mixture was then stirred in reflux for 8 h, cooled to room temperature, and filtered through a pad of Celite. The filtrate was concentrated in vacuo. The residue was extracted with EtOAc, and the organic extract was washed with saturated $NaHCO_3$, water, and brine and dried over anhydrous $MgSO_4$. It was then filtered and concentrated in vacuo to generate the compounds **17**. A solution of **17** (3 mmol), frmic acid (1.5 equiv) and PPA (1.5 equiv) in a mixture of DMF (10 mL) was stirred at 125°C for 4h. The volatiles were evaporated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2/MeOH 15:1) to generate the compounds **21a-t**, respectively.

*5.9.1. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((9-phenyl-9H-purin-2-yl)amino)phenyl)acrylamide (**21a**)*

Yield: 75.6%, M.p.:134.2-135.1°C ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H), 8.90 (d, *J* = 6.7 Hz, 2H), 8.69 (s, 1H), 8.22 (s, 1H), 7.96 (d, *J* = 7.8 Hz, 2H), 7.51 (t, *J* = 7.8 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 1H), 6.99 (s, 1H), 6.52 (s, 1H), 6.28 (d, *J* = 16.4 Hz, 1H), 5.80 (d, *J* = 10.1 Hz, 1H), 3.84 (s, 3H), 2.90 (s, 2H), 2.68 (s, 3H), 2.38 (s, 2H), 2.26 (s, 6H); MS (ESI) m/z(%): 487.2 $[M+H]^+$; Anal. calcd. for $C_{26}H_{30}N_8O_2$ (%): C, 64.18; H, 6.21; N, 23.03; Found (%): C, 64.22; H, 6.17; N, 23.07.

*5.9.2. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((9-(4-methoxyphenyl)-9H-purin-2-yl)amino)phenyl)acrylamide(**21b**)*

Yield: 65.5%, M.p.:143.7-144.5°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.57 (s, 1H), 9.32 (s, 1H), 8.80 (s, 1H), 8.17 (s, 1H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.04 (d, *J* = 8.9 Hz, 2H), 6.66 (s, 1H), 6.52 (dd, *J* = 16.8, 1.6 Hz, 1H), 5.80 (dd, *J* = 10.1, 1.6 Hz, 1H), 3.90 (s, 3H), 3.82 (s, 3H), 3.32 (s, 2H), 3.17 (s, 2H), 2.81 (s, 6H), 2.73 (s, 3H). MS (ESI) m/z(%): 517.3 $[M+H]^+$; Anal. calcd. For $C_{27}H_{32}N_8O_3$ (%): C, 62.77; H, 6.24; N, 21.69;. Found (%): C, 62.80; H, 6.27; N, 21.65.

*5.9.3. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((9-(p-tolyl)-9H-purin-2-yl)amino)phenyl)acrylamide(**21c**)*

Yield: 78.6%, M.p.: 162.2-163.1°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H), 8.89 (d, *J* = 4.7 Hz, 2H), 8.65 (s, 1H), 8.17 (s, 1H), 7.83 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 6.98 (s, 1H), 6.50 (dd, *J* = 17.0, 10.1 Hz, 1H), 6.29 (dd, *J* = 16.9, 1.6 Hz, 1H), 5.82 (dd, *J* = 10.1, 1.6 Hz, 1H), 3.85 (s, 3H), 2.89 (s, 2H), 2.69 (s, 3H), 2.32 (s, 5H), 2.24 (s, 6H). MS (ESI) m/z(%): 517.3 $[M+H]^+$; Anal. calcd. For $C_{27}H_{32}N_8O_2$ (%): C, 64.78; H, 6.44; N, 22.38; Found (%): C, 64.85; H, 6.58; N, 23.29.

*5.9.4. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((9-(4-isopropylphenyl)-9H-purin-2-yl)amino)-4-methoxyphenyl)acrylamide(**21d**)*

Yield: 63.2%, M.p.: 114.2-115.3°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.97 (s, 1H), 8.90 (s, 1H), 8.64 (s, 1H), 8.14 (s, 1H), 7.87 (d, *J* = 8.3 Hz, 2H), 7.37 (d, *J* = 8.2 Hz, 2H), 7.00 (s, 1H), 6.47 (dd, *J* = 16.7, 10.3 Hz, 1H), 6.33 (d, *J* = 16.8 Hz, 1H), 5.83 (d, *J* = 10.1 Hz, 1H), 3.84 (s, 3H), 2.88 – 2.82 (m, 2H), 2.70 (s, 3H), 2.29 – 2.24 (m, 2H), 2.21 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.38, 154.45, 153.03, 150.50, 150.40, 149.68, 146.35, 136.06, 134.99, 130.02, 128.24, 127.95, 125.00, 124.33, 123.54, 122.63, 115.33, 107.54, 57.31, 56.83, 52.23, 45.57, 39.57, 33.96, 23.38. MS (ESI) m/z(%): 529.2 $[M+H]^+$; Anal. calcd. For $C_{29}H_{36}N_8O_2$ (%): C, 65.89; H, 6.86; N, 21.20; Found (%): C, 65.80; H, 6.78; N, 21.29.

*5.9.5. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((9-(4-ethylphenyl)-9H-purin-2-yl)amino)-4-methoxyphenyl)acrylamide(**21e**)*

Yield: 71.2%, M.p.: 152.9-153.7°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 8.91 (d, *J* = 9.6 Hz, 2H), 8.65 (s, 1H), 8.17 (s, 1H), 7.86 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.3 Hz, 2H), 6.99 (s, 1H), 6.48 (dd, *J* = 16.8, 10.1 Hz, 1H), 6.31 (dd, *J* = 17.0, 1.8 Hz, 1H), 5.82 (dd, *J* = 10.1, 1.8 Hz, 1H), 3.84 (s, 3H), 2.87 (t, *J* = 5.5 Hz, 2H), 2.68 (d, *J* = 12.7 Hz, 3H), 2.61 (dd, *J* = 15.4, 7.8 Hz, 2H), 2.32 (d, *J* = 5.3 Hz, 2H), 2.23 (s, 6H), 1.18 (t, *J* = 7.6 Hz, 3H).MS (ESI) m/z(%): 515.2 $[M+H]^+$; Anal. calcd. For $C_{28}H_{34}N_8O_2$ (%): C, 65.35; H, 6.66; N, 21.77; Found (%): C, 65.42; H, 6.75; N, 21.60.

*5.9.6. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((9-(4-fluorophenyl)-9H-purin-2-yl)amino)-4-methoxyphenyl)acrylamide(**21f**)*

Yield: 62.6%, M.p.:186.6-187.5°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.90 (d, *J* = 6.1 Hz, 2H), 8.66 (s, 1H), 8.20 (s, 1H), 8.00 (s, 2H), 7.34 (t, *J* = 8.5 Hz, 2H), 6.99 (s, 1H), 6.49 (d, *J* = 9.8 Hz, 1H), 6.29 (dd, *J* = 17.0, 0.9 Hz, 1H), 5.88 – 5.75 (m, 1H), 3.85 (s, 3H), 2.90 (s, 2H), 2.69 (s, 3H), 2.37 (s, 2H), 2.29 (d, *J* = 25.9 Hz, 6H). MS (ESI) m/z(%): 505.2 $[M+H]^+$; Anal. calcd. For $C_{26}H_{29}FN_8O_2$ (%): C, 61.89; H, 5.79; N, 22.21. Found (%): C, 62.83; H, 5.84; N, 22.25.

*5.9.7. N-(5-((9-(4-chlorophenyl)-9H-purin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**21g**)*

Yield: 52.5%, M.p.:125.3-126.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.92 (d, *J* = 8.4 Hz, 2H), 8.71 (s, 1H), 8.23 (s, 1H), 8.04 (d, *J* = 8.7 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.00 (s, 1H), 6.49 (dd, *J* = 17.0, 9.9 Hz, 1H), 6.30 (dd, *J* = 16.9, 1.7 Hz, 1H), 5.83 (dd, *J* = 10.3, 1.4 Hz, 1H), 3.85 (s, 3H), 2.88 (t, *J* = 5.6 Hz, 2H), 2.70 (s, 3H), 2.33 (d, *J* = 5.2 Hz, 2H), 2.23 (s, 6H). MS (ESI) m/z(%): 521.3

$[M+H]^+$; Anal. calcd. For $C_{26}H_{29}ClN_8O_2$ (%): , C, 59.94; H, 5.61; N, 21.51; Found (%): C, 59.98; H, 5.57; N, 21.56

*5.9.8. N-(5-((9-(4-bromophenyl)-9H-purin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**21h**)*

Yield: 52.6%, M.p.:186.9-187.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 8.92 (d, *J* = 4.0 Hz, 2H), 8.71 (s, 1H), 8.23 (s, 1H), 7.98 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 2H), 7.01 (s, 1H), 6.47 (dd, *J* = 16.9, 10.0 Hz, 1H), 6.30 (d, *J* = 16.5 Hz, 1H), 5.83 (d, *J* = 10.8 Hz, 1H), 3.85 (s, 3H), 2.87 (s, 2H), 2.70 (s, 3H), 2.29 (s, 2H), 2.22 (s, 6H). MS (ESI) m/z(%): 565.2 $[M+H]^+$, 567.2 [M+H]; Anal. calcd. For $C_{26}H_{29}BrN_8O_2$ (%): , C, 55.23; H, 5.17; N, 19.82; Found (%): C, 55.27; H, 5.21; N, 19.86.

*5.9.9. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((9-(4-iodophenyl)-9H-purin-2-yl)amino)-4-methoxyphenyl)acrylamide(**21i**)*

Yield: 62.4%, M.p.:154.1-155.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.92 (d, *J* = 10.0 Hz, 2H), 8.70 (s, 1H), 8.22 (s, 1H), 7.84 (s, 4H), 7.13 – 6.86 (m, 1H), 6.70 – 6.03 (m, 2H), 5.84 (dd, *J* = 8.1, 1.6 Hz, 1H), 3.85 (s, 3H), 2.86 (s, 2H), 2.71 (s, 3H), 2.24 (d, *J* = 21.9 Hz, 8H). MS (ESI) m/z(%): 613.4 $[M+H]^+$; Anal. calcd. For $C_{26}H_{29}IN_8O_2$ (%): C, 50.99; H, 4.77; N, 18.30; Found (%): C, 50.87; H, 4.70; N, 18.18.

*5.9.10. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((9-(2-fluorophenyl)-9H-purin-2-yl)amino)-4-methoxyphenyl)acrylamide(**21j**)*

Yield: 66.2%, M.p.:154.1-155.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 9.51 (s, 1H), 8.84 (s, 1H), 8.24 (s, 2H), 7.45 (d, *J* = 61.5 Hz, 2H), 6.95 (s, 2H), 6.44 (s, 1H), 6.22 (d, *J* = 17.2 Hz, 1H), 5.79 – 5.66 (m, 1H), 3.83 (s, 3H), 2.89 (s, 2H), 2.66 (s, 3H), 2.25 (s, 8H). MS (ESI) m/z(%): 505.6 $[M+H]^+$; Anal. calcd. For $C_{26}H_{29}FN_8O_2$ (%): C, 61.89; H, 5.79;N, 22.21; Found (%): C, 61.81; H, 5.70; N, 22.15.

*5.9.11. N-(5-((9-(2-chlorophenyl)-9H-purin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**21k**)*

Yield: 71.2%, M.p.:164.1-165.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 9.51 (s, 1H), 8.84 (s, 1H), 8.24 (s, 2H), 7.45 (d, *J* = 61.5 Hz, 2H), 6.95 (s, 2H), 6.44 (s, 1H), 6.22 (dd, *J* = 16.9, 1.2 Hz, 1H), 5.79 – 5.66 (m, 1H), 3.83 (s, 3H), 2.89 (s, 2H), 2.66 (s, 3H), 2.25 (s, 8H). MS (ESI) m/z(%): 521.2 $[M+H]^+$; Anal. calcd. For $C_{26}H_{29}ClN_8O_2$(%): C, 59.94; H, 5.61; N, 21.51; Found (%): C, 59.82; H, 5.69; N, 21.71.

*5.9.12. N-(5-((9-(2-bromophenyl)-9H-purin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**21l**)*

Yield: 65.2%, M.p.:123.9-124.6°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.86 (s, 1H), 8.65 (s, 1H), 8.41 (s, 1H), 8.20 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.73 (d, *J* = 7.5 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.1 Hz, 1H), 6.93 (s, 1H), 6.41 (dd, *J* = 15.8, 9.7 Hz, 1H), 6.24 (d, *J* = 16.7 Hz, 1H), 5.76 (d, *J* = 9.9 Hz, 1H), 3.78 (s, 3H), 2.86 (s, 2H), 2.66 (s, 3H), 2.32 (s, 2H), 2.22 (s, 6H). MS (ESI) m/z(%): 565.2 $[M+H]^+$, 567.2 [M+H]; Anal. calcd. For $C_{26}H_{29}BrN_8O_2$(%): C, 55.23; H, 5.17; N, 19.82; Found (%): C, 55.34; H, 5.25; N, 19.73.

*5.9.13. N-(5-((9-(2,4-dichlorophenyl)-9H-purin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**21m**)*

Yield: 47.5%, M.p.:182.9-183.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.88 (s, 1H), 8.77 (s, 1H), 8.42 (s, 1H), 8.15 (s, 1H), 7.91 (d, *J* = 2.1 Hz, 1H), 7.80 (d, *J* = 8.6 Hz, 1H), 7.58 (dd, *J* = 8.6, 2.1 Hz, 1H), 6.95 (s, 1H), 6.37 (dd, *J* = 16.7, 10.0 Hz, 1H), 6.26 (dd, *J* = 16.7, 1.7 Hz, 1H), 5.77 (dd, *J* = 9.6, 2.0 Hz, 1H), 3.80 (s, 3H), 2.83 (t, *J* = 5.8 Hz, 2H), 2.67 (s, 3H), 2.25 (t, *J* = 5.7 Hz, 2H), 2.18 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ 162.86, 157.51, 153.31, 150.27, 147.07, 143.82, 138.86, 134.95, 132.69, 132.00, 131.58, 131.05, 130.40, 128.89, 127.80, 127.49, 126.58, 125.23, 105.59, 56.93, 56.46, 55.38, 49.06, 45.23, 42.95. MS (ESI) m/z(%): 555.5 $[M+H]^+$; Anal. calcd. For $C_{26}H_{28}C_{l2}N_8O_2$ (%): C, 56.22; H, 5.08; N, 20.17; Found (%): C, 56.12; H, 5.16; N, 20.26.

*5.9.14. N-(5-((9-(3-bromo-4-fluorophenyl)-9H-purin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**21n**)*

Yield: 57.5%, M.p.:114.9-115.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.89 (s, 1H), 8.80 (s, 1H), 8.68 (s, 1H), 8.36 – 8.26 (m, 2H), 8.13 – 8.05 (m, 1H), 7.48 (t, *J* = 8.8 Hz, 1H), 7.00 (s, 1H), 6.43 (s, 1H), 6.21 (dd, *J* = 16.8, 1.6 Hz, 1H), 5.76 (dd, *J* = 10.6, 1.5 Hz, 1H), 3.84 (s, 3H), 2.89 (s, 2H), 2.69 (s, 3H), 2.28 (d, *J* = 33.4 Hz, 8H).MS (ESI) m/z(%): 582.9 $[M+H]^+$， 584.9 $[M+H]^+$; Anal. calcd. For $C_{26}H_{28}BrFN_8O_2$ (%): C, 53.52; H, 4.84; N, 19.21; Found (%): C, 53.42; H, 4.90; N, 19.26.

*5.9.15. N-(5-((9-(3-chloro-4-fluorophenyl)-9H-purin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**21o**)*

Yield: 47.5%, M.p.:181.3-182.3°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.89 (s, 1H), 8.71 (s, 2H), 8.35 (s, 1H), 8.24 (dd, *J* = 6.3, 2.6 Hz, 2H), 8.09 – 8.01 (m, 2H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.54 (t, *J* = 9.0 Hz, 1H), 6.97 (s, 1H), 6.20 (dd, *J* = 16.7, 1.1 Hz, 1H), 5.73 (dd, *J* = 10.2, 1.2 Hz, 1H), 3.85 (s, 3H), 3.21 (s, 2H), 3.18 – 3.11 (m, 3H), 2.62 (d, *J* = 11.7 Hz, 8H).MS (ESI) m/z(%): 539.5 $[M+H]^+$; Anal. calcd. For $C_{26}H_{28}ClFN_8O_2$ (%): C, 57.94; H, 5.24; N, 20.79; Found (%): C, 57.82; H, 5.32; N, 20.68.

*5.9.16. N-(5-((9-cyclopentyl-9H-purin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxyphenyl)acrylamide(**21p**)*

Yield: 78.5%, M.p.:123.9-124.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H), 9.45 (s, 1H), 8.77 (s, 1H), 8.35 (s, 1H), 7.94 (s, 1H), 7.00 (s, 1H), 6.44 (dd, *J* = 16.7, 9.7 Hz, 1H), 6.23 (dd, *J* = 17.0, 1.6 Hz, 1H), 5.74 (dd, *J* = 10.3, 0.9 Hz, 1H), 3.88 (s, 3H), 2.92 – 2.83 (m, 2H), 2.69 (s, 3H), 2.38 – 2.29 (m, 2H), 2.23 (s, 6H). MS (ESI) m/z(%): 479.2 $[M+H]^+$; Anal. calcd. For $C_{25}H_{34}N_8O_2$ (%): C, 62.74; H, 7.16; N, 23.41; Found (%): C, 62.65; H, 7.23; N, 23.55.

*5.9.17. N-(5-((9-(4-isopropylphenyl)-9H-purin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide(**21q**)*

Yield: 57.7%, M.p.:215.5-216.4°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (s, 1H), 8.89 (s, 1H), 8.68 (s, 1H), 8.65 (s, 1H), 8.11 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.85 (s, 1H), 6.70 (dd, *J* = 16.9, 10.2 Hz, 1H), 6.31 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.80 (dd, *J* = 10.3, 1.3 Hz, 1H), 3.87 (s, 3H), 2.93 (dt, *J* = 13.7, 6.8 Hz, 1H), 2.84 (t, *J* = 4.4 Hz, 4H), 2.50 (s, 4H), 2.25 (s, 3H), 1.23 (d, *J* = 6.9 Hz, 6H). MS (ESI) m/z(%): 527.6 $[M+H]^+$; Anal. calcd. For $C_{29}H_{34}N_8O_2$(%): C, 66.14; H, 6.51; N, 21.28; Found (%): C, 66.23; H, 6.45; N, 21.36.

*5.9.18. N-(5-((9-(4-iodophenyl)-9H-purin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide(**21r**)*

Yield: 55.4%, M.p.:161.5-162.4°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.91 (s, 1H), 8.68 (d, *J* = 24.0 Hz, 2H), 8.17 (s, 1H), 7.90 (d, *J* = 7.4 Hz, 2H), 7.82 (d, *J* = 7.0 Hz, 2H), 6.85 (s, 1H), 6.69 (dd, *J* = 16.9, 9.3 Hz, 1H), 6.31 (d, *J* = 17.2 Hz, 1H), 5.83 (d, *J* = 9.5 Hz, 1H), 3.88 (s, 3H), 2.85 (s, 4H), 2.49 – 2.46 (m, 3H), 2.25 (s, 4H). MS (ESI) m/z(%): 611.4 $[M+H]^+$; Anal. calcd. For $C_{26}H_{27}IN_8O_2$(%): C, 51.16; H, 4.46; N, 18.36; Found (%): C, 51.23; H, 4.40; N, 18.46.

*5.9.19. N-(5-((9-(4-isopropylphenyl)-9H-purin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide(**21s**)*

Yield: 65.5%, M.p.:190.4-191.3°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 8.87 (s, 1H), 8.63 (d, *J* = 6.3 Hz, 1H), 8.13 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8.5 Hz, 2H), 6.88 (s, 1H), 6.79 (dd,J=16.5,1.7Hz, 1H), 5.75 (dd, *J* = 12.3, 1.5 Hz, 1H), 3.84 (s, 3H), 3.78 (d, *J* = 2.9 Hz, 4H), 2.88 – 2.77 (m, 4H), 2.29 (d, *J* = 3.8 Hz, 1H), 1.26 (d, *J* = 6.9 Hz, 6H). MS (ESI) m/z(%): 514.2 $[M+H]^+$; Anal. calcd. For $C_{28}H_{31}N_7O_3$(%): C, 65.48; H, 6.08; N, 19.09; Found (%): C, 65.34; H, 6.20; N, 18.86.

*5.9.20. N-(5-((9-(4-iodophenyl)-9H-purin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide(**21t**)*

Yield: 54.9%, M.p.:201.1-202.3°C; ^{1}H NMR (400 MHz, DMSO) δ 9.42 (s, 1H), 8.89 (s, 1H), 8.69 (s, 1H), 8.65 (s, 1H), 8.17 (s, 1H), 7.93 (d, *J* = 8.7 Hz, 2H), 7.81 (d, *J* = 8.8 Hz, 2H), 6.88 (s, 1H), 6.52 (dd, J=17.2, 1.5Hz, 1H), 5.76 (dd, J=10.6, 1.5Hz, 1H), 3.86 (s, 3H), 3.82 – 3.75 (m, 4H), 2.89 – 2.78 (m, 4H). MS (ESI) m/z(%): 598.1 $[M+H]^+$; Anal. calcd. For $C_{25}H_{24}IN_7O_3$(%): C, 50.26; H, 4.05; N, 16.41; Found (%): C, 50.34; H, 4.20; N, 16.56.

*5.10. General procedure for preparation of compounds (**22a-e**)*

To a solution of **17** (0.32 mmol, 1.0 eq.) in 3 mL of CH_2Cl_2 was added 115 mg (0.71 mmol, 2.2 eq.) of carbonyl diimidazole. The reaction mixture was stirred at room temperature for 16 h. The mixture was diluted with 20 mL of chloroform and 10 mL of sat. $NaHCO_3$ (aq) and the layers separated. the organic extract was washed with saturated $NaHCO_3$, water, and brine and dried over anhydrous $MgSO_4$. It was then filtered and concentrated in vacuo to generate the compounds **22a-e**, respectively.

*5.10.1. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((9-(4-iodophenyl)-8-oxo-8,9-dihydro-7H-purin-2-yl)amino)-4-methoxyphenyl)acrylamide(**22a**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.96 (s, 1H), 9.04 (s, 1H), 7.91 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.33 (s, 1H), 6.67 (s, 1H), 6.51 (d, *J* = 16.7 Hz, 1H), 5.83 (d, *J* = 11.1 Hz, 1H), 3.77 (s, 3H), 3.09 (s, 2H), 2.69 (s, 3H), 2.65 – 2.33 (m, 8H). MS (ESI) m/z(%): 629.1$[M+H]^+$; Anal. calcd. For $C_{26}H_{29}IN_8O_3$ (%): C, 49.69; H, 4.65; N, 17.83; Found (%): C, 49.55; H, 4.73; N, 17.92.

*5.10.2. N-(5-((9-(4-isopropylphenyl)-8-oxo-8,9-dihydro-7H-purin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide(**22b**)*

Yield: 53.7%, M.p.:186.4-187.3°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.97 (s, 1H), 8.50 (s, 1H), 8.01 (s, 1H), 7.66 (s, 1H), 7.55 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 6.80 (s, 1H), 6.64 (dd, J = 17.1, 10.0 Hz, 1H), 6.34 – 6.18 (m, 1H), 5.76 (d, J = 10.1 Hz, 1H), 3.82 (s, 3H), 2.97 – 2.89 (m, 1H), 2.80 (d, J = 4.0 Hz, 4H), 2.50 – 2.49 (m, 4H), 2.24 (s, 3H), 1.23 (d, J = 6.9 Hz, 6H). MS (ESI)

m/z(%): 543.6 $[M+H]^+$; Anal. calcd. For $C_{29}H_{34}N_8O_3$(%): C, 64.19; H, 6.32; N, 20.65; Found (%): C, 64.23; H, 6.45; N, 20.54.

*5.10.3. N-(5-((9-(4-iodophenyl)-8-oxo-8,9-dihydro-7H-purin-2-yl)amino)-4-methoxy-2-(4-methylpiperazin-1-yl)phenyl)acrylamide(**22c**)*

Yield: 52.1%, M.p.:256.4-257.4°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.51 (s, 1H), 8.00 (s, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 6.80 (s, 1H), 6.65 (dd, *J* = 16.9, 10.3 Hz, 1H), 6.32 – 6.20 (m, 1H), 5.83 – 5.73 (m, 1H), 3.84 (s, 3H), 2.81 (s, 4H), 2.50 – 2.47 (m, 4H), 2.24 (s, 3H). MS (ESI) m/z(%): 627.5 $[M+H]^+$; Anal. calcd. For $C_{26}H_{27}IN_8O_3$(%): C, 49.85; H, 4.34; N, 17.89; Found (%): C, 49.76; H, 4.25; N, 17.96.

*5.10.4. N-(5-((9-(4-isopropylphenyl)-8-oxo-8,9-dihydro-7H-purin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide(**22d**)*

Yield: 66.5%, M.p.:136.4-137.3°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (s, 1H), 8.45 (s, 1H), 8.27 (s, 1H), 7.53 (d, *J* = 15.0 Hz, 2H), 7.12 (s, 1H), 7.06 (d, *J* = 14.9 Hz, 2H), 6.43 (s, 1H), 6.29 (dd, *J* = 33.5, 19.6 Hz, 1H), 6.05 (dd, *J* = 33.5, 4.6 Hz, 1H), 5.69 (dd, *J* = 19.6, 4.6 Hz, 1H), 4.71 (s, 1H), 3.86 (s, 3H), 3.72 (t, *J* = 8.9 Hz, 4H), 3.18 (t, *J* = 9.0 Hz, 4H), 2.87 (dt, *J* = 25.4, 12.7 Hz, 1H), 1.20 (d, *J* = 12.8 Hz, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ 163.41, 155.31, 152.76, 151.16, 148.15, 146.82, 141.03, 139.27, 134.98, 132.90, 130.94, 127.13, 126.37, 125.29, 118.83, 116.01, 115.64, 103.86, 66.83, 56.47, 52.38, 33.68, 24.33. MS (ESI) m/z(%): 530.2 $[M+H]^+$; Anal. calcd. For $C_{28}H_{31}N_7O_4$(%): C, 63.50; H, 5.90; N, 18.51; Found (%): C,63.68; H, 5.83; N, 18.76.

*5.10.5. N-(5-((9-(4-iodophenyl)-8-oxo-8,9-dihydro-7H-purin-2-yl)amino)-4-methoxy-2-morpholinophenyl)acrylamide(**22e**)*

Yield: 59.5%, M.p.:168.4-169.3°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.54 (s, 1H), 8.04 (s, 1H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.71 (s, 1H), 7.53 (d, *J* = 8.7 Hz, 2H), 6.84 (s, 1H), 6.69 (dd, *J* = 16.8, 10.2 Hz, 1H), 6.27 (dd, *J* = 17.1, 1.6 Hz, 1H), 5.79 (dd, *J* = 10.4, 1.1 Hz, 1H), 3.84 (s, 3H), 3.76 (d, *J* = 4.3 Hz, 4H), 2.87 – 2.75 (m, 4H). MS (ESI) m/z(%): 614.1 $[M+H]^+$; Anal. calcd. For $C_{25}H_{24}IN_7O_4$(%): C, 48.95; H, 3.94; N, 15.98; Found (%): C,48.78; H, 3.83; N, 15.76.

*5.11. General procedure for preparation of compounds (**23a-c**)*

A suspension of compound **20a-c** (2.82 mmol), EDCI (0.6 g, 3.11 mmol), DIEA (0.5 mL, 3.78 mmol), and phenyl isothiocyanate (0.4 mL, 3.78 mmol) in CH_2Cl_2 (20 mL) was refluxed for 12 h until TLC showed that the reaction was complete. The reaction mixture was evaporated under reduced pressure, and the residue was chromatographed over silica gel eluting with CH_2Cl_2/MeOH (70:1) to give a brown solid, which was further washed with diethyl ether to afford pure product **23a-c**.

*5.11.1. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((9-methyl-8-(phenylamino)-9H-purin-2-yl)amino)phenyl)acrylamide(**23a**)*

Yield: 55.8%, M.p.:135.2-2136.1°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 9.17 (s, 1H), 8.36 (s, 1H), 7.90 (d, *J* = 7.9 Hz, 2H), 7.65 (s, 1H), 7.35 (t, *J* = 7.8 Hz, 2H), 7.01 (d, *J* = 9.4 Hz, 2H), 6.52 – 6.40 (m, 1H), 6.25 (dd, *J* = 16.7, 1.8 Hz, 1H), 5.75 (dd, *J* = 10.1, 1.6 Hz, 1H), 3.89 (s, 3H), 3.80 (s, 3H), 2.91 (s, 2H), 2.68 (s, 3H), 2.29 (d, *J* = 11.6 Hz, 8H). MS (ESI) m/z(%): 516.6 $[M+H]^+$; Anal. calcd. For $C_{27}H_{33}N_9O_2$ (%): C, 62.89; H, 6.45; N, 24.45; Found (%): C, 62.78; H, 6.55; N, 24.36.

*5.11.2. N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-5-((9-isopropyl-8-(phenylamino)-9H-purin-2-yl) amino)-4-methoxyphenyl)acrylamide(**23b**)*

Yield: 74.7%, M.p.:141.1-142.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 9.00 (d, *J* = 13.1 Hz, 2H), 8.35 (s, 1H), 7.82 (d, *J* = 7.8 Hz, 2H), 7.62 (s, 1H), 7.33 (t, *J* = 7.9 Hz, 2H), 7.02 – 6.93 (m, 2H), 6.45 (dd, *J* = 17.4, 10.4 Hz, 1H), 6.24 (dd, *J* = 16.9, 2.0 Hz, 1H), 5.73 (dd, *J* = 10.1, 1.8 Hz, 1H), 4.89 (dt, *J* = 13.5, 6.7 Hz, 1H), 4.26 (t, *J* = 5.2 Hz, 1H), 3.88 (s, 3H), 2.91 (s, 2H), 2.68 (s, 3H), 2.25 (s, 6H), 1.63 (d, *J* = 6.7 Hz, 6H). MS (ESI) m/z(%): 544.6 $[M+H]^+$; Anal. calcd. For $C_{29}H_{37}N_9O_2$ (%): C, 64.07; H, 6.86; N, 23.19; Found (%): C, 64.23; H, 6.82; N, 23.25.

*5.11.3. N-(5-((9-cyclopentyl-8-(phenylamino)-9H-purin-2-yl)amino)-2-((2-(dimethylamino)ethyl)(meth yl)amino)-4-methoxyphenyl)acrylamide(**23c**)*

Yield: 65.5%, M.p.:143.5-144.4°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H), 9.17 (s, 1H), 8.71 (s, 1H), 8.36 (s, 1H), 7.85 (d, *J* = 7.8 Hz, 2H), 7.66 (s, 1H), 7.32 (t, *J* = 7.9 Hz, 2H), 7.01 – 6.93 (m, 2H), 6.23 (dd, *J* = 16.8, 0.9 Hz, 1H), 5.76 – 5.67 (m, 1H), 4.99 (dd, *J* = 17.4, 8.8 Hz, 1H), 3.86 (s, 3H), 3.14 (s, 2H), 2.65 (s, 3H), 2.46 – 2.13 (m, 8H), 2.02 – 1.79 (m, 6H), 1.57 – 1.50 (m, 2H). MS (ESI) m/z(%): 570.6 $[M+H]^+$; Anal. calcd. For $C_{31}H_{39}N_9O_2$ (%): C, 65.36; H, 6.90; N, 22.13; Found (%): C, 65.46; H, 6.82; N, 22.25.

6. Pharmacology

6.1. In vitro enzymatic activity assay

The experiments were carried out by a well-established mobility shift assay, and EGFR kinases (EGFR T790M/L858R, EGFR T790M and WT EGFR) were purchased from Invitrogen. The kinase base buffer was consist of 50 mM HEPES (pH 7.5), 0.0015% Brij35 and 2 mM DTT, while the stop buffer contained a mixture of 100 mM HEPES (pH 7.5), 0.015% Brij-35, 0.2% Coating Reagent and 50 mM EDTA. Initially, the tested compounds were diluted to 50-fold of the final desired highest concentration in reaction by 100% DMSO. Subsequently, 30 mL of the solution was transferred to 60 mL of 100% DMSO in the next well and so forth for a total of 5 concentrations. No compound and no enzyme controls were prepared by adding 100 mL DMSO to two empty wells in the same 96-well plate. Then, 10 mL of compound was transferred to a new 96-well plate, which was marked as the intermediate plate. Additional 90 mL of kinase buffer was added to each well of intermediate plate. The mixture in intermediate plate was shaked for 10 min. The assay plate was prepared after transferring 5 mL of each well from the 96-well intermediate plate to a 384-well plate in duplicates. The prepared enzyme solution (appropriate EGFR in kinase base buffer) was added to the assay plate, which was then incubated at room temperature for 10 min, followed by the addition 10 mL of prepared peptide solution (FAM-labeled peptide and ATP in kinase base buffer). The mixture was incubated at 28℃ for another 1 h, then 25 mL of stop buffer was added. The conversion data was copied from Caliper program, and the values were converted to inhibition values. Percent inhibition ¼ (max -conversion)/ (max -min) ×100.

6.2. MTT assay

The target compounds were screened in A549, H1975 and HT-29 cells by a standard MTT assay. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximate 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37℃ for 24 h. The tested compounds

were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 mg/mL, and incubated with cells at 37℃ for 4 h. The formazan crystals were dissolved in 100 mL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of at least three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

Conflict of interest

The authors have declared no conflict of interest.

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Graphical Abstract