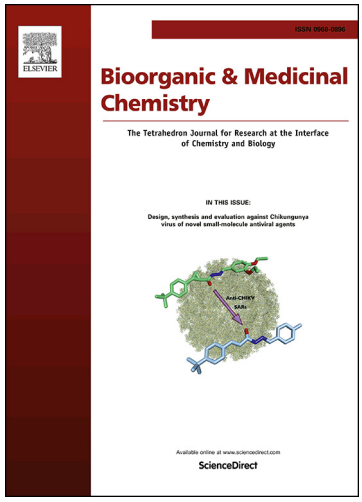


Synthesis and evaluation of 2,9-disubstituted 8-phenylthio/phenylsulfinyl-9*H*-purine as new EGFR Inhibitors

Yuan-Yuan Hei, Ying Shen, Jin Wang, Hao Zhang, Hong-Yi Zhao, Minhang Xin, Yong-Xiao Cao, Yan Li, San-Qi Zhang

PII: S0968-0896(18)30220-7
DOI: https://doi.org/10.1016/j.bmc.2018.03.025
Reference: BMC 14263

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 3 February 2018
Revised Date: 9 March 2018
Accepted Date: 14 March 2018

Please cite this article as: Hei, Y-Y., Shen, Y., Wang, J., Zhang, H., Zhao, H-Y., Xin, M., Cao, Y-X., Li, Y., Zhang, S-Q., Synthesis and evaluation of 2,9-disubstituted 8-phenylthio/phenylsulfinyl-9*H*-purine as new EGFR Inhibitors, *Bioorganic & Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.bmc.2018.03.025

Synthesis and evaluation of 2,9-disubstituted 8-phenylthio/phenylsulfinyl-9*H*-purine as new EGFR Inhibitors

Yuan-Yuan Hei[a], Ying Shen[a], Jin Wang[b], Hao Zhang[a], Hong-Yi Zhao[a], Minhang Xin[a], Yong-Xiao Cao[b], Yan Li,[a] San-Qi Zhang[a*]

[a] Department of Medicinal Chemistry, School of Pharmacy, Xi′an Jiaotong University, Xi′an, Shaanxi, 710061, PR China

[b] Department of Pharmacology, School of Basic Medical Sciences, Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, PR China

*Corresponding author: San-Qi Zhang, Ph. D., Professor, E-mail address: sqzhang@xjtu.edu.cn (S. Q. Zhang).

Abstract

In present study, we described the synthesis and biological evaluation of a new class of EGFR inhibitors containing 2, 9-disubstituted 8-phenylthio/ phenylsulfinyl-9*H*-purine scaffold. Thirty-one compounds were synthesized. Among them, compound **C9** displayed the IC_{50} of 29.4 nM against HCC827 cell line and 1.9 nM against $EGFR^{L858R}$. Compound **C12** showed moderate inhibitory activity against $EGFR^{L858R/T790M/C797S}$ (IC_{50} = 114 nM). Western bolt assay suggested that compound **C9** significantly inhibited EGFR phosphorylation. *In vivo* test, compound **C9** remarkably exhibited inhibitory effect on tumor growth at 5.0 mg/kg by oral administration in established nude mouse HCC827 xenograft model. These results indicate that the 2,9-disubstituted 8-phenylsulfinyl/phenylsulfinyl-9*H*-purine derivatives can act as potent EGFR(L858R) inhibitors and effective anticancer agents. Additionally, optimization of compound **C12** may result in discovering the fourth-generation EGFR-TKIs.

Keywords purine * EGFR-TK inhibitor * drug design * antiproliferative effects * anticancer agent

1. Introduction

The epidermal growth factor receptor (EGFR) belongs to the family of protein-tyrosine kinase receptor (RTK), which plays a key role in the regulation of cell growth, differentiation, and survival.[1] RTKs have been observed over-expression and/or constitutive activation in numerous types of human tumor, including colon, breast, ovarian, head and neck, and non-small cell lung cancers (NSCLC). Among the known RTKs, EGFR and human epidermal growth factor receptor have been extensively studied and clinically validated as targets for chemical therapies.[2, 3] The first-generation EGFR tyrosine kinase inhibitor (EGFR-TKI), gefitinib[3] and erlotinib[4] were approved by FDA for the treatment of NSCLC. For the population of NSCLC patients whose tumors harbor activating mutations in EGFR, targeted kinase inhibitors such as gefitinib and erlotinib have proven to be very effective treatments resulting in tumor regressions and improved progression-free survival.[5] However, the efficacy of small organic molecules inhibitors such as gefitinib, etc. is restricted to a small subset of patients due to molecular heterogeneity among and within tumors.[6, 7] The drug resistance caused by receptor mutation is another issue needed to pay attention.[8-10] Numbers of compounds with different structures were discovered as EGFR or multi-target inhibitors.[11-15] The essential pharmacophore for 4-anilinoquinazolines is the two nitrogen atoms at 1-posotion, 3-position and the N-aryl at 4-position as well.[16] The morpholine moiety in gefitinib is not involved in any interaction with EGFR and is randomly ordered due to its lower electron density. Consequently, the modification of the 6-substituted group in 4-anilinoquinazoline with the group bearing active group provide the inhibitors with improved potency.[17-20] Meanwhile, Numbers of compounds with different structures have been developed as EGFR or multi-target inhibitors.[21,22] Salicylanilide molecule and 2-aryl-8-hydroxyisoquinolin-1(2*H*)-one can construct an intramolecular hydrogen bond and form a pseudo six-membered ring to mimic the pyrimidine ring of quinazolines. Thus, N-aryl salicylamides and 2-aryl-8-hydroxyisoquinolin-1(2*H*)-one were studied as EGFR inhibitors.[23,24]

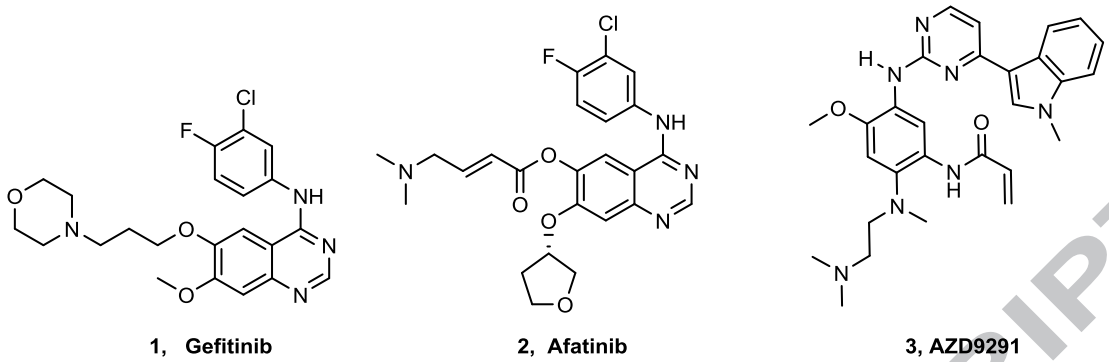


Figure 1. The structures of EGFR inhibitors

To overcome the resistance, the replacement of 6-substituted group in gefitinib with acrylamido group produced the second-generation irreversible EGFR inhibitors including afatinib[25] (Figure 1), dacomitinib[26] and neratinib.[27] Afatinib was demonstrated activity in preclinical studies against T790M mutations. But the clinical trial data of afatinib did not demonstrate distinctly improved efficacy.[28] The kinase active site domain is mutated in many NSCLC epithelial tumors and clinical studies suggest that these mutations aid in tumorigenesis. There are two main types of mutations. One is activating mutation (e.g., L858R or exon 19 deletion) and the other is drug resistance mutation (e.g., T790M).[29] The latter mutation (T790M) results in an increased affinity of the kinase toward ATP, rendering these ATP-competitive inhibitors less effective despite comparable affinities to the different mutant forms of the protein. The progress of 2-anilinopyrimidine-based small-molecule EGFR-T790M inhibitors AZD9291 and its analogues has been well summarized.[30] However, the effective treatment of patients that harbor the EGFR-T790M drug resistance mutation is limited by the emergence of new drug resistances to AZD9291 therapy.[31] Therefore, the development of the fourth-generation EGFR-TKIs has become a new research hotspot.[32,33]

Gefitinib, erlotinib and afatinib share the 4-arylaminoquinazoline as their common scaffold. Compound **4** (Figure 2) with a purine scaffold was discovered to be a reversible EGFR-TKI, which displayed potent inhibitory active toward EGFR (L858R).[34] However, the high CLogP value (6.40, predicted by ChemBioDraw 14.0) limited the development of compound **4** as a candidate. In this study, we intend to replace 8-phenylamino group in compound **4** with 8-phenylthio or phenylsulfinyl

moiety to discover new EGFR-TKI and anticancer agents (structures **C** and **D** in Figure 2). Meanwhile, oxygen atom or hydroxyl group is introduced to R^1 moiety to decrease CLogP value. Herein, we report the synthesis and biological activities evaluation of new compounds.

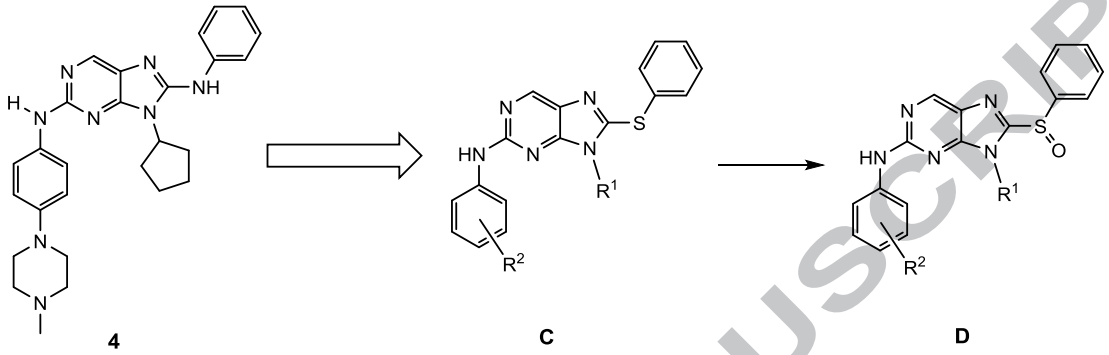
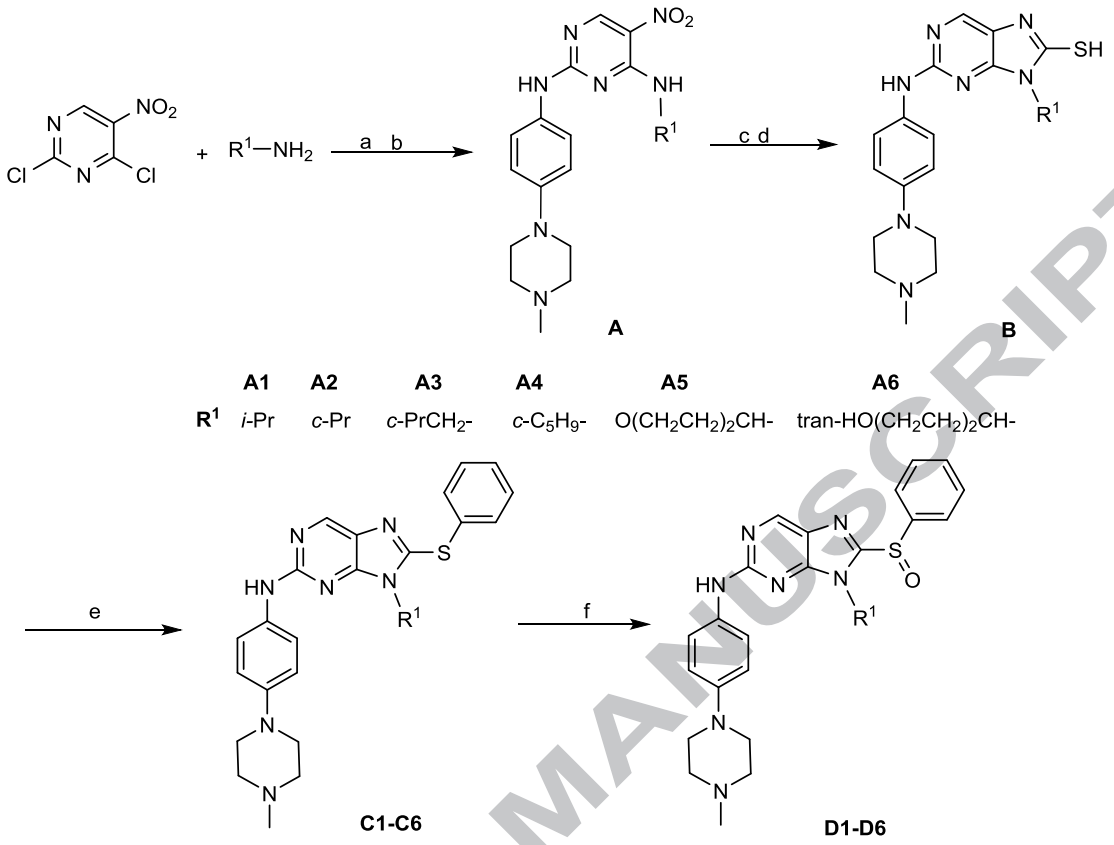


Figure 2. The design of target compounds

2. Results and discussion

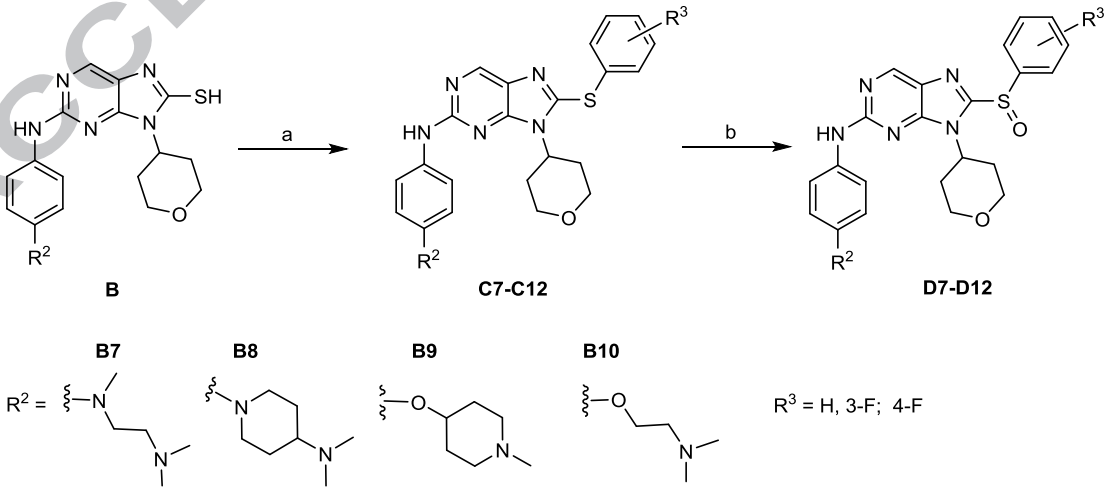
2.1. Chemistry

The synthetic route for the title compound **C** and **D** was outlined in Scheme 1. 2,4-Dichloro-5-nitropyrimidine was used as the starting material. The intermediates **A** were prepared as reported protocol.[34] Catalyzed by Pd/C (5%), the nitro group in intermediate **A** was performed hydrogenation-reduction to produce corresponding amine, which was used in the next step without further purification. The refluxing of the mixture containing the amine, CS_2, KOH, EtOH and H_2O afforded intermediates **B**. Catalyzed by CuI, the reaction of intermediates **B** with iodobenzene yielded products **C**, 2, 9-disubstituted 8-phenylthio-9*H*-purine. The oxidation of **C** with *m*-chloroperoxybenzoic acid produced compounds **D**, 2, 9-disubstituted 8-phenylsulfinyl-9*H*-purine.



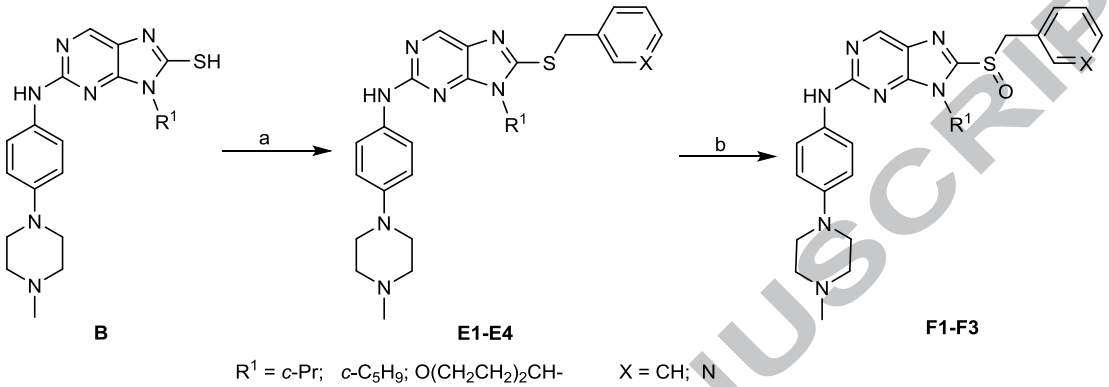
Scheme 1. Synthesis of 2-anilino-8-henylthio/phenylsulfinyl-9*H*-purine derivatives **C** and **D**. Reagents and conditions: a) CH_2Cl_2, DIEA, amine, -40°C to room temperature, 60-75%; b) n-butanol, 90 °C, 5 h, 70%-76%; c) H_2, 5% Pd/C, MeOH, 50°C, 6 h, 85%-90%; d) CS_2, KOH, EtOH:H_2O = 10:1, refluxed, 52.6-90.3%; e) PhI, CuI, 1,10-phenanthroline, K_2CO_3, DMSO, 140°C, 24 h, 55.2%-66.7%; f) *m*-chloroperoxybenzoic acid, CH_2Cl_2, 50.2-63.7%.

To elucidate the change of R^2 and R^3 on activity, compounds **C7**-**C12** and **D7**-**D12** were synthesized (Scheme 2).



Scheme 2. Synthesis of 2-anilino-8-henylthio/phenylsulfinyl-9*H*-purine derivatives **C** and **D**. Reagents and conditions: a) aryliodide, CuI, 1,10-phenanthroline, K_2CO_3, DMSO, 140℃, 24 h, 55.7%-63.6%; b) *m*-chloroperoxybenzoic acid, CH_2Cl_2, 49.2-63.2%.

To boost the structural diversity of the title compounds, 8-benzylthio/ benzylsulfinyl-9*H*-purine was synthesized. In the presence of K_2CO_3, the nucleophilic substitution of **B** with benzyl bromide yielded 8-benzylthio-9*H*-purine **E**, which was treated with *m*-chloroperoxybenzoic acid to produce compounds **F**.



Scheme 3. Synthesis of 2,8,9-trisubstitued purine derivatives **E** and **F**. Reagents and conditions: a) benzyl bromide or 3-chloromethylpyridine hydrochloride, acetone, K_2CO_3, 42.4%-72.9%; b) *m*-chloroperoxybenzoic acid, CH_2Cl_2, 46.3-68.2%.

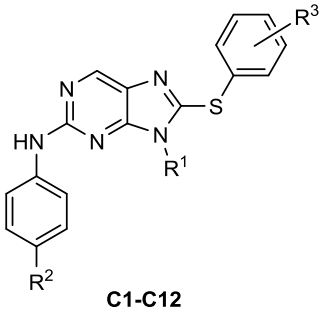
All the newly synthesized title compounds were characterized by ^{1}H-NMR, ^{13}C-NMR and MS.

2.2. The antiproliferative effects *in vitro*

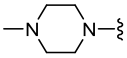
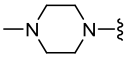
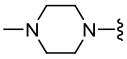
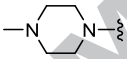
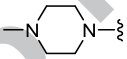
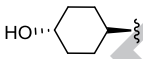
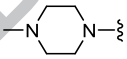
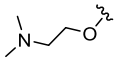
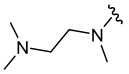
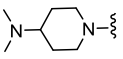
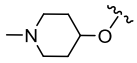
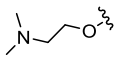
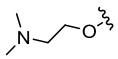
The antiproliferative effects of synthesized compounds were evaluated against human lung carcinoma cell line HCC827 (Del E746-A750) by applying the 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl-*2H*-tetrazolium bromide (MTT) colorimetric assay. The EGFR-TKI gefitinib and AZD9291 were used as the positive controls. The results of compounds **C** are summarized in Table 1. Firstly, to explore the effect of substituent at the *N*-9 position of purine scaffold, we synthesized compounds **C1**-**C6**, which contained substituents of different sizes at the *N*-9 purine position. Compounds **C1**, **C2** and **C3** displayed moderate activities against HCC827 with the IC_{50} values of 289.9-659.9 nM. Increasing the substituent volume, the antiproliferative effects of compounds **C4**, **C5** and **C6** significantly improved. Compound **C5** (IC_{50} = 42.6 nM) exhibited equipotent antiproliferative effect with AZD9291 (IC_{50} = 43.1 nM) and slightly increased in activity over compound **C4** (IC_{50} = 51.2 nM). Compound **C6** (IC_{50} = 47.9 nM), attached a hydrophilic group to the substitute group of *N*-9 position of the purine scaffold, did not improve its antiproliferative effect. These results

suggested that

Table 1. Antiproliferative effects of compounds C against HCC827 cell lines ($\bar{x} \pm s$, n = 3)



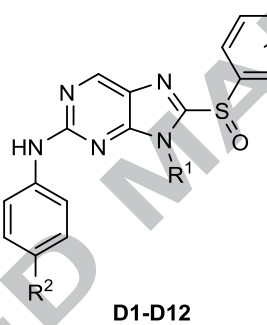
C1-C12

Compds	R^1	R^2	R^3	IC_{50} (nM)
C1			H	289.9±33.6
C2			H	304.5±33.5
C3			H	659.9±42.1
C4			H	51.2±10.3
C5			H	42.6±10.7
C6			H	47.9±9.3
C7			H	44.3±0.9
C8			H	498.8±32.4
C9			H	29.4±4.4
C10			H	139.8±23.9
C11			3-F	51.4±4.2
C12			4-F	47.8±0.7
gefitinib				33.3±4.2
AZD9291				43.1±1.1

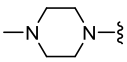
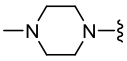
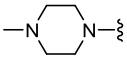
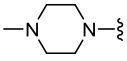
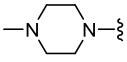
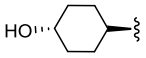
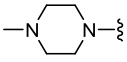
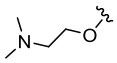
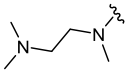
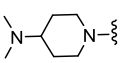
Next, compounds **C7**-**C10** were synthesized to explore the influence of the substituent at position of R^2 on the antiproliferative effect. Compound **C7** (IC_{50} = 44.3 nM) with 2-dimethylaminoethoxy at R^2 position displayed the same potency

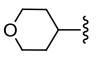
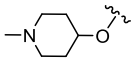
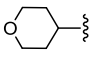
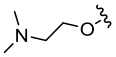
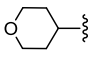
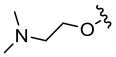
compared with **C5** (IC_{50} = 42.6 nM). The replacement of *N*-methylpiperazinyl in **C5** with *N*-(2-(dimethylamino)ethyl)-*N*-methylamino (**C8**) or 1-methylpiperidin-4-yloxy group (**C10**) resulted in a decrease in cell-based effect. However, compound **C9** with 4-*N*, *N*-dimethylaminopiperidin-1-yl at position of R^2 showed the most potent antiproliferative effect against HCC827 (IC_{50} = 29.4 nM), which was comparable to the positive controls gefitinib and AZD9291. When fluorine atom was presented at the meta-position (**C11**, IC_{50} = 51.4 nM) and para-position (**C12**, IC_{50} = 47.8 nM) of the phenyl ring of the 8-phenylthio, the antiproliferative effect of the two compounds was not changed distinctly against HCC827.

Table 2. Antiproliferative effects of compounds **D** against HCC827 cell lines ($\bar{x} \pm s$, n = 3)



D1-D12

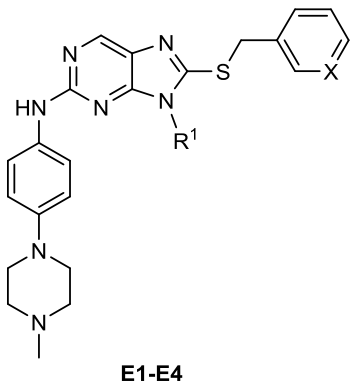
Compds	R^1	R^2	R^3	IC_{50} (nM)
D1			H	214.5±25.5
D2			H	1627.8±208.4
D3			H	838.3±26.3
D4			H	64.7±18.1
D5			H	410.6±20.1
D6			H	268.3±80.7
D7			H	50.3±8.8
D8			H	1329.3±56.8
D9			H	1287.6±61.0

D10			H	852.7±17.2
D11			3-F	358.7±30.7
D12			4-F	553.1±62.2
gefitinib				33.3±4.2
AZD9291				43.1±1.1

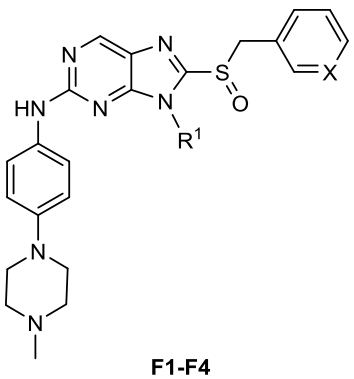
The antiproliferative effects of 2,9-disubstituted 8-phenylsulfinyl-9*H*-purine, compounds **D** were listed in table 2. Compounds **D4** and **D7** showed potent antiproliferative effects on HCC827 with IC_{50} values of 64.7 nM and 50.3 nM, respectively. However, the rest compounds **D** displayed moderate antiproliferative effects in the HCC827 cellular assay with IC_{50} values of 214.5-1627.8 nM. Compared with compounds **C** with a phenylthio at 8-position of the purine scaffold, compounds **D** with a phenylsulfinyl at 8-position of the purine scaffold were less effective against HCC827.

To further study structure-activity relationship of compounds, we changed the 8-phenylthio/phenylsulfinyl to 8-benzylthio/benzylsulfinyl to produce compound **E** and **F**. The antiproliferative effects of compounds **E1-E4** and **F1-F3** against HCC827 are summarized in Table 3. Compared with compounds **C** and positive controls, these compounds showed a significant drop in the cell-based activity, indicating that benzylthio/benzylsulfinyl at the C-8 position of purine scaffold was not suitable.

Table 3. Antiproliferative effects of 8-benzylthio/ benzylsulfinyl-9H-purine **E** and **F** against HCC827 cell lines ($\bar{x} \pm s$, n = 3)



E1-E4



F1-F4

Compds	R	X	IC_{50} (nM)
E1		N	8832.6±243.5
E2		N	517.3±112.1
E3		N	499.6±20.5
E4		CH	106.5±9.9
F1		N	2353.3±532.4
F2		N	>10000
F3		CH	433.1±11.1
gefitinib			33.3±4.2
AZD9291			43.1±1.1

In order to investigate the cell-based selectivity, compounds with potent antiproliferative effects were selected to measure their antiproliferative effects against human lung carcinoma cell lines H1975 and A549 cell lines. The results were summarized in Table 4. The data in Table 4 indicated that compounds **C4** and **C6** showed moderate antiproliferative effects against A549 cell line. The other tested compounds exhibited weak antiproliferative effects against H1975 and A549 cell lines. In addition, the most active compound **C9** displayed 340-fold selectivity against HCC827 cell lines over A549 cell line. Overall, these data suggested that tested compounds were significantly more active against HCC827 cell lines compared to the H1975 and A549 cell lines.

Table 4. Antiproliferative effects and cell-based selectivity of compounds against HCC827, H1975 and A549 cell lines ((IC_{50}, nM), $\bar{x} \pm s$, n = 3)

Compds	HCC827	H1975	A549	H1975/ HCC827	A549/ HCC827
C4	51.2±10.3	5580±190	237±10	109	5
C5	42.6±10.7	2260±120	2945±123	53	69
C6	42.6±9.3	1320±220	426±16	31	10
C7	44.3±0.9	9610±360	>10000	217	>226

C9	29.4±4.4	4990±147	>10000	170	>340
C11	51.4±4.2	2770±280	3362±820	54	65
C12	47.8±0.71	6260±680	4850±313	131	101
D4	64.7±18.1	5110±980	>10000	79	>154
D7	50.3±8.8	>10000	>10000	>198	>198
gefitinib	33.3±4.2	>10000	1260	-	-
AZD9291	43.1±1.1	47.2±5.8	486 ± 170	-	-

2.3. EGFR enzymatic activity assay

Then, compounds **C4-C7**, **C9**, **C11**, **C12**, **D4** and **D7** exhibited nanomolar antiproliferative effects and were selected to evaluate their inhibitory activity against EGFR to elucidate the mechanism of antiproliferative effects. **AZD9291** was used as the positive drug. The data are listed in Table 5.

Table 5. Enzymatic activity of **C4-C7**, **C9**, **C11**, **C12**, **D4** and **D7** (IC_{50}, nM, n = 2)

Compds	EGFR (WT)	EGFR (L858R)	EGFR (L858R/T790M)	EGFR (L858R/T790M/C797S)
C4	nd	4.2	nd	nd
C5	nd	2.0	nd	892
C6	nd	2.4	nd	529
C7	nd	1.7	nd	556
C9	1.6	1.9	104	331
C11	nd	5.8	nd	805
C12	2.5	1.2	189	114
D4	nd	4.2	nd	nd
D7	nd	2.6	nd	nd
AZD9291	1.4	2.1	1.8	120

"nd": not determined.

The data in Table 5 indicated that all the tested compounds displayed potent inhibitory activity against EGFR (L858R), weak activity toward EGFR (L858R/T790M) and EGFR (L858R/T790M/C797S). At the same time, compounds **C9**, **C12** and AZD9291 exhibited potent inhibitory activity against EGFR (WT) at the

same test conditions. It should be noticed that compound **C7** and **C12** displayed the IC_{50} of 556 nM and 114 nM against EGFR triple mutant (C797S), respectively. The data suggested that a compound (**C12**) with 8-(4-fluorophenylthio) moiety may improve its activity on EGFR triple mutant. This hints that optimization of compound **C12** may result in discovering potent EGFR (L858R/T790M/C797S) inhibitors.

So far, thirty-one compounds were synthesized and their antiproliferative effects were evaluated. Nine compounds were determined their inhibitory activities against EGFR. The property comparison of compounds **C7**, **C9**, **4** and AZD9291 was listed in Table 6. The data in Table 6 indicated that compounds **C7** and **C9** displayed close antiproliferative effects against HCC827 and enzyme inhibitory on EGFR (L858R) compared with compound **4** and AZD9291. However, compounds **C7** and **C9**, presented in our study, showed higher growth inhibition ratio on HCC827 cell than compound **4** and AZD9291 at 1 μM. In the meantime, predicted CLogP value was less than 5.

Table 6. Property comparison of compounds **C7**, **C9**, **4**, and AZD9291

compds	MW[a]	CLogP[b]	IC_{50}(nM) (HCC827)	E_{max}[c] (%, 1 μM)	IC_{50} (nM), EGFR(L858R)
C7	491	4.89	44.4±0.9	78	1.7
C9	530	4.41	29.4±4.4	81	1.9
4	469	6.40	35.9±4.2	70	nd
AZD9291	500	4.60	25.4±1.5	73	2.1

"nd": not determined. a: molecule weight (MW) was calculated from ChemBioDraw 14.0. b: CLogP was predicted by ChemBioDraw 14.0. c: growth inhibition rate on HCC827 cell at 1 μM.

2.4. Western blot assay of inhibition of EGFR autophosphorylation

To further verify the mechanism of the antiproliferative activity of compound **C9**, Western blot assay was employed to evaluate the inhibition of EGFR autophosphorylation of compound **C9** in HCC827 cell line (Figure 3). The results suggested that compound **C9** can block EGFR phosphorylation in a dose-dependent manner in the HCC827 cell line.

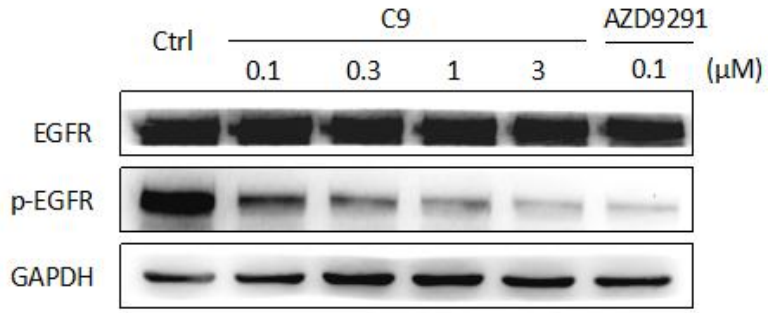


Figure 3. Inhibition of EGFR autophosphorylation of compound **C9** in HCC827 cell line

2.5. Anticancer effects *in vivo*

Finally, we explored whether potent compound could inhibit tumor growth in established mouse xenografts models. Compound **C9** displayed potent inhibitory activity against EGFR (L858R) and antiproliferative effect on HCC827. Then **C9** was selected to evaluate its anticancer effect *in vivo*. A study using mice bearing HCC827 xenografts was performed. Compound **C9** was dosed orally at 1 mg/kg or 5 mg/kg once a day for 20 days. The results were shown in Figure 4.

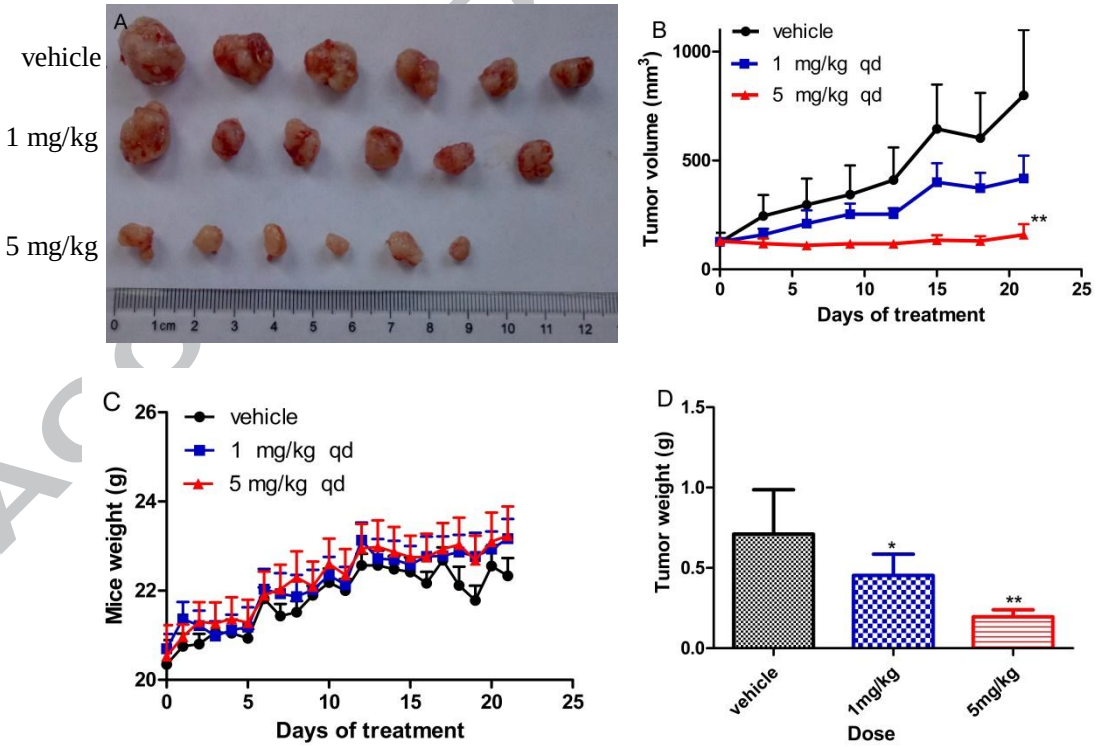


Figure 4. The anticancer effect of compound **C9** on HCC827 xenografts model (n = 6 in each group). (A) The photograph of tumors in each group after **C9** and vehicle treatment. (B) Tumor growth curves during treatment with **C9** and vehicle. $^{**}p < 0.01$ *vs* vehicle. (C) Body weight of

mice during treatment with **C9** and vehicle. (D) Comparison of the final tumor weights in each group after the 20-day treatment of **C9**. Numbers in columns indicate the mean tumor weight in each group. *$p < 0.05$, **$p < 0.01$ *vs* vehicle.

The results indicated that compound **C9** can significantly inhibit tumor growth at a dosage of 5 mg/kg. Meanwhile, mouse body weights of tested animals were increased during treatment.

2.6. Molecular docking studies

To further explain the EGFR inhibitory activity of compounds **C7**, **D7** and **C9**, we performed a docking analysis utilizing the C-DOCKER program within Discovery Studio 2.5 software packages. The binding mode of compounds **C7**, **D7** and **C9** within the active site of the T790M mutant predicted by molecular docking were depicted in Figure 5. From the docking results, we observed that compounds **C7**, **D7** and **C9** were favorably located into the receptor pocket and had three same binding characteristics: (1) the purine ring was located in the ATP-binging pocket and sandwiched between the N- and C-lobes of the kinase. (2) The hydrogen atom of aniline at the C-2 position of purine scaffold and the 1-nitrogen atom from purine core formed a hydrogen bond with Met793, respectively. (3) The 2-aniline branch extended the solvent and the phenyl from 8-phenylthio moiety or 8-phenylsulfinyl was directed into the hydrophobic pocket of the ATP-binding domain. The sp^3 hybridization of sulfur atom in 8-phenylthio moiety or 8-phenylsulfinyl may be conducive to this combination. It was noted that the 8-phenylsulfinyl in compound **D7** could form two extra hydrogen bonds with Lys745 and Asp855, respectively. However, compound **D9** did not produce a suitable docking result. These could explain why compound **D7** displayed more potent inhibitory activity than other compounds **D** in enzyme and cell-based evaluation.

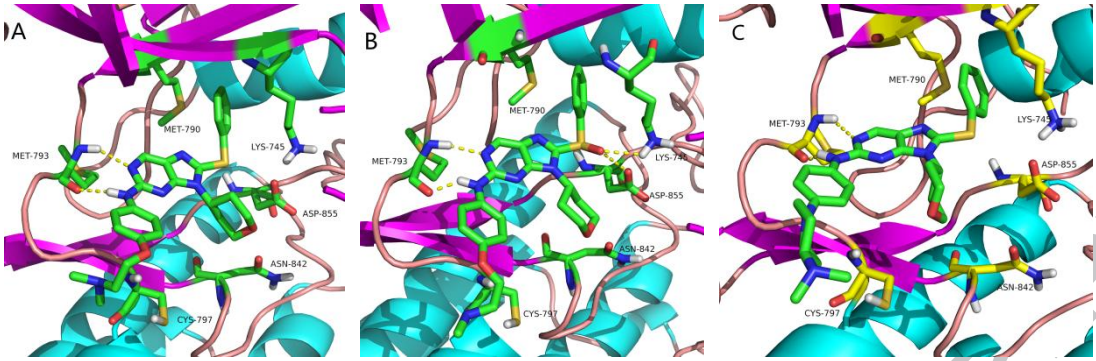


Figure 5. The docking mode of Compounds **C7**, **D7** and **C9** with the T790M EGFR mutant (PDB code: 2JIU). (A) The docking pose of compound **C7** with EGFR. (B) The docking pose of compound **D7** with EGFR. (C) The docking pose of compound **C9** with EGFR. Compounds **C7**, **D7** and **C9** were shown as sticks. Hydrogen bonds within 3.0 Å were shown as yellow dashed lines.

3. Conclusions

In summary, thirty-one 2, 9-disubstituted 8-phenylthio/phenylsulfinyl /benzylthio-9H-purine derivatives were synthesized. Some compounds displayed potent antiproliferative effects against HCC827 cancer cell line and compound **C9** exhibited potent inhibitory activities against the $EGFR^{L858R}$ (IC_{50} = 1.9 nM). Compound **C12** showed moderate inhibitory activity against $EGFR^{L858R/T790M/C797S}$ (IC_{50} = 114 nM). Western bolt assay suggested that compound **C9** significantly inhibited EGFR phosphorylation. Additionally, compound **C9** remarkably exhibited inhibitory effect on tumor growth at 5.0 mg/kg by oral administration in established nude mouse HCC827 xenograft model. These results indicate that 2,9-disubstituted 8-phenylsulfinyl-9H-purine derivatives can act as potent EGFR(L858R) inhibitors and effective anticancer agents. Additionally, optimization of compound **C12** may result in discovering the fourth-generation EGFR-TKIs.

4. Experimental protocols

4.1. Chemistry and chemical methods

Unless specified otherwise, all starting materials, reagents and solvents were commercially available. All the reactions were monitored by thin-layer

chromatography on silica gel plates (GF-254) and visualized with UV light. All the melting points were determined on a Shanghai micro melting-point apparatus (model: SGW® X-4B) and thermometer was uncorrected. NMR spectra were recorded on a 400 Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal reference. All chemical shifts were reported in parts per million (ppm). Mass measurements were performed using electrospray ionization (positive mode) on a quadrupole time-of-flight (QTOF) mass spectrometer (Skyray Instrument).

4.1.1. General procedure for the synthesis of intermediates B1-B10.

The reaction of 2, 4-dichloro-5-nitropyrimidine with isopropylamine produced intermediate 2-chloro-*N*-isopropyl-5-nitropyrimidin-4-amine. 4-Fluoronitrobenzene reacted with 1-methylpiperazine in DMSO yielded the intermediate 1-methyl-4-(4-nitrophenyl)piperazine in the presence of K_2CO_3. The catalytic hydrogenation of 1-methyl-4-(4-nitrophenyl)piperazine with palladium on carbon (Pd/C, 5%) quantificationally provided the desired 4-(4-methylpiperazin-1-yl) aniline. Refluxing of the 2-chloro-*N*-isopropyl-5-nitropyrimidin-4-amine with 4-(4-methyl piperazin-1-yl)aniline in *n*-butanol yielded N^4-isopropyl-N^2-(4-(4-methylpiperazin-1-yl)phenyl)-5-nitropyrimidine-2,4-diamine, which was reduced to intermediate **A1** with a good yield by catalytic hydrogenation using Pd/C as a catalyst. Intermediates **A** were prepared as these steps and used for the next step without further purification. These processes were carried out as reported.[34]

The mixture of **A** (0.88 mmol), CS_2 (85 μL, 0.88 mmol) and KOH (51 mg, 0.88 mmol) in EtOH (30 mL) and H_2O (3 mL) was refluxed for 4 h, evaporated under reduced pressure. The resulting crude product was purified by silica gel column chromatography using DCM/MeOH = 20:1 (V/V) as eluent to afford the compound **B** as a solid.

4.1.1.1. 9-Isopropyl-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-9*H*-purine-8-thiol (**B1**). Off-white solid; Yield 66.7%; mp 239.9-241.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H, Ar-H), 7.51 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.13 (s, 1H, NH), 6.98 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.38 (dt, *J* = 13.9, 6.9 Hz, 1H, CH), 3.26 – 3.21 (m, 4H, $CH_2\times2$),

2.69 – 2.64 (m, 4H, $CH_2\times2$), 2.41 (s, 3H, CH_3), 1.68 (d, J = 6.9 Hz, 6H, $CH_3\times2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.1, 156.3, 152.0, 146.4, 137.6, 133.4, 120.2 (2C), 117.1, 116.3(2C), 55.1 (2C), 49.3(2C), 47.8, 46.2, 19.8 (2C). MS (ESI, m/z): 384.6 $[M + H]^+$.

4.1.1.2. 9-Cyclopropyl-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-9*H*-purine-8-thiol (**B2**). Off-white solid; Yield 63.8%; mp >250 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H, Ar-H), 7.53 (d, J = 8.9 Hz, 2H, Ar-H), 7.02 (s, 1H, NH), 6.98 (d, J = 9.0 Hz, 2H, Ar-H), 3.28 (s, 1H, CH), 3.24 – 3.20 (m, 4H, $CH_2\times2$), 2.64 (s, 4H, $CH_2\times2$), 2.40 (s, 3H, CH_3), 1.43 (s, 2H, $CH\times2$), 1.26 (d, J = 6.5 Hz, 2H, $CH\times2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.4, 156.5, 153.0, 146.3, 137.2, 133.5, 120.1 (2C), 117.0, 116.4 (2C), 55.1 (2C), 49.3 (2C), 46.1, 26.1, 6.7 (2C). MS (ESI, m/z): 382.6 $[M + H]^+$.

4.1.1.3. 9-(Cyclopropylmethyl)-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-9*H*-purine-8-thiol (**B3**). Gray solid; Yield 52.6%; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H, NH), 8.19 (s, 1H, Ar-H), 7.59 (d, J = 9.0 Hz, 2H, Ar-H), 6.88 (d, J = 9.1 Hz, 2H, Ar-H), 4.02 (d, J = 7.2 Hz, 2H, CH_2), 3.10 – 3.04 (m, 4H, $CH_2\times2$), 2.49 – 2.45 (m, 4H, $CH_2\times2$), 2.23 (s, 3H, CH_3), 1.39 (dd, J = 13.7, 6.2 Hz, 1H, CH), 0.51 (dd, J = 8.1, 6.6 Hz, 4H, $CH_2\times2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 156.9, 152.5, 146.4, 137.4, 133.4, 120.2 (2C), 117.3, 116.3 (2C), 55.2 (2C), 49.3 (2C), 46.7, 46.2, 10.3, 4.1 (2C). MS (ESI, m/z): 395.2 $[M + H]^+$.

4.1.1.4. 9-Cyclopentyl-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-9*H*-purine-8-thiol (**B4**). Off-white solid; Yield 70.9%; mp >250 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H, Ar-H), 7.50 (d, J = 8.9 Hz, 2H, Ar-H), 7.36 (s, 1H, NH), 6.95 (d, J = 8.9 Hz, 2H, Ar-H), 5.54 – 5.40 (m, 1H, CH), 3.28 – 3.23 (m, 4H, $CH_2\times2$), 2.79 – 2.73 (m, 4H, $CH_2\times2$), 2.47 – 2.39 (m, 5H, CH_3, $CH\times2$), 2.05 (d, J = 6.3 Hz, 4H, $CH\times4$), 1.77 – 1.69 (m, 2H, $CH\times2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 156.3, 151.7, 146.5, 137.7, 133.2, 120.4 (2C), 117.2, 116.3 (2C), 55.7, 55.1 (2C), 49.3 (2C), 46.1, 28.7 (2C), 25.1 (2C). MS (ESI, m/z): 410.9 $[M + H]^+$.

4.1.1.5. 2-((4-(4-Methylpiperazin-1-yl)phenyl)amino)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purine-8-thiol (**B5**). Off-white solid; Yield 90.3%; mp >250 °C; ^{1}H NMR (400

MHz, $CDCl_3$) δ 8.15 (s, 1H, Ar-H), 7.53 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.18 (s, 1H, NH), 6.98 (d, *J* = 8.7 Hz, 2H, Ar-H), 5.27 (t, *J* = 12.3 Hz, 1H, CH), 4.18 (d, *J* = 7.1 Hz, 2H, CH×2), 3.62 (t, *J* = 11.7 Hz, 2H, CH×2), 3.25 (s, 4H, CH_2×2), 3.04 – 2.89 (m, 2H, CH×2), 2.71 (s, 4H, CH_2×2), 2.44 (s, 3H, CH_3), 1.81 (d, *J* = 10.1 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.3, 156.4, 152.1, 146.3, 142.3, 137.8, 132.9, 120.2 (2C), 116.3 (2C), 67.1 (2C), 55.2 (2C), 52.7, 49.4 (2C), 46.2, 29.3(2C). MS (ESI, m/z): 426.6 $[M + H]^+$.

4.1.1.6. (1*S*, 4*S*)-4-(8-Mercapto-2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-9*H*-purin-9-yl)cyclohexan-1-ol (**B6**). Yellow solid; Yield 73.6%; mp 235.7-239.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H, Ar-H), 7.50 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.11 (s, 1H, NH), 6.97 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.02 (t, *J* = 12.4 Hz, 1H, CH), 3.80 (dd, *J* = 20.6, 9.7 Hz, 1H, CH), 3.25 – 3.22 (m, 4H, CH_2×2), 2.73 – 2.69 (m, 4H, CH_2×2), 2.44 (s, 3H, CH_3), 2.18 (s, 2H, CH×2), 1.89 (d, *J* = 11.5 Hz, 2H, CH×2), 1.56 (d, *J* = 13.6 Hz, 2H, CH×2), 1.29 (d, *J* = 13.2 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 156.2, 151.9, 146.4, 137.8, 133.4, 120.2 (2C), 117.0, 116.3 (2C), 68.7, 55.2 (2C), 54.6, 49.4 (2C), 46.2, 35.1 (2C), 27.1 (2C). MS (ESI, m/z): 440.6 $[M + H]^+$.

4.1.1.7. 2-((4-((2-(Dimethylamino)ethyl)(methyl)amino)phenyl)amino)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purine-8-thiol (**B7**). Off-white solid; Yield 63.6%; mp 184.2-187.7℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H, Ar-H), 7.34 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.04 (d, *J* = 15.1 Hz, 1H, NH), 6.64 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.33 – 5.24 (m, 1H, CH), 4.17 (dd, *J* = 11.4, 4.2 Hz, 2H, CH_2), 3.64 – 3.52 (m, 4H, CH_2, CH×2), 3.01 – 2.93 (m, 5H, CH_3, CH×2), 2.69 (t, *J* = 6.6 Hz, 2H, CH×2), 2.46 (s, 6H, CH_3 ×2), 1.82 (d, *J* = 9.6 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.1, 156.5, 152.2, 144.9, 137.6, 130.6, 121.2 (2C), 117.1, 112.7 (2C), 67.2 (2C), 55.9, 52.7, 50.8, 45.9 (2C), 38.9, 29.3 (2C). MS (ESI, m/z): 428.6 $[M + H]^+$.

4.1.1.8. 2-((4-(4-(Dimethylamino)piperidin-1-yl)phenyl)amino)-9-(tetrahydro-2*H*-pyran-4-yl) -9*H*-purine-8-thiol (**B8**). Off-white solid; Yield 55.7%; mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H, NH), 8.19 (s, 1H, Ar-H), 7.59 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.89 (d, *J* = 9.1 Hz, 2H, Ar-H), 5.12 (s, 1H, CH), 4.37 (s, 1H, CH),

4.03 (d, *J* = 7.0 Hz, 2H, CH×2), 3.64 (d, *J* = 12.1 Hz, 2H, CH×2), 3.45 (d, *J* = 6.7 Hz, 2H, CH×2), 2.85 – 2.73 (m, 2H, CH×2), 2.62 (d, *J* = 11.7 Hz, 2H, CH×2), 2.43 (s, 6H, CH_3×2), 1.92 (d, *J* = 12.4 Hz, 2H, CH×2), 1.67 (d, *J* = 9.3 Hz, 2H, CH×2), 1.58 (d, *J* = 8.5 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.1, 153.8, 148.9, 147.9, 147.2, 132.5, 131.7 (2C), 130.8, 129.6 (2C), 67.5 (2C), 62.1, 54.2, 50.1 (2C), 41.7 (2C), 30.4 (2C), 28.4 (2C). MS (ESI, m/z): 454.6 $[M + H]^+$.

4.1.1.9. 2-((4-((1-Methylpiperidin-4-yl)oxy)phenyl)amino)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purine-8-thiol (**B9**). Off-white solid; Yield 55.7%; mp 247.6-249.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H, Ar-H), 7.54 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.20 (s, 1H, NH), 6.95 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.27 (ddd, *J* = 12.3, 7.9, 4.2 Hz, 1H, CH), 4.35 (s, 1H, CH), 4.18 (dd, *J* = 11.5, 4.3 Hz, 2H, CH×2), 3.62 (t, *J* = 11.5 Hz, 2H, CH×2), 2.93 (qd, *J* = 12.5, 4.7 Hz, 2H, CH×2), 2.82 (s, 2H, CH×2), 2.48 (s, 2H, CH×2), 2.41 (s, 3H, CH_3), 2.09 (dd, *J* = 10.3, 6.4 Hz, 2H, CH×2), 1.93 (s, 2H, CH×2), 1.81 (d, *J* = 9.7 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 156.1, 153.8, 152.2, 137.6, 134.4, 120.6 (2C), 117.4, 114.8 (2C), 67.1 (2C), 66.3, 58.2 (2C), 52.7, 45.9 (3C), 29.3 (2C). MS (ESI, m/z): 441.6 $[M + H]^+$.

4.1.1.10. 2-((4-(2-(Dimethylamino)ethoxy)phenyl)amino)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purine-8-thiol (**B10**). Gray solid; Yield 56.2%; mp 228.6-229.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H, Ar-H), 7.46 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.16 (s, 1H, NH), 6.83 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.34 – 5.22 (m, 1H, CH), 4.19 – 4.13 (m, 4H, CH_2, CH×2), 3.63 (d, *J* = 11.7 Hz, 2H, CH_2), 2.97 – 2.86 (m, 4H, CH×4), 2.50 (s, 6H, CH_3×2), 1.80 (d, *J* = 9.4 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 156.1, 152.2, 152.0, 137.6, 134.6, 120.7 (2C), 117.7, 116.6 (2C), 74.5, 72.5, 67.1 (2C), 52.7 (2C), 46.1, 30.9 (2C), 29.3 (2C). MS (ESI, m/z): 415.6 $[M + H]^+$.

4.1.2. General procedure for the synthesis of compounds C1-C12.

The mixture of **B** (0.52 mmol), iodobenzene (80 μL, 0.78 mmol), CuI (8.2 mg, 0.026 mmol), 1,10-phenanthroline (16.3 mg, 0.052 mmol), K_2CO_3 (160.4 mg, 1.04 mmol) and DMSO (5 mL) was stirred at 140 °C for 24 h under argon atmosphere, cooled to room temperature. To the mixture was added 20 mL of DCM. The organic phase was washed three times with water, and dried over Na_2SO_4, filtered,

concentrated in vacuum. The residue was purified by column chromatography on silica gel using DCM/MeOH = 10:1 (V/V) as the eluent to afford the compound **C** as a solid.

4.1.2.1. 9-Isopropyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-(phenylthio)-9*H*-purin-2–amine (**C1**). Off-white solid; Yield 66.7%; mp 190.1-191.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H, Ar-H), 7.56 (t, *J* = 6.1 Hz, 2H, Ar-H), 7.48 (dt, *J* = 8.6, 2.3 Hz, 2H, Ar-H), 7.43 – 7.34 (m, 3H, Ar-H), 7.34 – 7.30 (m, 1H, NH), 6.97 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.98 – 4.82 (m, 1H, CH), 3.24 – 3.19 (m, 4H, $CH_2\times2$), 2.66 – 2.61 (m, 4H, $CH_2\times2$), 2.39 (s, 3H, CH_3), 1.63 (d, *J* = 6.9 Hz, 6H, $CH_3\times2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 153.8, 148.6, 146.8, 132.8, 131.7 (2C), 130.8, 129.6 (2C), 129.1, 128.5, 120.3 (2C), 116.9 (2C), 55.2 (2C), 49.9 (2C), 49.3, 46.1, 20.7 (2C). MS (ESI, m/z): 415.6 $[M + H]^+$.

4.1.2.2. 9-Cyclopropyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-(phenylthio)-9*H*-purin-2-amine (**C2**). Off-white solid; Yield 69.7%; mp 210.4-213.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H, Ar-H), 7.62 (s, 4H, Ar-H), 7.44 (s, 3H, Ar-H), 7.25 (s, 1H, NH), 6.97 (d, *J* = 7.2 Hz, 2H, Ar-H), 3.23 (s, 4H, $CH_2\times2$), 3.05 (s, 1H, CH), 2.67 (s, 4H, $CH_2\times2$), 2.43 (d, *J* = 17.4 Hz, 3H, CH_3), 1.36 (s, 2H, CH_2), 1.24 (s, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 155.3, 152.7, 147.5, 146.7, 133.4 (2C), 133.0, 129.6 (2C), 129.1, 128.9, 128.5, 120.2 (2C), 117.2 (2C), 55.1 (2C), 49.9 (2C), 46.0, 24.4, 7.2 (2C). MS (ESI, m/z): 458.2 $[M + H]^+$.

4.1.2.3. 9-(Cyclopropylmethyl)-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-(phenylthio)-9*H*-purin-2-amine (**C3**). Off-white solid; Yield 58.3%; mp 172.7-173.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H, Ar-H), 7.57 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.53 (d, *J* = 6.8 Hz, 2H, Ar-H), 7.39 (d, *J* = 7.1 Hz, 3H, Ar-H), 6.97 (d, *J* = 8.5 Hz, 2H, Ar-H), 4.06 (d, *J* = 6.9 Hz, 2H, CH_2), 3.22 (s, 4H, $CH_2\times2$), 2.65 (s, 4H, $CH_2\times2$), 2.40 (s, 3H, CH_3), 1.33 (s, 1H, CH), 0.58 – 0.49 (m, 4H, $CH_2\times2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 154.3, 148.6, 148.3, 146.9, 132.7, 131.9 (2C), 130.2, 129.6 (2C), 128.8, 128.6, 120.5 (2C), 117.1 (2C), 55.2 (2C), 49.8 (2C), 47.7, 46.1, 11.0, 4.3 (2C). MS (ESI, m/z): 472.6 $[M + H]^+$.

4.1.2.4. 9-Cyclopentyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-(phenylthio)-9*H*-purin

-2-amine (**C4**). Off-white solid; Yield 57.6%; mp 179.4-181.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H, Ar-H), 7.55 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.50 – 7.47 (m, 1H, Ar-H), 7.46 (d, *J* = 1.4 Hz, 1H, Ar-H), 7.41 – 7.34 (m, 3H, Ar-H), 7.17 (s, 1H, NH), 6.95 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.96 (t, *J* = 8.7 Hz, 1H, CH), 3.26 – 3.19 (m, 4H, CH_2 ×2), 2.68 – 2.61 (m, 4H, CH_2×2), 2.50 – 2.42 (m, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.05 (dd, *J* = 7.1, 5.4 Hz, 2H, CH_2), 1.94 – 1.85 (m, 2H. CH_2), 1.68 (dd, *J* = 11.3, 5.6 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 148.9, 148.7, 146.8, 132.9, 131.9, 131.6 (2C), 130.9, 129.7, 129.6 (2C), 128.4, 120.4 (2C), 117.1 (2C), 57.5, 55.1 (2C), 49.8 (2C), 45.9, 30.1 (2C), 25.1 (2C). MS (ESI, m/z): 486.6 $[M + H]^+$.

4.1.2.5. *N*-(4-(4-Methylpiperazin-1-yl)phenyl)-8-(phenylthio)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**C5**). Off-white solid; Yield 56.7%; mp 235.6-236.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, Ar-H), 7.58 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.50 (dd, *J* = 7.9, 1.6 Hz, 2H, Ar-H), 7.44 – 7.31 (m, 3H, Ar-H), 7.29 (s, 1H, NH), 6.98 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.72 – 4.62 (m, 1H, CH), 4.12 (dd, *J* = 11.5, 4.2 Hz, 2H, CH× 2), 3.44 (t, *J* = 11.4 Hz, 2H, CH×2), 3.27 – 3.14 (m, 4H, CH_2×2), 2.93 (qd, *J* = 12.5, 4.5 Hz, 2H, CH×2), 2.69 – 2.55 (m, 4H, CH_2×2), 2.38 (s, 3H, CH_3), 1.55 (dd, *J* = 12.4, 2.5 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 153.8, 148.9, 147.9, 146.9, 132.6, 131.8 (2C), 130.8, 129.7 (2C), 128.8, 128.7, 120.2 (2C), 117.0 (2C), 67.5 (2C), 55.2 (2C), 54.2, 49.8 (2C), 46.1, 30.4 (2C). MS (ESI, m/z): 502.6 $[M + H]^+$.

4.1.2.6. (1*S*, 4*S*)-4-(2-((4-(4-methylpiperazin-1-yl)phenyl)amino)-8-(phenylthio)-9*H*-purin-9-yl)cyclohexan-1-ol (**C6**). Off-white solid; Yield 55.2%; mp 203.9-207.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H, Ar-H), 7.55 (d, *J* = 6.8 Hz, 2H, Ar-H), 7.49 (s, 2H, Ar-H), 7.37 (s, 4H, Ar-H, NH), 6.96 (d, *J* = 6.6 Hz, 2H, Ar-H), 4.46 (s, 1H, CH), 3.80 (s, 1H, CH), 3.21 (s, 4H, CH_2×2), 2.63 (s, 4H, CH_2×2), 2.39 (s, 3H, CH_3), 2.14 (s, 4H, CH_2×2), 1.67 (d, *J* = 9.5 Hz, 2H, CH×2), 1.40 (d, *J* = 10.9 Hz, 2H, CH ×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 153.6, 148.8, 148.2, 146.9, 132.7, 131.9 (2C), 130.7, 129.6 (2C), 128.7, 128.9, 120.4 (2C), 116.9 (2C), 69.6, 56.0, 55.2 (2C), 49.9 (2C), 46.1, 34.8 (2C), 27.9 (2C). MS (ESI, m/z): 516.1 $[M + H]^+$.

4.1.2.7. *N*-(4-(2-(Dimethylamino)ethoxy)phenyl)-8-(phenylthio)-9-(tetrahydro-2*H*-py-

ran-4-yl)-9*H*-purin-2-amine (**C7**). Off-white solid; Yield 47.3%; mp 172.7-173.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, Ar-H), 7.57 (t, *J* = 6.1 Hz, 2H, Ar-H), 7.50 (dd, *J* = 7.9, 1.6 Hz, 2H, Ar-H), 7.42 – 7.32 (m, 4H, Ar-H, NH), 6.95 (d, *J* = 8.9 Hz, 2H, Ar-H), 4.66 (ddd, *J* = 12.1, 7.9, 4.2 Hz, 1H, CH), 4.14 – 4.07 (m, 4H, CH×4), 3.44 (t, *J* = 11.6 Hz, 2H, CH_2), 2.92 (qd, *J* = 12.5, 4.5 Hz, 2H, CH×2), 2.75 (t, *J* = 5.7 Hz, 2H, CH_2), 2.37 (s, 6H, CH_3×2), 1.56 (dd, *J* = 12.4, 2.4 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 154.4, 153.8, 148.8, 148.1, 133.2, 131.8 (2C), 130.7, 129.7 (2C), 128.9, 128.7, 120.7 (2C), 115.0 (2C), 67.5 (2C), 66.4, 58.4, 54.2, 45.9 (2C), 30.4 (2C). MS (ESI, m/z): 491.1 $[M + H]^+$.

4.1.2.8. N^1-(2-(Dimethylamino)ethyl)-N^1-methyl-N^4-(8-(phenylthio)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-yl)benzene-1,4-diamine (**C8**). Off-white solid; Yield 55.3%; mp 164.4-165.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 7.50 (d, *J* = 2.3 Hz, 2H, Ar-H), 7.49 – 7.47 (m, 1H, Ar-H), 7.41 – 7.34 (m, 3H, Ar-H), 6.77 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.71 – 4.62 (m, 1H, CH), 4.11 (dd, *J* = 11.5, 4.2 Hz, 2H, CH×2), 3.48 – 3.45 (m, 2H, CH_2), 3.42 (d, *J* = 11.7 Hz, 2H, CH×2), 2.96 (s, 3H, CH_3), 2.95 – 2.86 (m, 2H,), 2.55 – 2.47 (m, 2H, CH_2), 2.31 (s, 6H, CH_3×2), 1.55 (dd, *J* = 12.4, 2.5 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 153.95, 148.95, 147.6, 145.6, 131.6 (2C), 130.9, 129.7 (2C), 129.6, 128.7, 128.6, 121.3 (2C), 113.0 (2C), 67.5 (2C), 55.9, 54.2, 51.6, 45.9 (2C), 38.9, 30.5 (2C). MS (ESI, m/z): 504.2 $[M + H]^+$.

4.1.2.9. *N*-(4-(4-(Dimethylamino)piperidin-1-yl)phenyl)-8-(phenylthio)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**C9**). Off-white solid; Yield 51.4%; mp 242.3-244.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, Ar-H), 7.57 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.49 (dd, *J* = 7.9, 1.6 Hz, 2H, Ar-H), 7.38 (tdd, *J* = 7.0, 5.0, 1.9 Hz, 3H, Ar-H), 7.31 (s, 1H, NH), 6.98 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.66 (dd, *J* = 10.1, 6.0 Hz, 1H, CH), 4.12 (dd, *J* = 11.5, 4.2 Hz, 2H, CH×2), 3.68 (d, *J* = 12.3 Hz, 2H, CH×2), 3.44 (t, *J* = 11.4 Hz, 2H, CH×2), 2.93 (td, *J* = 12.4, 7.9 Hz, 2H, CH×2), 2.70 (td, *J* = 12.1, 1.9 Hz, 2H, CH×2), 2.35 (s, 6H, CH_3×2), 2.28 (dd, *J* = 9.2, 5.7 Hz, 1H, CH), 1.95 (d, *J* = 12.4 Hz, 2H, CH×2), 1.69 (tt, *J* = 12.0, 6.1 Hz, 2H, CH×2), 1.55 (dd, *J*

= 12.4, 2.5 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 153.8, 148.9, 147.9, 147.2, 132.5, 131.7 (2C), 130.8, 129.6 (2C), 128.7, 120.2 (2C), 117.5 (2C), 67.5 (2C), 62.1, 54.2, 50.1 (2C), 41.7 (2C), 30.4 (2C), 28.4 (2C). MS (ESI, m/z): 530.2 $[M + H]^+$.

4.1.2.10. *N*-(4-((1-Methylpiperidin-4-yl)oxy)phenyl)-8-(phenylthio)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**C10**). Off-white solid; Yield 59.6%; mp 177.9-179.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, Ar-H), 7.58 (s, 1H, Ar-H), 7.56 (d, *J* = 3.4 Hz, 1H, Ar-H), 7.51 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.49 (d, *J* = 1.4 Hz, 1H, Ar-H), 7.43 – 7.34 (m, 3H, Ar-H), 7.32 (s, 1H, NH), 6.97 – 6.92 (m, 2H, Ar-H), 4.67 (ddd, *J* = 12.1, 7.9, 4.2 Hz, 1H, CH×2), 4.35 – 4.24 (m, 1H, CH×2), 4.12 (dd, *J* = 11.5, 4.2 Hz, 2H, CH×2), 3.44 (t, *J* = 11.4 Hz, 2H, CH×2), 2.92 (qd, *J* = 12.5, 4.6 Hz, 2H, CH×2), 2.72 (s, 2H, CH×2), 2.33 (s, 3H, CH_3), 2.29 (s, 2H, CH×2), 2.07 – 1.97 (m, 2H, CH×2), 1.91 – 1.81 (m, 2H, CH×2), 1.56 (dd, *J* = 12.4, 2.5 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 153.8, 152.9, 148.8, 148.1, 133.4, 131.8 (2C), 130.6, 129.7 (2C), 128.9, 128.7, 120.6 (2C), 116.9 (2C), 67.5 (2C), 54.2 (2C), 52.7, 46.2 (2C), 30.9 (2C), 30.4 (2C). MS (ESI, m/z): 517.1 $[M + H]^+$.

4.1.2.11. *N*-(4-(2-(Dimethylamino)ethoxy)phenyl)-8-((3-fluorophenyl)thio)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**C11**). Off-white solid; Yield 55.2%; mp 146.2-150.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H, Ar-H), 7.58 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.36 (td, *J* = 8.0, 5.9 Hz, 1H, Ar-H), 7.29 – 7.18 (m, 3H, NH, Ar-H), 7.12 – 7.02 (m, 1H, Ar-H), 6.96 (d, *J* = 8.9 Hz, 2H, Ar-H), 4.74 – 4.60 (m, 1H, CH), 4.13 (dd, *J* = 12.1, 6.7 Hz, 4H, CH_2, CH×2), 3.47 (t, *J* = 12.0 Hz, 2H, CH×2), 3.02 – 2.88 (m, 2H, CH×2), 2.81 (t, *J* = 5.5 Hz, 2H, CH_2), 2.41 (s, 6H, CH_3×2), 1.62 (d, *J* = 12.6 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6 (d, J_{C-F} = 250), 156.2, 154.4, 153.7, 149.2, 146.7, 133.1, 130.9, 126.8, 120.7 (2C), 118.3, 118.1, 115.8, 115.6, 114.9 (2C), 67.5 (2C), 66.2, 58.3, 54.2, 45.8 (2C), 30.5 (2C). MS (ESI, m/z): 509.2 $[M + H]^+$.

4.1.2.12. *N*-(4-(2-(Dimethylamino)ethoxy)phenyl)-8-((4-fluorophenyl)thio)-9-(tetrahy-dro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**C12**). Off-white solid; Yield 47.2%; mp 142.7-147.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H, Ar-H), 7.62 – 7.53 (m,

4H, Ar-H), 7.18 (s, 1H, NH), 7.13 (td, *J* = 8.5, 1.5 Hz, 2H, Ar-H), 7.00 – 6.92 (m, 2H, Ar-H), 4.71 – 4.57 (m, 1H, CH), 4.16 (dd, *J* = 11.6, 3.9 Hz, 2H, CH×2), 4.10 (t, *J* = 5.7 Hz, 2H, CH_2), 3.51 (t, *J* = 12.0 Hz, 2H, CH×2), 2.95 (qd, *J* = 12.4, 4.2 Hz, 2H, CH×2), 2.77 (t, *J* = 5.6 Hz, 2H, CH_2), 2.38 (t, *J* = 3.8 Hz, 6H, CH_3×2), 1.66 (d, *J* = 12.2 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3 (d, J_{C-F} = 265 Hz), 155.9, 154.5, 153.9, 148.8, 148.4, 135.0, 134.9, 133.1, 128.9, 124.7, 120.6 (2C), 117.1, 116.9, 115.0 (2C), 67.5 (2C), 66.3, 58.4, 54.1, 45.9 (2C), 30.5 (2C). MS (ESI, m/z): 509.2 $[M + H]^+$.

4.1.3. General procedure for the synthesis of compounds E1-E4.

The mixture of **B** (0.52 mmol), 3-chloromethylpyridine hydrochloride (87 mg, 0.52 mmol), K_2CO_3 (180 mg, 1.30 mmol) and acetone (10 mL) was refluxed for 6 h. The mixture was concentrated in vacuum. The residue was purified by column chromatography on silica gel using DCM/MeOH = 10:1 as the eluent to afford the compound **E** as an off-white solid.

4.1.3.1. 9-Cyclopropyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-((pyridin-3-ylmethyl) thio)-9*H*-purin-2-amine (**E1**). Off-white solid; Yield 42.4%; mp 164.6-165.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J* = 1.7 Hz, 1H, Ar-H), 8.59 (s, 1H, Ar-H), 8.55 (dd, *J* = 4.7, 1.3 Hz, 1H, Ar-H), 7.84 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.60 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.29 (d, *J* = 5.5 Hz, 1H, Ar-H), 7.20 (s, 1H, NH), 6.97 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.57 (s, 2H, CH_2), 3.24 – 3.19 (m, 4H, CH_2×2), 3.07 – 2.98 (m, 1H, CH), 2.67 – 2.62 (m, 4H, CH_2×2), 2.40 (s, 3H, CH_3), 1.25 (t, *J* = 6.5 Hz, 2H, CH_2), 1.23 – 1.18 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 155.7, 153.7, 150.4, 149.0, 146.7, 146.1, 136.7, 133.1, 132.7, 128.6, 123.5, 120.2 (2C), 117.2 (2C), 55.1 (2C), 49.9 (2C), 46.0, 32.8, 23.7, 6.9 (2C). MS (ESI, m/z): 473.5 $[M + H]^+$.

4.1.3.2. 9-Cyclopentyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-((pyridin-3-ylmethyl) thio)-9*H*-purin-2-amine (**E2**). Off-white solid; Yield 72.9%; mp 178.4-179.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.61 (s, 1H, Ar-H), 8.54 (dd, *J* = 4.8, 1.5 Hz, 1H, Ar-H), 7.81 (dt, *J* = 7.8, 1.8 Hz, 1H, Ar-H), 7.54 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.28 – 7.25 (m, 1H, Ar-H), 7.10 (s, 1H, NH), 6.96 (d, *J* = 9.0 Hz, 2H,

Ar-H), 4.71 – 4.62 (m, 1H, CH), 4.58 (s, 2H, CH_2), 3.23 – 3.18 (m, 4H, $CH_2\times2$), 2.67 – 2.62 (m, 4H, $CH_2\times2$), 2.44 – 2.38 (m, 5H, CH_3, CH_2), 2.04 (dd, *J* = 15.6, 9.4 Hz, 4H, $CH_2\times2$), 1.70 (d, *J* = 5.3 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 154.4, 151.1, 150.3, 149.0, 146.7, 146.4, 136.6, 132.7, 129.3, 123.5, 120.4 (2C), 117.1 (2C), 56.9, 55.2 (2C), 49.9 (2C), 45.9, 33.7, 30.1 (2C), 24.9 (2C). MS (ESI, m/z): 501.7 $[M + H]^+$.

4.1.3.3. *N*-(4-(4-Methylpiperazin-1-yl)phenyl)-8-((pyridin-3-ylmethyl)thio)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**E3**). Off-white solid; Yield 64.5%; mp 193.4-196.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, Ar-H), 8.62 (s, 1H, Ar-H), 8.57 – 8.50 (m, 1H, Ar-H), 7.83 – 7.75 (m, 1H, Ar-H), 7.56 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.25 (dd, *J* = 7.7, 4.9 Hz, 1H, Ar-H), 6.96 (d, *J* = 8.9 Hz, 2H, Ar-H), 4.57 (s, 2H, CH_2), 4.41 – 4.30 (m, 1H, CH), 4.19 – 4.09 (m, 2H, $CH\times2$), 3.49 (t, *J* = 11.5 Hz, 2H, $CH\times2$), 3.26 – 3.13 (m, 4H, $CH_2\times2$), 2.86 (qd, *J* = 12.4, 4.5 Hz, 2H, $CH\times2$), 2.65 – 2.56 (m, 4H, $CH_2\times2$), 2.36 (s, 3H, CH_3), 1.71 (d, *J* = 12.5 Hz, 2H, $CH\times2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7, 154.5, 150.3, 150.1, 149.1, 146.8, 146.6, 136.6, 132.9, 132.5, 128.9, 123.5, 120.2 (2C), 117.0 (2C), 67.4 (2C), 55.2 (2C), 53.5, 49.9 (2C), 46.1, 33.8, 30.5 (2C). MS (ESI, m/z): 517.2 $[M + H]^+$.

4.1.3.4. 8-(Benzylthio)-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**E4**). Off-white solid; Yield 56.3%; mp 179.0-182.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H, Ar-H), 7.58 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.43 (d, *J* = 6.8 Hz, 2H, Ar-H), 7.40 – 7.29 (m, 3H, Ar-H), 7.27 (s, 1H, NH), 6.98 (d, *J* = 8.9 Hz, 2H, Ar-H), 4.59 (s, 2H, CH_2), 4.47 – 4.33 (m, 1H, CH), 4.14 (dd, *J* = 11.6, 4.1 Hz, 2H, $CH\times2$), 3.49 (t, *J* = 11.7 Hz, 2H, $CH\times2$), 3.30 – 3.18 (m, 4H, $CH_2\times2$), 2.89 (qd, *J* = 12.5, 4.6 Hz, 2H, $CH\times2$), 2.72 – 2.62 (m, 4H, $CH_2\times2$), 2.41 (s, 3H, CH_3), 1.70 (dd, *J* = 12.5, 2.5 Hz, 2H, $CH\times2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 154.5, 151.1, 146.6, 146.5, 136.1, 133.1, 129.1 (2C), 128.8 (3C), 127.9, 120.1 (2C), 117.2 (2C), 67.5 (2C), 55.1 (2C), 53.5, 49.8 (2C), 46.1, 37.2, 30.4 (2C). MS (ESI, m/z): 516.2 $[M + H]^+$.

4.1.4. General procedure for the synthesis of compounds D1-D12 and F1-F3.

A solution of 3-chloroperbenzoic acid (45.3 mg, 0.22 mmol) in DCM (10 mL)

was added dropwise to a mixture of **C** or **E** (0.22 mmol) in DCM (10 mL) at -15 °C. The reaction mixture was stirred at room temperature for 30 min and concentrated under reduced pressure. The resulting crude product was purified by silica gel chromatography using DCM/MeOH = 5:1 (V/V) as the eluent to afford **D** or **F** as a yellow solid.

4.1.4.1. 9-Isopropyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-(phenylsulfinyl)-9*H*-purin-2-amine (**D1**). Yellow solid; Yield 55.6%; mp 176.6-177.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H, Ar-H), 7.60 (d, *J* = 9.0 Hz, 3H, Ar-H), 7.48 (d, *J* = 6.4 Hz, 2H, Ar-H), 7.37 (q, *J* = 5.8 Hz, 3H, Ar-H, NH), 6.97 (t, *J* = 7.6 Hz, 2H, Ar-H), 4.88 (dt, *J* = 13.6, 6.8 Hz, 1H, CH), 3.76 (t, *J* = 11.0 Hz, 2H, CH×2), 3.52 (t, *J* = 9.8 Hz, 2H, CH×2), 3.37 (d, *J* = 3.5 Hz, 4H, CH_2×2), 3.33 (s, 3H, CH_3), 1.62 (d, *J* = 6.8 Hz, 6H, CH_3×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 153.8, 148.5, 148.3, 145.2, 134.1, 131.8 (2C), 130.6, 129.6 (2C), 129.1, 128.6, 120.2 (2C), 117.9 (2C), 65.8 (2C), 60.5, 49.3, 45.2 (2C), 20.7 (2C). MS (ESI, m/z): 476.2 $[M + H]^+$.

4.1.4.2. 9-Cyclopropyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-(phenylsulfinyl)-9*H*-purin-2-amine (**D2**). Yellow solid; Yield 60.3%; mp 140.7-143.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H, Ar-H), 7.69 – 7.57 (m, 4H, Ar-H), 7.43 (d, *J* = 3.6 Hz, 3H, Ar-H), 6.98 (d, *J* = 8.3 Hz, 2H, Ar-H), 3.75 (t, *J* = 10.9 Hz, 2H, CH×2), 3.54 (s, 2H, CH×2), 3.39 (d, *J* = 32.6 Hz, 7H, CH_2×2, CH_3), 3.04 (d, *J* = 3.5 Hz, 1H, CH), 1.31 – 1.19 (m, 4H, CH_2×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 155.2, 152.9, 147.4, 145.2, 140.1, 134.2, 133.6 (2C), 129.6 (2C), 129.2, 128.8, 120.1 (2C), 118.0 (2C), 65.8, 60.3, 45.3 (2C), 24.4 (2C), 7.2 (2C). MS (ESI, m/z): 474.1 $[M + H]^+$.

4.1.4.3. 9-(Cyclopropylmethyl)-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-(phenylsulfinyl)-9*H*-purin-2-amine (**D3**). White solid; Yield 58.8%; mp 136.1-140.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H, Ar-H), 7.60 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.53 (d, *J* = 5.6 Hz, 2H, Ar-H), 7.38 (s, 4H, Ar-H, NH), 6.97 (d, *J* = 7.1 Hz, 2H, Ar-H), 4.05 (d, *J* = 5.9 Hz, 2H, CH_2), 3.46 (d, *J* = 41.9 Hz, 11H, CH_2×4, CH_3), 1.32 (s, 1H, CH), 0.53 (d, *J* = 11.9 Hz, 4H, CH_2×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 154.3, 149.0, 148.1, 145.1, 134.1, 132.0 (2C), 129.9, 129.6 (2C), 128.8, 128.7, 120.4 (2C), 117.9 (2C), 65.5, 60.0, 47.7, 45.1 (2C), 11.0 (2C), 4.3 (2C). MS (ESI, m/z): 488.2 $[M + H]^+$.

4.1.4.4. 9-Cyclopentyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-(phenylsulfinyl)-9*H*-purin-2-amine (**D4**). White solid; Yield 54.3%; mp 192.0-194.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H, Ar-H), 7.59 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.48 (dd, *J* = 7.9, 1.7 Hz, 2H, Ar-H), 7.41 – 7.35 (m, 3H, Ar-H), 7.28 (s, 1H, NH), 6.98 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.96 (p, *J* = 8.7 Hz, 1H. CH), 3.80 (dd, *J* = 17.5, 7.0 Hz, 2H, CH_2), 3.54 (td, *J* = 11.5, 3.3 Hz, 2H, CH_2), 3.41 – 3.34 (m, 7H, CH_3, $CH_2\times2$), 2.44 (td, *J* = 16.5, 8.2 Hz, 2H, CH_2), 2.04 (dd, *J* = 13.8, 7.8 Hz, 2H, CH_2), 1.97 – 1.86 (m, 2H, CH_2), 1.76 – 1.64 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7, 153.5, 149.2, 148.6, 145.2, 134.1, 131.7 (2C), 130.7, 129.6 (2C), 129.3, 128.6, 120.3 (2C), 117.9 (2C), 65.7 (2C), 60.1, 57.5, 45.2 (2C), 30.1 (2C), 25.1 (2C). MS (ESI, m/z): 502.5 $[M + H]^+$.

4.1.4.5. *N*-(4-(4-Methylpiperazin-1-yl)phenyl)-8-(phenylsulfinyl)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**D5**). White solid; Yield 63.7%; mp 170.9-171.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H, Ar-H), 7.62 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.55 (s, 1H, Ar-H), 7.49 (d, *J* = 6.5 Hz, 2H, Ar-H), 7.37 (d, *J* = 6.9 Hz, 3H, Ar-H, NH), 6.99 (d, *J* = 8.5 Hz, 2H, Ar-H), 4.64 (d, *J* = 11.8 Hz, 1H, CH), 4.11 (d, *J* = 8.1 Hz, 2H, CH×2), 3.74 (t, *J* = 11.1 Hz, 2H, CH×2), 3.57 – 3.37 (m, 8H, $CH_2\times4$), 3.34 (s, 3H, CH_3), 2.91 (dt, *J* = 12.0, 8.4 Hz, 2H, CH×2), 1.55 (d, *J* = 11.0 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 153.8, 148.8, 148.2, 145.2, 133.9, 131.8 (2C), 130.6, 129.7 (2C), 128.9, 128.7, 120.2 (2C), 117.9 (2C), 67.5 (2C), 65.8, 60.4, 54.2 (2C), 45.1 (2C), 30.4 (2C). MS (ESI, m/z): 518.6 $[M + H]^+$.

4.1.4.6. (1*S*, 4*S*)-4-(2-((4-(4-Methylpiperazin-1-yl)phenyl)amino)-8-(phenylsulfinyl)-9*H*-purin-9-yl)cyclohexan-1-ol (**D6**). Brown oil; Yield 58.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H, NH), 8.72 (s, 1H, Ar-H), 7.69 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.53 – 7.40 (m, 5H, Ar-H), 6.98 (d, *J* = 8.9 Hz, 2H, Ar-H), 4.38 (t, *J* = 12.0 Hz, 1H, CH), 3.72 – 3.61 (m, 4H, $CH_2\times2$), 3.52 (d, *J* = 11.7 Hz, 4H, $CH_2\times2$), 3.32 (s, 4H, CH, CH_3), 2.55 (s, 2H, CH×2), 1.93 (d, *J* = 10.2 Hz, 2H, CH×2), 1.58 (d, *J* = 10.8 Hz, 2H, CH×2), 1.26 (s, 2H, CH×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.1, 153.4, 149.1, 147.2, 144.9, 134.2, 131.9 (2C), 131.1, 130.2 (2C), 129.1, 128.5, 120.1 (2C), 116.9 (2C), 68.5, 64.3 (2C), 58.4, 56.1, 44.3 (2C), 34.9 (2C), 28.2 (2C). MS

(ESI, m/z): 532.1 $[M + H]^+$.

4.1.4.7. *N*-(4-(2-(Dimethylamino)ethoxy)phenyl)-8-(phenylsulfinyl)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**D7**). White solid; Yield 59.4%; mp 159.5-161.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, Ar-H), 7.61 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.50 (d, *J* = 6.3 Hz, 2H, Ar-H), 7.39 (d, *J* = 7.0 Hz, 3H, Ar-H), 7.26 (s, 1H, NH), 6.95 (d, *J* = 8.7 Hz, 2H, Ar-H), 4.67 (t, *J* = 12.0 Hz, 1H, CH), 4.59 (s, 2H, CH_2), 4.12 (dd, *J* = 11.3, 3.5 Hz, 2H, CH×2), 3.78 (s, 2H, CH×2), 3.51 – 3.26 (m, 8H, CH_2, CH_3×2), 2.92 (tt, *J* = 12.1, 6.2 Hz, 2H, CH×2), 1.57 (d, *J* = 12.2 Hz, 2H, CH ×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.2, 153.7, 153.0, 148.9, 134.9, 132.2 (2C), 130.9, 130.2 (3C), 129.1, 128.6, 120.6 (2C), 114.9 (2C), 68.9, 66.9 (2C), 62.8, 59.6, 54.0 (2C), 30.5 (2C). MS (ESI, m/z): 507.1 $[M + H]^+$.

4.1.4.8. N^1-(2-(Dimethylamino)ethyl)-N^1-methyl-N^4-(8-(phenylsulfinyl)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-yl)benzene-1,4-diamine (**D8**). Yellow solid; Yield 61.2%; mp 144.8-146.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H, Ar-H), 7.54 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.48 (dd, *J* = 7.8, 1.5 Hz, 2H, Ar-H), 7.41 – 7.34 (m, 4H, Ar-H), 6.84 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.65 (tt, *J* = 11.9, 4.0 Hz, 1H, CH), 4.09 (dd, *J* = 11.6, 4.0 Hz, 2H, CH×2), 3.97 (t, *J* = 6.7 Hz, 2H, CH_2), 3.47 – 3.44 (m, 2H, CH_2), 3.41 (d, *J* = 12.4 Hz, 2H), 3.27 (s, 6H, CH_3×2), 2.99 (s, 3H, CH_3), 2.91 (dd, *J* = 12.5, 4.4 Hz, 2H, CH×2), 1.58 – 1.50 (m, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 153.9, 148.9, 147.8, 144.8, 131.7 (2C), 130.8, 130.7, 129.6 (2C), 128.8, 128.7, 121.4 (2C), 113.9 (2C), 67.5 (2C), 66.6, 59.6 (2C), 54.1, 47.7, 39.6, 30.4 (2C). MS (ESI, m/z): 520.2 $[M + H]^+$.

4.1.4.9. *N*-(4-(4-(Dimethylamino)piperidin-1-yl)phenyl)-8-(phenylsulfinyl)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**D9**). Yellow solid; Yield 49.2%; mp 151.6-152.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H, Ar-H), 7.56 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.48 (d, *J* = 6.2 Hz, 2H, Ar-H), 7.42 (s, 1H, NH), 7.36 (d, *J* = 7.1 Hz, 3H, Ar-H), 6.93 (d, *J* = 8.8 Hz, 2H, Ar-H), 4.64 (t, *J* = 11.8 Hz, 1H, CH), 4.13 – 4.07 (m, 2H, CH×2), 3.75 (d, *J* = 10.9 Hz, 2H, CH×2), 3.42 (t, *J* = 11.7 Hz, 3H, CH×2, CH), 3.26 (s, 6H, CH_3×2), 2.96 – 2.82 (m, 2H, CH×2), 2.75 (t, *J* = 11.4 Hz, 2H, CH×2),

2.38 (d, *J* = 8.4 Hz, 2H, CH×2), 1.89 (d, *J* = 9.6 Hz, 2H, CH×2), 1.53 (d, *J* = 10.3 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 153.8, 148.8, 148.1, 145.9, 133.3, 131.8 (2C), 130.6, 129.7 (2C), 128.9, 128.7, 120.20 (2C), 117.8 (2C), 76.38, 67.5 (2C), 55.1 (2C), 54.2, 49.7 (2C), 30.4 (2C), 26.6 (2C). MS (ESI, m/z): 546.2 $[M + H]^+$.

4.1.4.10. *N*-(4-((1-Methylpiperidin-4-yl)oxy)phenyl)-8-(phenylsulfinyl)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**D10**). Yellow solid; Yield 63.2%; mp 83.1-85.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, Ar-H), 7.61 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.54 – 7.44 (m, 2H, Ar-H), 7.40 (dt, *J* = 11.6, 4.2 Hz, 3H, Ar-H), 7.28 (s, 1H, NH), 6.94 (d, *J* = 8.9 Hz, 2H, Ar-H), 4.74 – 4.57 (m, 2H, OCH, NCH), 4.12 (dd, *J* = 11.4, 4.1 Hz, 2H, CH×2), 3.60 (t, *J* = 11.2 Hz, 2H, CH×2), 3.45 (t, *J* = 11.6 Hz, 2H, CH×2), 3.32 (s, 3H, CH_3), 3.24 (d, *J* = 11.1 Hz, 2H, CH×2), 2.91 (dd, *J* = 12.4, 4.4 Hz, 2H, CH×2), 2.72 (t, *J* = 13.4 Hz, 2H, CH×2), 1.99 (d, *J* = 14.7 Hz, 2H, CH×2), 1.57 (d, *J* = 9.8 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 153.8, 151.9, 148.8, 148.5, 133.9, 131.9 (2C), 130.4, 129.7 (2C), 129.1, 128.9, 120.6 (2C), 116.7 (2C), 67.5 (2C), 67.4, 61.5 (2C), 60.9, 54.2, 30.4 (2C), 24.9 (2C). MS (ESI, m/z): 533.3 $[M + H]^+$.

4.1.4.11. *N*-(4-(2-(Dimethylamino)ethoxy)phenyl)-8-((3-fluorophenyl)sulfinyl)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**D11**). White solid; Yield 63.9%; mp 166.8-167.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H, Ar-H), 7.61 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.51 (s, 1H, NH), 7.35 (dd, *J* = 14.0, 7.8 Hz, 1H, Ar-H), 7.21 (dd, *J* = 17.9, 8.3 Hz, 2H, Ar-H), 7.05 (t, *J* = 8.3 Hz, 1H, Ar-H), 6.94 (d, *J* = 8.5 Hz, 2H, Ar-H), 4.65 (t, *J* = 12.0 Hz, 1H, CH), 4.58 (s, 2H, CH_2), 4.13 (d, *J* = 8.1 Hz, 2H, CH×2), 3.70 (s, 2H, CH_2), 3.46 (t, *J* = 11.9 Hz, 2H, CH×2), 3.34 (s, 6H, CH_3×2), 3.00 – 2.89 (m, 2H, CH×2), 1.60 (d, *J* = 10.7 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.6 (d, $J_{C\text{-}F}$ = 250), 156.3, 153.6, 153.1, 149.3, 134.8, 131.9, 128.6, 127.6, 120.7 (2C), 118.3, 118.1, 116.0, 115.8, 114.9 (2C), 68.8, 66.9, 62.9 (2C), 60.1 (2C), 54.1, 30.57 (2C). MS (ESI, m/z): 525.2 $[M + H]^+$.

4.1.4.12. *N*-(4-(2-(Dimethylamino)ethoxy)phenyl)-8-((4-fluorophenyl)sulfinyl)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**D12**). White solid; Yield 58.6%; mp

168.1-169.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.62 (s, 1H, NH), 8.70 (s, 1H, Ar-H), 7.71 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.64 (dd, *J* = 8.8, 5.3 Hz, 2H, Ar-H), 7.32 (t, *J* = 8.8 Hz, 2H, Ar-H), 6.92 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.73 – 4.58 (m, 1H, CH), 4.52 – 4.45 (m, 2H, CH_2), 4.01 (dd, *J* = 11.2, 3.8 Hz, 2H, CH×2), 3.55 (s, 2H, CH_2), 3.47 (s, 2H, CH×2), 3.12 (s, 6H, CH_3×2), 2.83 – 2.65 (m, 2H, CH×2), 1.64 (d, *J* = 9.7 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.9 (d, J_{C-F} = 234), 156.1, 153.8, 153.0, 148.6, 148.2, 142.5, 135.6, 135.5, 134.9, 128.6, 125.8, 120.6, 117.4, 117.2, 114.9 (2C), 68.8, 66.9 (2C), 62.9, 60.0 (2C), 53.9, 30.6 (2C). MS (ESI, m/z): 525.2 $[M + H]^+$.

4.1.4.13. 9-Cyclopentyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-((pyridin-3-ylmethyl) sulfinyl)-9*H*-purin-2-amine (**F1**). Yellow solid; Yield 68.2%; mp 181.9-183.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.62 (s, 1H, Ar-H), 8.54 (dd, *J* = 4.8, 1.5 Hz, 1H, Ar-H), 7.84 – 7.78 (m, 1H, Ar-H), 7.58 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.28 – 7.25 (m, 1H, Ar-H), 7.18 (s, 1H, NH), 6.99 (d, *J* = 8.9 Hz, 2H, Ar-H), 4.71 – 4.62 (m, 1H, CH), 4.58 (s, 2H, CH_2), 3.77 (t, *J* = 10.9 Hz, 2H, CH_2), 3.55 (td, *J* = 11.3, 2.9 Hz, 2H, CH_2), 3.46 – 3.33 (m, 7H, CH_3, CH_2×2), 2.51 – 2.33 (m, 2H, CH_2), 2.11 – 1.97 (m, 4H, CH_2), 1.71 (d, *J* = 5.3 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.3, 149.1, 146.4, 145.1, 136.6, 134.3, 132.7, 132.6, 129.4, 129.3, 127.3, 123.5, 120.2 (2C), 118.0 (2C), 65.8 (2C), 60.2, 56.8, 45.2 (2C), 33.7, 30.1 (2C), 24.9 (2C). MS (ESI, m/z): 517.4 $[M + H]^+$.

4.1.4.14. *N*-(4-(4-Methylpiperazin-1-yl)phenyl)-8-((pyridin-3-ylmethyl)sulfinyl)-9-(t etrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**F2**). Yellow solid; Yield 48.2%; mp 101.9-102.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H, Ar-H), 8.60 (s, 1H, Ar-H), 8.52 (s, 1H, Ar-H), 7.78 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.56 (d, *J* = 7.3 Hz, 2H, Ar-H), 7.28 – 7.18 (m, 1H, Ar-H), 6.95 (d, *J* = 8.1 Hz, 2H, Ar-H), 4.55 (s, 2H, CH_2), 4.32 (s, 1H, CH), 4.11 (d, *J* = 7.9 Hz, 2H, CH×2), 3.84 (s, 4H, CH_2×2), 3.64 (d, *J* = 10.0 Hz, 2H, CH×2), 3.54 – 3.42 (m, 4H, CH_2×2), 3.33 (s, 3H, CH_3), 2.83 (d, *J* = 9.2 Hz, 2H, CH×2), 1.68 (d, *J* = 9.8 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 154.5, 150.2, 149.1, 146.5, 145.1, 136.7 (2C), 134.0, 132.6, 129.0, 123.5, 120.1 (2C), 117.9 (2C), 67.4 (2C), 65.6 (2C), 59.9, 53.5, 45.1 (2C), 33.7, 30.4 (2C). MS (ESI,

m/z): 533.2 $[M + H]^+$.

4.1.4.15. 8-(Benzylsulfinyl)-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-9-(tetrahydro-2*H*-pyran-4-yl)-9*H*-purin-2-amine (**F3**). Yellow solid; Yield 46.4%; mp 152.1-154.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H, Ar-H), 7.60 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.43 (d, *J* = 6.9 Hz, 2H, Ar-H), 7.38 – 7.30 (m, 3H, Ar-H), 7.26 (s, 1H, NH), 6.98 (d, *J* = 8.4 Hz, 2H, Ar-H), 4.59 (s, 2H, CH_2), 4.37 (t, *J* = 12.0 Hz, 1H, CH), 4.14 (d, *J* = 8.1 Hz, 2H, CH×2), 3.74 – 3.28 (m, 14H, CH_2×2, CH_2×2, CH×2, CH_3), 2.87 (d, *J* = 8.6 Hz, 2H, CH×2), 1.70 (d, *J* = 10.7 Hz, 2H, CH×2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.3, 154.4, 151.4, 146.4, 144.9, 136.1, 134.2, 129.3, 129.1 (2C), 128.8 (2C), 127.9, 120.1 (2C), 117.9 (2C), 67.5 (2C), 65.5, 59.5, 53.5, 45.1 (2C), 37.1 (2C), 30.4 (2C). MS (ESI, m/z): 532.2 $[M + H]^+$.

4.1.5. Procedure for the synthesis of compound 4.

9-cyclopentyl-*N*-(4-(4-methylpiperazin-1-yl)phenyl)-8-phenylamino-9*H*-purin-2 –amine (**4**). Compound **4** was prepared as reported protocol.[32] mp 223.7-226.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H, NH), 8.96 (s, 1H, NH), 8.33 (s, 1H, Ar-H), 7.79 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.59 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.37–7.26 (m, 2H, Ar-H), 6.97 (t, *J* = 7.3 Hz, 1H, Ar-H), 6.85 (d, *J* = 9.1 Hz, 2H, Ar-H), 5.05–4.82 (m, 1H, CH), 3.09–2.96 (m, 4H, NCH_2), 2.48–2.38 (m, 6H, NCH_2, CH_2), 2.11–1.93 (m, 3H, CH_3), 2.11–1.93 (m, 4H, CH_2), 1.77–1.58 (m, 2H, CH_2). MS (ESI, m/z): 469.9 $[M + H]^+$.

4.2. Biology assay

4.2.1. Antiproliferative assays

Cellular chemosensitivity was determined by using a modified MTT method assay in vitro. In brief, HCC827, H1975 or A549 cells in 200 μL culture medium were seeded into 96-well microplates at 3000-5000 cells per well respectively and cultured in RPMI 1640 10% FBS, incubated at 37 ℃ for 24 h prior to drug exposure. Cell numbers were titrated to keep control cells growing in the exponential phase throughout the 48 h incubation period. Cells were treated with final concentrations of 10.0, 1.0, 0.1 0.01 and 0.01 μM of tested compounds simultaneously and incubated

for 72 h and then 20 μL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 h. The formed blue formazan crystals were pelleted to the bottom of the well by centrifugation, separated from the supernatant, and dissolved in 200 μL of DMSO. The optical density at 490 nm was determined by Varioskan Flash Multimode Reader (Thermo scientific). Three separate experiments with triplicate data were performed to obtain mean cell viability. The IC_{50} value, that is, the concentration (μM) of a compound was able to cause 50% cell death with respect to the control culture, was calculated according to the inhibition ratios.

4.2.2. EGFR enzymatic activities assay

Theses assays were carried out as reported previously.[33,35] All of the enzymatic reactions were conducted at 30℃ for 40 minutes. The 50 μL reaction mixture contains 40 mM Tris, pH 7.4, 10 mM $MgCl_2$, 0.1 mg/ml BSA, 1 mM DTT, 10 μM ATP, EGFR TK and the enzyme substrate (0.2 mg/mL Poly (Glu, Tyr)). The compounds were diluted in 10% DMSO and 5 μL of the dilution was added to a 50 μL reaction so that the final concentration of DMSO is 1% in all of reactions. The assay was performed using Kinase-Glo Plus luminescence kinase assay kit. It measures kinase activity by quantitating the amount of ATP remaining in solution following a kinase reaction. The luminescent signal from the assay is correlated with the amount of ATP present and is inversely correlated with the amount of kinase activity. The IC_{50} values were calculated using nonlinear regression with normalized dose–response fit using Prism GraphPad sofeware.

4.2.3. Western blotting analysis

HCC827 cells were seeded in 6-well plates at 1×10^6 cells per well, incubated at 37 ℃ for 16 h prior to drug exposure. Cells were treated with final concentrations of compound **C9** at 0.1, 3.0, 1.0 and 3.0 μM or AZD9291 at 0.1 μM for 8 h, collected and suspended in lysis buffer (Beyotime) and centrifuged for 20 min at 12000 rpm, then removed the insoluble material. The same amounts of proteins were loaded and separated by 8% SDS-PAGE and transferred to polyvinylidene fluoride membranes

(Millpore). The anti-EGFR, anti-pEGFR (Tyr1068) were purchased from Cell Signaling Technology and diluted at 1:1000, while the anti-GAPDH was diluted at 1:2000. All secondary antibodies were used at 1:10000. The results were detected by an Enhanced Chemiluminescene System (Millpore).

4.2.4. Anticancer effects in HCC827 xenografts model *in vivo*

Nude mice (Balb/c, female, 4 weeks old, 19.1 ± 1.5 g) were purchased from Beijing Vitong Lihua experimental animal technology co. LTD and fed in Animal Center of Xi′an Jiaotong University College of Medicine. The experimental protocol was approved by Ethic Committee of Xi′an Jiaotong University.

HCC827 cells at 2×10^6 were injected subcutaneously into the flank of mice. The tumors volume reached to 200 mm^3 after 19 days. All tumor-bearing mice were randomly divided into three groups, with 6 mice in each group. In the solvent group, the same volume of solvent was administered orally. Compound **C9** was dissolved in DMSO/PEG400/H_2O (volume ratio, 5:55:40) and dosed orally at 1 mg/kg and 5 mg/kg for the low and high dosage groups once a day for 20 days, respectively. Body weights were recorded per day. Tumor length (a) and width (b) were measured once the three days. Tumor volume is equal to $a \times b^2 \times 0.5$. The mice were anesthetized and sacrificed on Day 21. The neoplasm was stripped.

4.3. Molecular modeling

The molecular docking procedure was performed by using C-DOCKER protocol within Discovery Studio 2.5. The structure of the T790M EGFR mutant derived from the protein database (PDB entry: 2JIU) was prepared by removal of ligand, and the hydrogen atoms added. The whole EGFR enzyme was defined as a receptor and the site sphere was selected based on the ligand binding location. Ligand was removed and compounds **C7**, **D7** or **C9** was placed, other parameters were set as default. After accomplishment of the molecular docking procedure, ten docking poses were scored and selected based on calculated C-DOCKER energy.

4.4. Statistical analysis

The data are reported as mean ± standard deviation (SD) for at least three experiments. Statistical differences were analyzed according to one way ANOVA test wherein the differences were considered to be significant at $P<0.05$. All statistics were calculated using a statistical program PRISM 5, Graph Pad software.

Acknowledgments Financial support from The National Natural Science Foundation of China (Grant No. 81673354) is gratefully acknowledged.

References

1. Olayioye MA, Neve RM, Lane HA, Hynes NE. *EMBO J*. 2000; 19: 3159-3167.
2. Moasser MM. *Oncogene*. 2007; 26: 6577-6592.
3. Geyer CE, Forster J, Lindquist D, et al. *N. Engl. J. Med*. 2006; 355: 2733-2743.
4. Press MF, Lenz H-J. *Drugs*. 2007; 67: 2045-2075.
5. Carey KD, Garton AJ, Romero MS, et al. *Cancer Res*. 2006; 66: 8163-8171.
6. Rewcastle GW, Palmer BD, Thompson AM, et al. *J. Med. Chem*. 1996; 39: 1823-1835.
7. Rewcastle GW, Bridges AJ, Fry DW, Rubin JR, Denny WA. *J. Med. Chem*. 1997; 40: 1820-1826.
8. Sun L, Cui J, Liang C, Zhou Y, et al. *Bioorg. Med. Chem*. 2016; 24: 3359–3370.
9. Fink BE, Norris D, Mastalerz H, et al. *Bioorg. Med. Chem. Lett*. 2011; 21: 781-785.
10. Rewcastle GW, Palmer BD, Bridges AJ, et al. *J. Med. Chem*. 1996; 39: 918-928.
11. Showalter HD, Bridges AJ, Zhou H, et al. *J. Med. Chem*. 1999; 42: 5464-5474.
12. Zhang X, Li RD, Qiao K, et al. *Archiv. Pharm*. 2013; 346: 44-52.
13. Chilin A, Conconi MT, Marzaro G, et al. *J. Med. Chem*. 2010; 53: 1862-1866.
14. Fukuda T, Umeki T, Tokushima K, et al. *Bioorg. Med. Chem*. 2017; 25: 6563–6580.
15. Sharma SV, Bell DW, Settleman J, Haber DA. *Nat. Rev. Cancer*, 2007; 7: 169-181.
16. Zhang H, Berezov A, Wang Q, et al. *J. Clin. Invest*. 2007; 117: 2051-2063.
17. Tu Y, Wang C, Xu S, et al. *Bioorg. Med. Chem*. 2017; 25: 3148–3157.
18. Hamed MM, Abou El Ella DA, Keeton AB, et al. *ChemMedChem*. 2013; 8: 1495-1504.

19. Huang S, Li C, Armstrong EA, et al. *Cancer Res.* 2013; 73: 824-833.

20. Zuo SJ, Zhang S, Mao S, et al. *Bioorg. Med. Chem.* 2016; 24: 179-190.

21. Song A, Zhang J, Ge Y, et al. *Bioorg. Med. Chem.* 2017; 25: 2724–2729.

22. Rewcastle GW, Palmer BD, Bridges AJ, et al. *J. Med. Chem.* 1996; 39: 918-928.

23. Deng W, Guo Z, Guo Y, et al. *Bioorg. Med. Chem. Lett.* 2006; 16: 469-472.

24. Kang BR, Shan AL, Li YP, et al. *Bioorg. Med. Chem.* 2013; 21: 6956-6964.

25. Li D, Ambrogio. L, Shimamura T, et al. *Oncogene*. 2008; 27: 4702-4711.

26. Kalous O, Conklin D, Desai AJ, et al. *Mol. Cancer Ther.* 2012; 11: 1978-1987.

27. Deeks ED. *Drugs.* 2017; 77: 1695-1704.

28. Kim Y, Ko J, Cui Z, et al. *Mol. Cancer Ther.* 2012; 11: 784-791.

29. Yun CH, Boggon TJ, Li Y, et al. *Cancer Cell.* 2007; 11: 217-227.

30. Song Z, Ge Y, Wang C, et al. *J. Med. Chem.* 2016; 59: 6580-6594

31. Thress KS, Paweletz CP, Felip E, et al. *Nat. Med.* 2015; 21: 560-562.

32. Park H, Jung HY, Mah S, et al. Angew. Chem. Int. Edit. 2017; 56: 7634-7638.

33. Zhang H, Wang J, Shen Y, et al. *Euro J. Med. Chem*, 2018; 148: 221-237.

34. Yang J, Wang L-J, Liu J-J, et al. *J. Med. Chem.* 2012; 55: 10685-10699.

35. Kashem MA, Nelson RM, Yingling JD, et al. *J Biomol Screen.* 2007; 12: 70-83.

Figure and scheme Legends

Figure 1. The structures of EGFR inhibitors

Figure 2. The design of target compounds

Figure 3. Inhibition of EGFR autophosphorylation of compound **C9** in HCC827 cell line

Figure 4. The anticancer effect of compound **C9** on HCC827 xenografts model (n = 6 in each group). (A) The photograph of tumors in each group after **C9** and vehicle treatment. (B) Tumor growth curves during treatment with **C9** and vehicle. $^{**}p < 0.01$ *vs* vehicle. (C) Body weight of mice during treatment with **C9** and vehicle. (D) Comparison of the final tumor weights in each group after the 20-day treatment of **C9**. Numbers in columns indicate the mean tumor weight in each group. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* vehicle.

Figure 5. The docking mode of Compounds **C7**, **D7** and **C9** with the T790M EGFR mutant (PDB code: 2JIU). (A) The docking pose of compound **C7** with EGFR. (B) The docking pose of compound **D7** with EGFR. (C) The docking pose of compound **C9** with EGFR. Compounds **C7**,

D7 and **C9** were shown as sticks. Hydrogen bonds within 3.0 Å were shown as yellow dashed lines.

Scheme 1. Synthesis of 2-anilino-8-henylthio/phenylsulfinyl-9*H*-purine derivatives **C** and **D**. Reagents and conditions: a) CH_2Cl_2, DIEA, amine, -40°C to room temperature, 60-75%; b) n-butanol, 90 °C, 5 h, 76%; c) H_2, 5% Pd/C, MeOH, 50°C, 6 h, 85%; d) CS_2, KOH, EtOH:H_2O = 10:1, refluxed, 60-70%; e) PhI, CuI, 1,10-phenanthroline, K_2CO_3, DMSO, 140°C, 24 h, 60%; f) *m*-chloroperoxybenzoic, CH_2Cl_2, 70-80%.

Scheme 2. Synthesis of 8,9-Dianilinopurine Derivatives **C** and **D**. Reagents and conditions: a) aryliodide, CuI, 1,10-phenanthroline, K_2CO_3, DMSO, 140℃, 24h, 60%; b) *m*-chloroperoxybenzoic, CH_2Cl_2, 70-80%.

Table 1. Antiproliferative effects of compounds **C** against HCC827 cell lines ($\overline{x} \pm s$, n = 3)

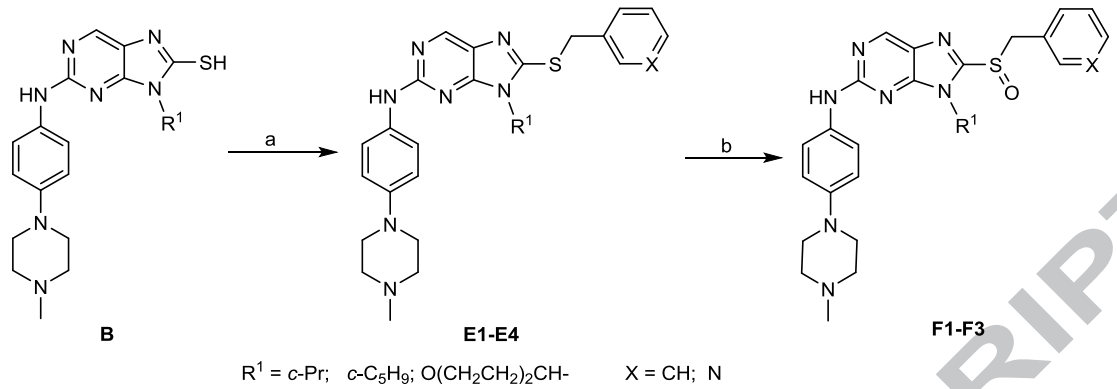
Table 2. Antiproliferative effects of compounds **D** against HCC827 cell lines ($\overline{x} \pm s$, n = 3)

Table 3. Antiproliferative effects of 8-benzylthio/ benzylsulfinyl-9H-purine **E** and **F** against HCC827 cell lines ($\overline{x} \pm s$, n = 3)

Table 4. Antiproliferative effects and cell-based selectivity of compounds against HCC827, H1975 and A549 cell lines ((IC_{50}, nM), $\overline{x} \pm s$, n = 3)

Table 5. Enzymatic activity of **C4-C7**, **C9**, **C11**, **C12**, **D4** and **D7** (IC_{50}, nM, n = 2)

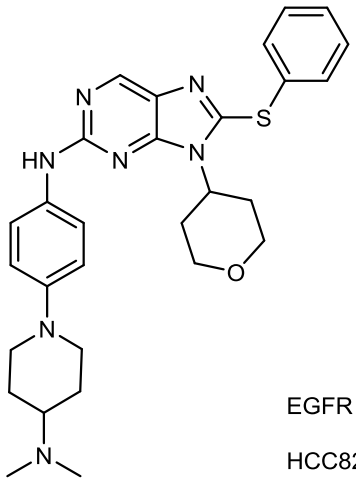
Table 6. Property comparison of compounds **C7**, **C9**, **4**, and AZD9291



Scheme 3. Synthesis of 2,8,9-trisubstitued purine derivatives **E** and **F**. Reagents and conditions: a) benzyl bromide or 3-chloromethylpyridine hydrochloride, acetone, K_2CO_3, 42.4%-72.9%; b) *m*-chloroperoxybenzoic acid, CH_2Cl_2, 46.3-68.2%.

Graphical abstract

A series of 2,9-disubstituted 8-phenylthio/phenylsulfinyl-9*H*-purine were synthesized and characterized. **C9** displayed potent activity *in vitro* and *in vivo*.



C9

	IC_{50} (nM)
EGFR (L858R):	1.9
HCC827 Cell	29.4