Journal of
Medicinal Chemistry

Article

Identification of N-phenyl-7H-pyrrolo[2,3-d] pyrimidin-4-amine Derivatives as Novel, Potent, and Selective NF-#B Inducing Kinase (NIK) Inhibitors for the Treatment of Psoriasis

Yuqin Zhu, Yuxiang ma, Weidong Zu, Jianing Song, Hua Wang, You Zhong, Hongmei Li, Yanmin Zhang, Qianqian Gao, Bo Kong, Junyu Xu, fei jiang, Xinren Wang, Shuwen Li, Chenhe Liu, Haichun Liu, Tao Lu, and Yadong Chen

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.0c00055 • Publication Date (Web): 01 Jun 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Identification of N-phenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-amine Derivatives as Novel, Potent, and Selective NF-κB Inducing Kinase (NIK) Inhibitors for the Treatment of Psoriasis

Yuqin Zhu[†,⊥], *Yuxiang Ma*[§,⊥], *Weidong Zu*[†], *Jianing Song*[†], *Hua Wang*[§], *You Zhong*[§], *Hongmei Li*[†], *Yanmin Zhang*[†], *Qianqian Gao*[†], *Bo, Kong*[†], *Junyu Xu*[†], *Fei Jang*[†], *Xinren Wang*[†], *Shuwen Li*[†], *Chenhe Liu*[†], *Haichun Liu*[†], *Tao Lu**[†,§], *Yadong Chen**[†].

[†]School of Sciences, China Pharmaceutical University, 639 Longmian Avenue, Nanjing 211198, PR China.

[§]State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, PR China.

ABSTRACT

A series of N-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine derivatives with NF-κB inducing kinase (NIK) inhibitory activity were obtained through structure-based drug design and synthetic chemistry. Among them, 4-(3-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-4-morpholinophenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (**12f**) was identified as a highly potent NIK inhibitor, along with satisfactory selectivity. The pharmacokinetics of **12f** and its ability to inhibit interleukin 6 secretion in BEAS-2B cells were better than compound **1** developed by Amgen. Oral administration of different doses of **12f** in an imiquimod-induced psoriasis mouse model showed effective alleviation of psoriasis, including invasive erythema, swelling, skin thickening, and scales. The underlying pathological mechanism involved attenuation of pro-inflammatory cytokine and chemokine gene expression, and the infiltration of macrophages after the treatment of **12f**. This work provides a foundation for the development of NIK inhibitors, highlighting the potential of developing NIK inhibitors as a new strategy for the treatment of psoriasis.

INTRODUCTION

As a common chronic inflammatory skin disease, psoriasis affects approximately 2-4% of the worldwide population. Psoriasis can be divided into psoriasis vulgaris, pustular psoriasis, erythropoietic psoriasis, and arthritic psoriasis.[1-3] Regardless of the type, it is characterized by associated histological and pathological features, including exaggerated angiogenesis, abnormal growth, differentiation of keratinocytes, and prominent immune cell infiltration, such as T cells, neutrophils, and macrophages.[4-7] Like other autoimmune and inflammatory diseases, the complex pathogenesis of psoriasis determines the multidirectional and multipath nature of drug development.

Indeed, biomacromolecules targeting autoimmune and inflammatory processes, particularly belimumab (Benlysta), has contributed significantly in patient care.[8-10] Despite substantial advances in the treatment of autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), there is still an urgent need for effective psoriasis treatment strategies.[11] In arthritic psoriasis, monoclonal antibodies (mAbs) and recombinant proteins targeting cytokines and receptors, such as IL-12, IL-17, IL-23, TNFα, and TNFR II, have become a research hotspot for major pharmaceutical giants represented by Pfizer Inc. and Janssen Biotech Inc.[12-14] Small molecule inhibitors yet provide an alternative when biomacromolecules targeting specific extracellular cytokines cannot reliably achieve disease remission.[15] Janus kinase (JAK) is a popular target that has been studied for the treatment of psoriasis. There exists non- or low-selective JAK inhibitors tofacitinib[16], ruxolitinib[17] and baricitinib[18], but anemia and other hematological toxicities (e.g., dose-dependent neutropenia and significant lymphopenia) are often caused by inhibition of the JAK2 subunit which prompts researchers to develop more selective JAK inhibitors.[19] Peficitinib as a selective JAK1/3 inhibitor is in phase II clinical trials for the treatment of psoriasis.[20-21] BMS-986165 developed by Bristol-Myers Squibb is currently in phase III clinical trials as a potential treatment for psoriasis.[22] Besides, other types of inhibitors are developed to target autoimmune-disease associated with intracellular proteins such as BTK and SYK.[23-25] It was believed that patients would benefit from combination therapies where multiple pathways or synergies are involved in improving efficacy.[15, 26]

NF-κB inducing kinase (NIK, also known as MAP3K14) is an essential noncanonical NF-κB signaling mediator downstream of the corresponding ligands of TNF superfamily receptors, including CD40, CD27, lymphotoxin β receptor, BAFF-R, receptor activator of NF-κB (RANK), OX40, and Fn14 (TWEAK-R). In the basal or resting state, TRAF3 links NIK to TRAF2-cIAPs E3 complex, promoting cIAPs-mediated Lys48 ubiquitination and proteasomal degradation of NIK by the E3 ligase. Once the homeostasis is perturbed, such as the stimulation of CD40L or BAFF, NIK would accumulate in the cell, leading to phosphorylation of IKKα, p100 processing as well as increased p52 protein levels. p52 then interacts with RelB to form p52/RelB heterodimer, which translocates into the nucleus and triggers the expression of inflammaory cytokines and chemokines.[27-31] Dysfunction of the NIK protein drives the development of many autoimmune and inflammatory diseases. In synovial endothelial cells of RA patients, NIK is highly expressed, along with the increased BAFF serum levels.[32-33] Furthermore, *Peli1*-deficiency mice exhibited splenic $CD19^{+}$ B cell hyperproliferation and increased production of its antigen-specific antibodies (IgM, IgG2a, and IgG3), due to accumulation of NIK in the cell, leading to spontaneous SLE.[27, 30] Related liver disease studies also show that one of the common pathological features in patients with primary biliary cirrhosis is hepatocyte-specific overexpression of NIK, which further promotes liver inflammation and injury.[34-36] Similarly, NIK plays a direct or indirect role in the pathogenesis of psoriasis.[37-42] In 2015, Etemadi et al. reported that $TRAF2^{-/-}$ but not wild-type keratinocytes showed increased levels of NIK and p52, which led to many psoriasis associated-inflammatory gene expression, including Csf1 (M-CSF), IL-23, TNFSF9 (4-1BBL), Cr1l and Cxcl16.[37] In addition to local tissue cell-associated inflammatory regulation, dysfunctional NIK also mediates the behavior of immune T cells in autoimmune-like disease. It has recently been established that NIK signaling not only promotes the differentiation of αβ T and γδ T cells but also acts as the hardwiring in thymic epithelial cells to mediate γδ T cell cytokine production.[38-40] Furthermore, in TRAF2-ablated DKO mice, constitutive NF-κB2 activity resulting from NIK accumulation triggers aberrant enhancement of T cell activation and premature differentiation of Th17 effector T cells, but not T-helper 1 (Th1), and eventually develops autoimmune diseases such as psoriasis. Subsequent research confirmed that the corresponding inflammatory phenotype

of the DKO mice could be reversed by a haplodeficiency of NIK.[41] Accordingly, development of novel NIK inhibitors for the treatment of psoriasis has extremely high research value.

Currently, the design and application of NIK inhibitors are scarce, and only a few cases of NIK inhibitors have been reported. Amgen is the first to design a series of compounds containing alkynol fragments, which have good NIK inhibitory potency with a typical representative being compound **1** (K_i = 0.4 ± 0.3 nM). In 2016, Chen's group confirmed the efficacy of **1** on toxin-induced acute hepatitis and liver injury.[36] After the X-ray crystal structure of the NIK kinase domain was determined, Janssen Pharmaceuticals and Genentech discovered another series of NIK inhibitors with high NIK inhibitory activity and selectivity (compounds **2**-**4**).[29-30] Compounds **2** and **3** can effectively inhibit LTβR-mediated p52/RelB nuclear translocation and the expression of related target genes. Both are in the preclinical stage, and the initial indication is the same as SLE.

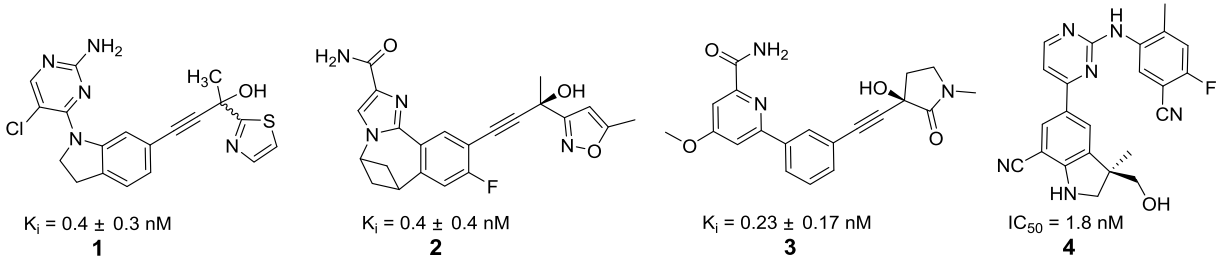


Figure 1 Representative NIK inhibitors.

Although the efficacy of NIK small molecule inhibitors against SLE and toxin-induced liver inflammation and injury has been well established (compounds **3** and **1**, respectively), there is no reported case of NIK small molecule inhibitor as potential therapeutics for psoriasis.[28, 36] In this study, we report the design and synthesis of N-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine derivatives as novel NIK inhibitors. A promising lead molecule **12f** was identified from this series after a joint effort of medicinal chemistry synthesis and bioassay evaluation of kinase selectivity, macrophage nitric oxide secreted inhibition and T cell anti-proliferation screening. The pharmacokinetics (PK) properties of **12f** were also evaluated, and it is more stable than the starting compound **1** developed by Amgen. The in vivo study in an imiquimod (IMQ)-induced

psoriasis mouse model demonstrated that compound **12f** could significantly improve the disease phenotype, and it also showed evidence of the underlying pathological characteristics.

RESULTS AND DISCUSSION

Structure-Based Design Strategy

Indolinoline-based compound **1** (developed by Amgen) is the first NIK inhibitor that has been shown to be effective in vivo when administered by injection in acute hepatitis and liver injury models.[43] Analyzing the binding pattern of ligand **1** to NIK indicated that the interaction surface in the solvent accessible region showing onset of van der waals clashes. The size of this region may allow us to open the tetrahydropyrrole ring and grow new substituents at the appropriate site. Moreover, we hypothesized that the pyrimidine ring grows into a pyrrolopyrimidine fragment that could engage the hinge region with potential H-bond (**Figure 2A**). Starting from compound **1**, a structure-based design including ring-opening of tetrahydropyrrole, cyclization of the aminopyrimidine ring, and the introduction of hydrophilic groups, was utilized, resulting in a new molecule **12k** with a distinct scaffold (**Figure 2B**).

Subsequently, compound **12k** was docked into the NIK crystal structure (RCSB PDB code 4G3E) to investigate the compatibility of **12k** with the target. As shown in **Figure 2C**, overlay of compounds **1** and **12k** in the active site looks reasonable. Besides, a similar binding pattern of **12k** with NIK protein was surfaced, compared to that of compound **1**. The 4-amino-pyrrolo[2,3-*d*]pyrimidine unit forms two conserved hydrogen bonds with Leu474 in the hinge region of NIK. The acetylenic alcohol unit forms two hydrogen bonds with Glu442 and Phe537 in the alpha-C helix and the DFG region, respectively. The thiazole nitrogen atom captures Asp536 to form hydrogen-bond interaction. The methyl-piperazine fits into the solvent accessible region near the G-loop. The next work demonstrated moderate NIK inhibitory activity (IC_{50} = 78.3 ± 5.53 nM, **Figure 2B**). Following this design idea, structural optimization was performed on compound **12k**, and a series of molecules were synthesized and evaluated in our kinase assay.

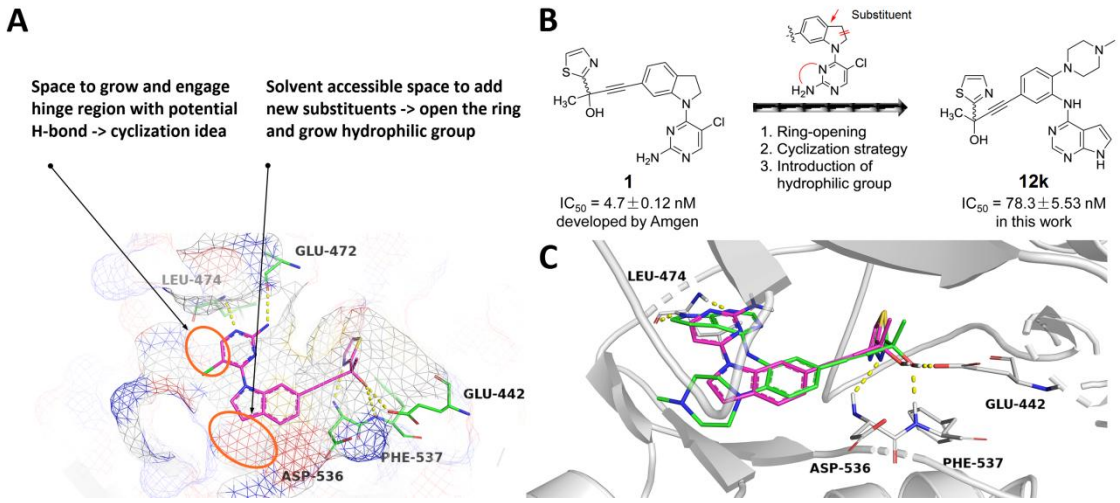


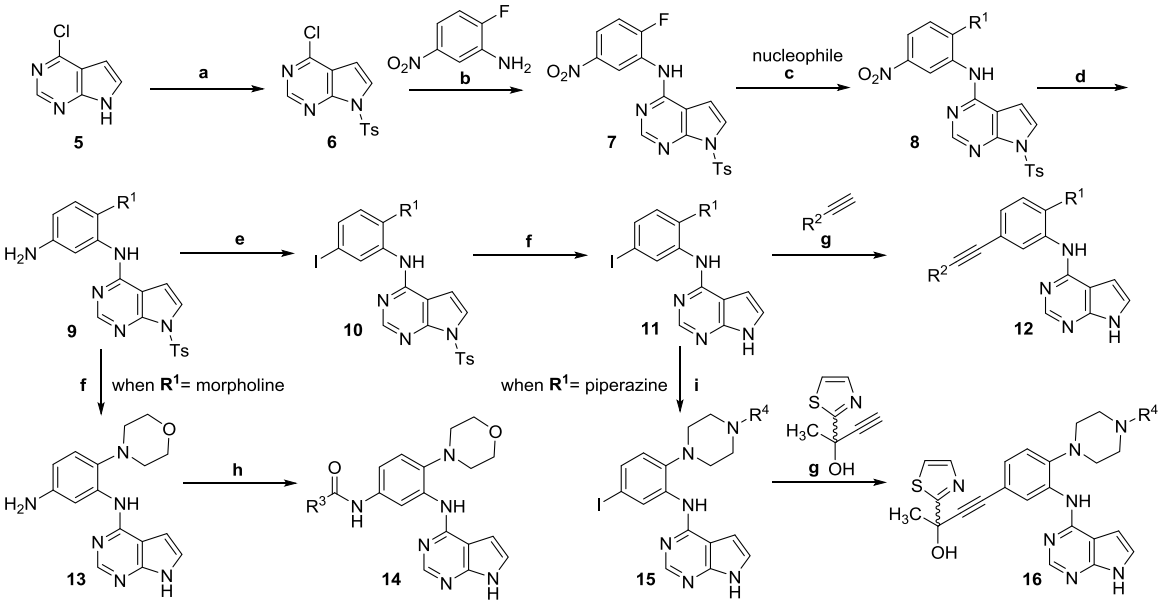
Figure 2. (A) The binding mode of ligand **1** with NIK and the assumptions of design strategies. (B) The design strategy from compound **1** to **12k**. The corresponding NIK inhibitory activities were displayed (C) The overlay of compound **12k** to ligand **1** (PDB code 4G3E). Only the hydrogen bonding interaction between **12k** and NIK is presented.

Chemsitry

Synthetic routes to the N-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine analogs are depicted in **Scheme 1**. Compound **7** was obtained in two steps starting with 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine. In the second step, AgOTf was used as a catalyst and the electron-withdrawing tosyl protecting group allowed the nucleophilic aromatic reaction to occur readily. After the nitro reduction reaction with Fe/NH_4Cl, subsequent diazotization reaction afforded **10** in acceptable yield. Removal of the *N*-tosyl group was carried out to provide **11** using LiOH or NaOH in EtOH. Target compounds N-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine analogs **12** were obtained via the sonogashira coupling reaction. Conversion of intermediate **9** to target analogs **14** were then achieved in two steps: the deprotection step, and the condensation reaction of **13** with acyl chloride or isocyanate in the presence of Et_3N. Similarly, using Et_3N as a base, the nucleophilic attack of the piperazine nitrogen atom to appropriate acyl chloride gave **15**. Then, we constructed the alkynol fragment on the core skeleton via the coupling reaction and obtained the target compound **16**. As shown in **Scheme 2**, compounds **21a-f** were prepared in good overall yields via a six-steps conversion, similar to the

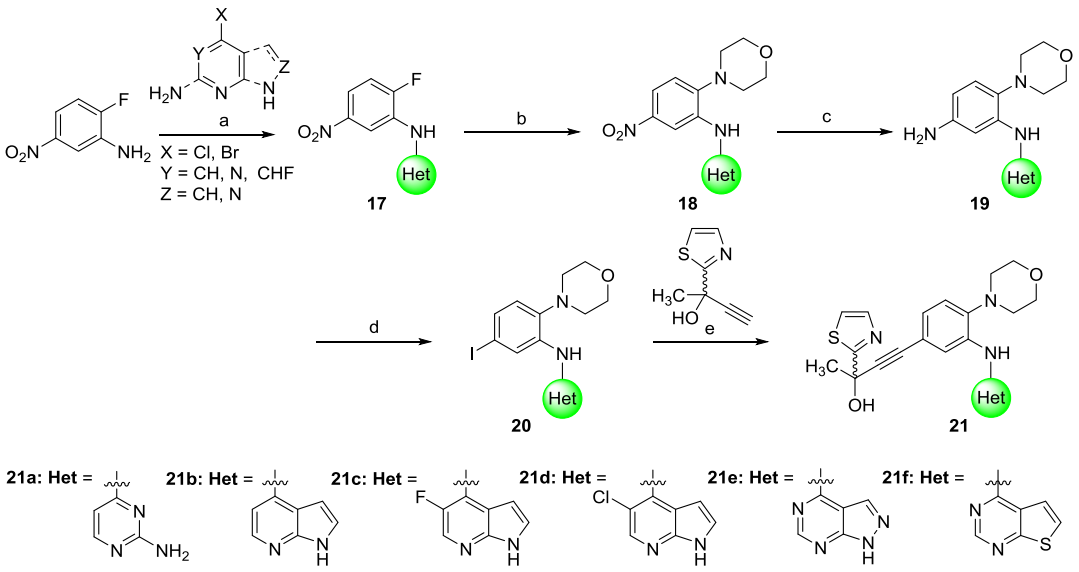
reactions shown in **Scheme 1**. The difference was in the reaction step **a**: using AgOTf or $Pd_2(dba)_3$ as a catalyst and 2-fluoro-5-nitroaniline as a starting material to react with the appropriate nitrogen-containing aromatic heterocyclic compounds.

Scheme 1. Synthesis of N-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine analogues[a]



[a]Reagents and conditions: (a) TsCl, NaH, THF, 0 °C; (b) AgOTf, Dioxane, 130 °C; (c) corresponding amine, K_2CO_3, DMF, 90 °C or relative alcohol, NaH, DMF, 0 °C; (d) Fe, NH_4Cl, 75% EtOH; (e) $NaNO_2$, KI, *p*-TsOH, CH_3CN/H_2O, 0 °C – r.t; (f) NaOH, 95% EtOH, 40 °C; (g) $PdCl_2(PPh_3)_2$, CuI, Et_3N/THF = 1:3; (h) appropriate acyl chloride or isocyanate, Et_3N, DMF, 0 °C; (i) appropriate sulfonyl chloride or acetyl chloride, Et_3N, DMF, 0 °C.

Scheme 2. Synthesis of compounds **21a-21f**[a]



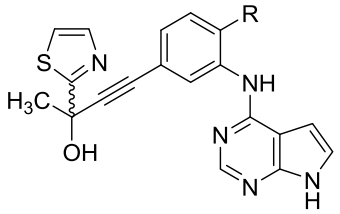
[a]Reagents and conditions: (a) when X = Cl, AgOTf, Dioxane, 130 °C or when X = Br, $Pd_2(dba)_3$, K_2CO_3, *t*-BuOH, 100 °C; (b) morpholine, K_2CO_3, DMF, 90 °C; (c) Fe, NH_4Cl, 75% EtOH; (d) $NaNO_2$, KI, *p*-TsOH, CH_3CN/H_2O, 0 °C – room temperature; (e) $PdCl_2(PPh_3)_2$, CuI, THF/ Et_3N = 3:1, 40 °C.

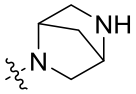
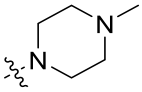
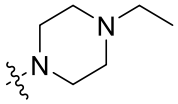
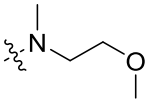
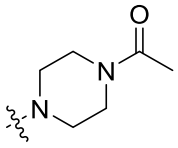
Structure-Activity-Relationship (SAR) Studies

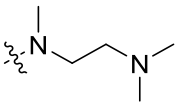
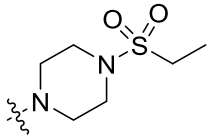
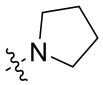
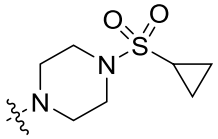
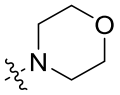
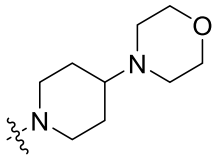
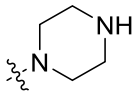
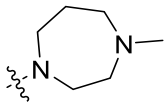
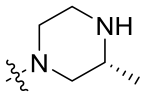
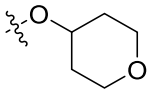
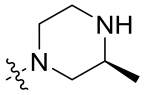
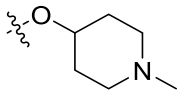
The structural modification starting with the 1-methylpiperazine ring in **12k** as hydrophilic groups at this position may help improve potency and modulate physical properties. Removal of 1-methylpiperazinyl is not tolerated and **12a** loses NIK inhibitory activity. The introduction of "-$N(CH_3)_2$" to the 2-position of the phenyl ring (**12b**) (IC_{50} = 22.5 ± 3.18 nM) is well tolerated and showed enhanced activity compared to **12k**. To investigate whether substitution of flexible chain-like hydrophilic groups has an impact on the potency, analogs **12c** and **12d** were synthesized and we observed that the methyl ether group could retain the activity. We also examined 5 or 6-membered cyclic hydrophilic groups including: pyrrole (**12e**), morpholine (**12f**), piperazine (**12g**), functionalized piperazine rings with different substituents (**12h-12l**). Among these compounds, it is noteworthy that **12f** stood out for its enhanced NIK inhibitory activity (IC_{50}: 9.87 ± 0.15 nM) compared to compound **12k**. Besides, (*S*)-3-methyl-piperazine (**12i**, IC_{50}: 69.16 ± 5.19 nM) showed better activity than that of **12h** and **12j**. The introduction of a larger ethyl group than the methyl group on the "-*N*-" atom of the piperazine ring had no effect on the inhibitory activity against NIK (**12k**-**12l**). In order to obtain

compounds with different TPSA values (forecast with ChemBioDraw software), we introduced functional groups containing different numbers of the oxygen atom on the piperazine nitrogen site. Fortunately, the corresponding compounds (**16a-16c**) exhibited good inhibitory potency similar to that of **12f**. Bulkier rings such as 4-(piperidin-4-yl)-morpholine (**12m**, IC_{50}: 15.3 ± 2.25 nM) and 1-methyl-1,4-diazepane (**12n**, IC_{50}: 60.1 ± 5.62 nM) retained the activity, suggesting that the cavity of the solvent accessible area could accommodate larger substituents. An oxygen atom was introduced to link the phenyl and six-membered cyclic hydrophilic group, **12o** and **12p**, but showed decreased inhibitory efficiency against NIK. Taken together, exploration of the solvent accessible area showed that a hydrophilic group at 2-position of phenyl is necessary for the activity.

Table 1. Optimization of the hydrophilic substituents in the solvent accessible area



Compounds	R	NIK IC_{50}(nM)[a]	Compounds	R	NIK IC_{50}(nM)[a]
1	/	4.7±0.12	**12j**		199.9±7.03
12a	H	1639±85	**12k**		78.3±5.53
12b		22.5±3.18	**12l**		34.5±7.20
12c		72.4±3.12	**16a**		17.8±1.49

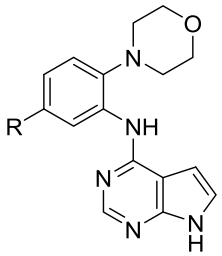
12d		484±19.85	**16b**		8.22±0.26
12e		106.7±23.53	**16c**		16.2±1.07
12f		9.87±0.15	**12m**		15.3±2.25
12g		47.9±3.48	**12n**		60.1±5.62
12h		209.5±13.44	**12o**		112±8.94
12i		69.16±5.19	**12p**		705±9.80

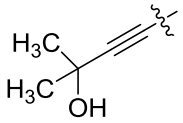
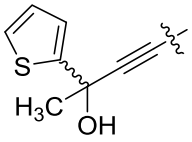
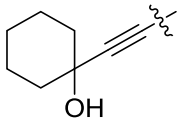
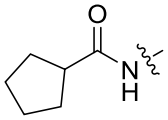
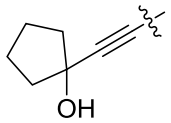
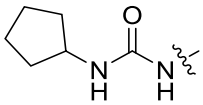
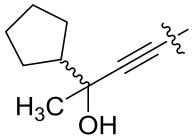
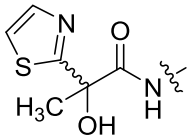
[a]Kinase inhibition assay was performed at 10 μM ATP concentration, and the values are the mean ± SD from two independent experiments.

Further investigation focused on the interactions of the alkynol fragment with the back pocket, as presented in **Table 2**. Several tertiary alcohol fragments were examined, based on their shapes, sizes and electrical properties. The inhibitory activity towards NIK of **12q** was largely lost. This may be due to the loss of the afore-mentioned H-bond interactions formed between thiazole and pocket residues. Comparing the affinity of **12r** and **12s** with NIK protein, the five-membered ring seems to be more suitable for the volume of the back pocket than the six-membered ring. Interestingly, replacement of the cyclopentyl with thiophene substituent resulted in an approximately 40-fold increase in potency of the inhibitors (**12t** and **12u**), suggesting the importance of electrical properties of aromatic heterocycles. Deduced from our design strategy shown in **Figure 2**, the molecular flexibility of the N-phenylpyrimidine-4-amine skeleton was higher

than the 4-(indolin-1-yl)pyrimidine-2-amine, which may be harmful to the orientation of the alkyne. Considering this, we hypothesized that a more flexible linker such as an amide bond could perform better when the corresponding fragment passes through the methioine gatekeeper into the back pocket. However, the results are negative (compare **12s** with **14b** or **14c** with **12f**, respectively). Specifically, compounds **14a** and **14b** exhibited the same level of inhibition against NIK, despite the different lengths of respective linkers. Based on the principle of electronic isosterism, the introduction of an amide bond to replace alkynyl linker of **12f** yields **14c,** leading to a large decrease in its inhibitory activity against NIK.

Table 2. Structures and their inhibitory activities against NIK of target compounds

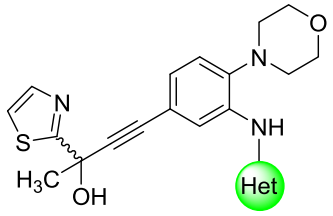


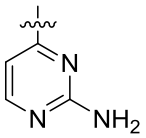
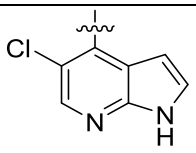
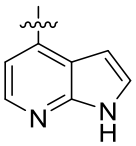
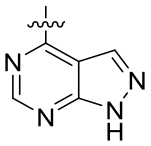
Compounds	R	(%enzyme activity at 1.0 μM)[b] IC_{50}(nM)[a]	Compounds	R	(% enzyme activity at 1.0 μM)[b] IC_{50}(nM)[a]
12q		2714±37	**12u**		50.4±1.59
12r		1270±64	**14a**		(77.27±0.44%) nd
12s		645±14.2	**14b**		(76.40±1.05%) nd
12t		2240±77	**14c**		1404±62

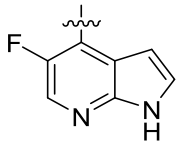
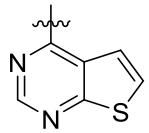
[a]Kinase inhibition assay was performed at 10 μM ATP concentration, and the values are the mean ± SD from two independent experiments.

As showed in **Table 3**, the 2-aminopyrimidine unit was introduced into the backbone to replace the pyrrolopyrimidine fragment of **12k**, and compound **21a** exhibited a similar excellent inhibitory activity to **12f**. Subsequently, different nitrogen-containing aromatic heterocycle fragments were introduced to investigate the binding tolerance of NIK the hinge region. Indeed, the ring-opening treatment of the dihydroindole skeleton increases the flexibility of the molecule, and this is not conducive to the formation of crucial hydrogen bonds in the hinge region. Interestingly, 7-azaindole (**21b**) and 1*H*-pyrazolo[3,4-*d*]pyrimidine (**21e**) structural units (IC_{50}: 6.59 ± 0.09 nM, and 8.63 ± 0.31 nM, respectively) can still capture the hinge region interactions, which confirms the feasibility of cyclization strategy. Based on the prediction that the 5-position of 7-azaindole could be a potential metabolic site, we introduced fluorine or chlorine atom to the position. The corresponding compounds were then excluded because of their reduced inhibition towards NIK, which might be due to clashes with the binding pocket (**21c-21d**). Reduced potency of compound **21f** (IC_{50}: 179 ± 5.68 nM) confirms the importance of the "-NH-" unit to maintain the optimal "two-anchor" type hydrogen bonding interactions at the hinge binding mode.

Table 3. Optimization of the nitrogen heterocyclic aromatic substituents in the hinge area



Compounds	Het	NIK IC_{50}(nM)[a]	Compounds	Het	NIK IC_{50}(nM)[a]
21a		11.4±0.81	**21d**		>10000
21b		6.59±0.09	**21e**		8.63±0.31

21c		1378±29	**21f**		179±5.68

[a]Kinase inhibition assay was performed at 10 μM ATP concentration, and the values are the mean ± SD from two independent experiments.

Taken together, we investigated the NIK inhibitory activities of a series of N-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine derivatives by exploring hydrophilic groups in the solvent accessible region, scaffolds engaging the hinge region as well as back pocket binding fragments. Compound **12f** showed optimal NIK inhibitory activity, and thus the binding mode of compound **12f** within NIK was elucidated using a docking model (**Figure 3**). Similarly, the pyrrolopyrimidine unit forms two conserved hydrogen bonds with Leu474. What is noticeable is that the generation of a new hydrogen bond between the oxygen atom of the morpholine ring and Arg410 or Gly411, respectively (**Figure 3A and 3B**). This interaction further anchors the spatial conformation of the ligand in the pocket, which likely contributes to the inhibitory activity against NIK (compare **12f** with **12k**).

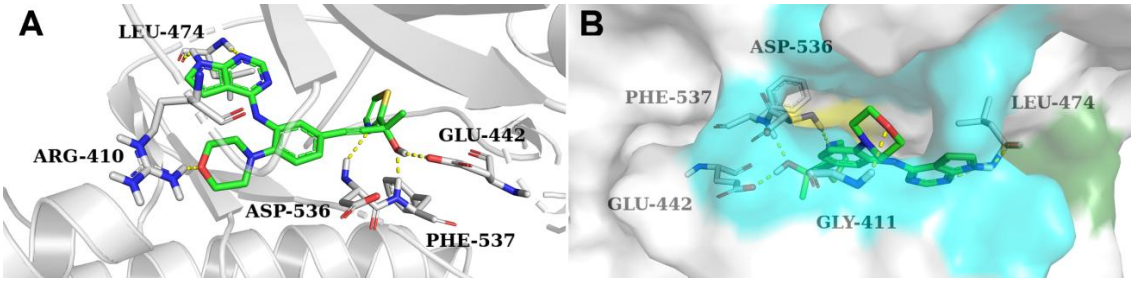


Figure 3. Binding mode analysis of compound **12f** to NIK protein. The solvent accessible region of NIK protein occupied by hydrophilic fragment of the ligand is particularly highlighted. (A) Compound **12f** docked into NIK (PDB code 4G3E). (B) Compound **12f** docked into NIK (PDB code 6G4Y).

Kinase-Inhibition Profile of Compound 12f

The pyrrolopyrimidine fragment has been widely used as a core moiety to construct hydrogen-bonding interactions between the small molecule and the hinge region. Thus, a wild-type screening (Reaction Biology Corp.) was performed to investigate the selectivity of **12f** against 268 kinases (**Figure 4A** and **Table S1**). To

better estimate the binding potency against six types of selected kinases, the IC_{50} value of compound **12f** was determined (**Figure 4B**). Compound **12f** displayed around 4-20 fold higher inhibitory activity against **NIK** (IC_{50}: 9.7 ± 1.7 nM) than **Aurora B** (IC_{50}: 43.2 ± 3.4 nM), **TRKC** (IC_{50}: 50.1 ± 4.7 nM), **LRRK2** (IC_{50}: 94.6 ± 8.4 nM) and **ACK1** (IC_{50}: 191.7 ± 5.3 nM). It should be mentioned that **12f** also has weak inhibitory activities against **JAK1** (IC_{50}: 182.6 ± 6.4 nM) and **JAK3** (IC_{50}: 892 ± 5.2 nM). Taken together, these data establish **12f** as a selective and highly potent inhibitor of NIK with satisfactory selectivity.

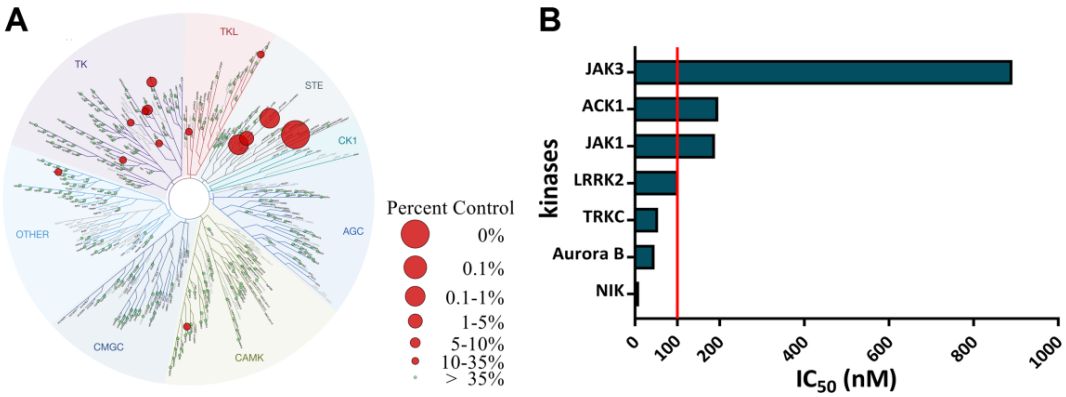


Figure 4. (A) A wild-type screening (Reaction Biology Corp.) was performed to investigate the selectivity of **12f** against 268 kinases in a single-dose duplicate mode at a concentration of 1 μM. The 10 μM ATP concentration was conducted. Staurosporine served as a positive control. The % Ctrl = [(test compound signal − positive control signal)/(negative control signal − positive control signal)] × 100%. (B) Further inhibitory activity of compound **12f** against selected kinases using the P^{33}-radiolabeled assay. The values are the mean from two independent experiments.

Nitric Oxide Production-Inhibitory and Anti-Proliferative Activity of Selected Compounds in Vitro

Previous studies indicate that NIK inhibitors have essential effects on B cell survival, T cell proliferation, and macrophage infiltration in the pathogenesis of autoimmune diseases such as SLE and psoriasis.[28, 38, 41] Thus, the nitric oxide (NO) production-inhibitory activity of J774 peritoneal macrophages and the anti-proliferative activity against primary T cells isolated from the spleen were considered for the next screening. A total of fifteen compounds were selected for cell activity assay. Using compound **1** as a positive control, we

can see that most of the compounds showed satisfactory activities, and eight compounds (**12f**, **12g**, **12i**, **12k**, **12l**, **12n**, **12o** and **21b**) presented higher inhibition rates than 60%. Encouragingly, with the stimulation of 1μM LPS, the inhibition rate of compound **12f** on macrophage NO production was as high as 86.28±0.09%, and its low toxicity was further proven in **Figure S1**. Besides, we also determined whether the selected compounds affect the proliferation of primary T cells, which were isolated from the spleen. Consistently, compounds **12f**, **12i** and **21b** showed better anti-proliferative activities against splenic T cells than others, at different concentrations of 2 μM and 0.4 μM. These data confirmed that compound **12f** to be a highly potent anti-inflammatory NIK inhibitor, which was considered for further studies.

Table 4. Anti-inflammatory activity screening of selected compounds in vitro.

Cpds	NIK IC_{50} (nM)	NO Inhibition rate (%)[a]	Proliferation-Inhibitory rate (%)[b]	
		At 1 μM	At 2 μM	At 0.4 μM
1	4.7±0.12	43.90±0.12	59.57±0.09	19.04±0.03
12c	72.4±3.12	47.97±0.13	27.85±0.10	13.31±0.04
12f	9.87±0.15	86.28±0.09	62.29±0.04	35.96±0.07
12g	47.9±3.48	67.99±0.07	39.87±0.06	20.33±0.14
12i	69.16±5.19	62.86±0.24	49.51±0.19	38.05±0.24
12k	78.3±5.53	78.46±0.04	43.70±0.13	33.03±0.05
12l	34.5±7.20	67.36±0.16	33.67±0.19	24.12±0.24
12m	15.3±2.25	57.69±0.20	34.52±0.09	32.17±0.11
12n	60.1±5.62	67.34±0.13	47.73±0.16	35.54±0.21
12o	112±8.94	68.29±0.11	42.90±0.18	19.93±0.15
16a	17.8±1.49	29.27±0.07	31.46±0.08	16.23±0.04
16b	8.22±0.26	34.45±0.08	32.52±0.12	14.02±0.10
16c	16.2±1.07	37.19±0.13	32.26±0.20	16.91±0.14
21a	11.4±0.81	58.53±0.16	31.44±0.25	27.39±0.09
21b	6.59±0.09	60.37±0.18	50.23±0.06	19.01±0.13
21e	8.63±0.31	35.85±0.25	24.70±0.14	14.77±0.21

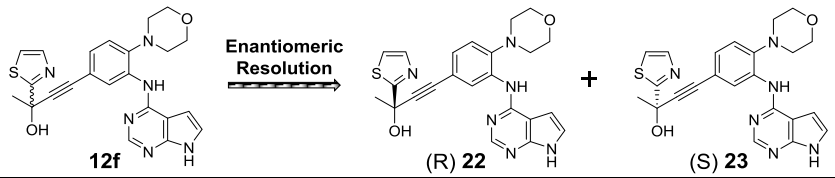
[a]Using Griess reagents method, NO inhibition rate % = (model group NO concentration – compound group NO concentration) / (model group NO concentration – control group NO concentration) × 100%. [b]Con A-induced primary T cell proliferation was investigated under the treatment of selected compounds.

Proliferation -Inhibitory rate (%) = (test hole absorbance – blank hole absorbance) / (control hole absorbance – blank hole absorbance) × 100%. [a,b]The values are the mean ± SD from three independent experiments, n = 3.

Resolution of Enantiomers and Determination of the Dominant Configuration

Given the outstanding enzyme and cell inhibitory activities of **12f**, further work focused on the resolution of its enantiomers and determination of respective activities. Under the specific chiral separation conditions, compound **12f** was resolved into **22** and **23**. As presented in **Scheme 3**, compound **22** displayed about 11 fold higher inhibitory activity against **NIK** (IC_{50}: 11.98 ± 3.13 nM) than that of **23** (IC_{50}: 135.70 ± 7.96 nM). Besides, **22** was more effective in inhibiting NO release of LPS-induced J774 macrophages. Inconsistently, at high and low dosing concentrations, these three compounds had similar inhibitory potency on the proliferation of T cells stimulated by Con A in vitro. It is also interesting that **12f** and its enantiomer **23** were both less toxic to normal T cell growth than **22** (**Figure 5**). In summary, compound **12f**, as a racemate, can exhibit similar anti-T cell proliferation activity and lower toxicity compared to that of the dominant enantiomer **22**. Thus, **12f** was considered for further studies on the biological mechanism and efficacy evaluation in vivo.

Scheme 3. Compound **12f** enantiomeric resolution and determination of the dominant configuration [a]



Cpds	NIK IC_{50} (nM)	NO Inhibition rate (%)[b]	Proliferation-Inhibitory rate (%)[c]	
		At 1 μM	At 2 μM	At 0.4 μM
12f	9.87±0.15	86.28±0.09	62.29±0.04	35.96±0.07
22	11.98±3.13	100.05±0.22	61.17±0.18	37.60±0.10
23	135.70±7.96	74.35±0.17	60.12±0.06	39.50±0.08

[a]Enantiomeric resolution of compound **12f** was preformed. Mobile phase: Hexane : EtOH = 50 : 50, v / v. [b]Using Griess reagents method**,** NO inhibition rate % = (model group NO concentration – compound group NO concentration) / (model group NO concentration – control group NO concentration) × 100%. [b]Con A-induced primary T cell proliferation was investigated under the treatment of selected compounds.

Proliferation - inhibitory rate (%) = (test hole absorbance – blank hole absorbance) / (control hole absorbance – blank hole absorbance) × 100%. [b,c]The values are the mean ± SD from three independent experiments, n = 3.

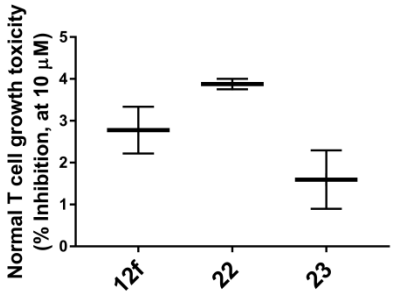


Figure 5. Toxicity comparison between **12f** and its enantiomers (**22**, **23**) against normal growth of T cells at 10 μM concentration. Proliferation - inhibitory rate (%) = (test hole absorbance – blank hole absorbance) / (control hole absorbance – blank hole absorbance) × 100%. The values are the mean ± SD from three independent experiments, n = 3.

PK Data of Selected Compounds Across Multiple Species in Vitro and Vivo

A note to mention is that compound **1** was developed by Amgen as a NIK inhibitor, and then Chen's group disclosed its efficacy on acute hepatitis liver injury in 2016.[36] In addition, as shown in **Table 5**, our work revealed its moderate liver microsomal metabolic stability in human species, but poor metabolic-stability for monkey, dog, and rat liver microsomes ($\mathbf{T_{1/2}}$ ranged from 6.6 to 9.8 min). Conversely, compound **12f** exhibited better metabolic stability in human liver microsomes (HLM), monkey liver microsomes (MLM), dog liver microsomes (DLM), rat liver microsomes (RLM), consistent with the lower clearance data in vitro (ranged between 32.4 μL/min/mg and 76.8 μL/min/mg).

Inspired by the favorable in vitro stability properties of compound **12f**, we performed in vivo pharmacokinetic assessment using intravenous (IV) and per os (PO) administration to Sprague Dawley Rat (shown in **Table 6**). After IV administration of **12f** at a dose of 1.0 mg/kg, maximum concentration (C_{max}) reached 97.3 μg/L, area under the curve ($AUC_{0-\infty}$) was 419 ng·h/mL, and the half-life was 0.944 h with low clearance. Besides, compound **12f** exhibited compatible pharmacokinetic properties under PO administration (C_{max} reached 772 ng/mL at 0.833 h post-dosing, $AUC_{0-\infty}$ = 2680 ng/mL·h, $T_{1/2}$ = 1.39 h, and F% = 64.0%).

Overall, these data, especially high oral bioavailability, demonstrate better pharmacokinetic properties of **12f** than compound **1** in rat.

Table 5. Pharmacokinetics of compounds **1** and **12f** in liver microsomes of multiple species

Compound	Species	$R^{2\,a}$	$T_{1/2}$ (min)[b]	$CL_{int,\ microsome}$ (μL/min/mg proteins)[c]	$CL_{int,\ in\ vivo}$ (mL/min/kg)[d]
1	**Human**	0.9956	33.940	40.844	38.598
	Monkey	0.9996	6.659	208.147	299.732
	Dog	0.9982	7.219	192.014	276.501
	Rat	0.9687	9.816	141.205	254.169
12f	**Human**	0.9060	42.882	32.422	30.639
	Monkey	0.9809	18.048	76.797	110.588
	Dog	0.9765	32.385	43.312	62.369
	Rat	0.9215	30.790	45.016	81.028

[a]R^2 is the correlation coefficient of the linear regression for determination of the kinetic constant. [b]Elimination half-life ($T_{1/2}$). [c]Intrinsic clearance in LMs ($CL_{int,\ in\ vitro}$), [d]Intrinsic hepatic clearance ($\mathbf{CL}_{int,\ in\ vivo}$). $\mathbf{CL}_{int,\ in\ vivo}$ = $\mathbf{CL}_{int,\ in\ vitro}$ (mL/min/mg microsomal protein) × Scaling factor (physiological parameter) = $\mathbf{CL}_{int,\ in\ vitro}$ (mL/min/mg microsomal protein) × Microsomal protein (mg protein/g liver) × Liver weight (g liver/kg body weight).

Table 6. The PK parameter of compound **12f** in Sprague Dawley Rat[a]

PK parameters (n=3)[b]	IV (1.0 mg/Kg)	SD	PK parameters (n=3)[a]	PO (10 mg/Kg)	SD
AUC (0-∞)(ng ħ/mL)	419	54.8	**AUC(0-∞)(ng ħ/mL)**	2680	111
C_0 (ng/mL)	377	56.1	**C_{max} (ng/mL)**	772	60
$T_{1/2}$ (h)	0.944	0.112	**$T_{1/2}$ (h)**	1.39	0.0987
V_d (L/kg)	2.73	0.393	**T_{max} (h)**	0.833	0.289

CL (mL/kg/min)	40.3	4.93	**F(%)**	64.0	2.7

[a]Individual plasma concentration-time data of **12f**, after an i.v./p.o. dose of 1/10 mg kg^{-1} in Sprague Dawley Rat, respectively. Corresponding in vivo pharmacokinetic parameters are given. Injection (i.v.) formulation (prescription, DMSO : PEG200 : Saline = 20 : 20 : 60, v / v / v. concentration, 0.2 $mg \cdot mL^{-1}$). Oral formulation (0.5% CMC-Na / 0.2% Tween 80. concentration, 1 $mg \cdot mL^{-1}$). [b]PK parameters are the mean ± SD from three independent experimental rats, n = 3.

Compound 12f Blocks LPS-Induced Secretion of IL-6 in BEAS-2B Cells and Expression of Inflammation-Related Genes in J774 Macrophages

The most noticeable features in the pathogenesis of psoriasis are excessive secretion of various cell chemokines and pro-inflammatory factors in epidermal and dermal cells.[44] Thus, we further figured out the effects of compounds **12f** and **1** on the secretion of interleukin-6 (IL-6) in BEAS-2B cells. As seen in **Figure 6A**, within a specific concentration range, both compounds exhibited a dose-dependent inhibition of IL-6 secretion against inflammatory model cells. Compound **12f** has a relatively higher inhibitory activity than compound **1,** and it inhibits IL-6 secretion at concentrations ranging from 14 nM to 10 μM. Next, we determined the effectiveness of **12f** in regulating inflammation-related gene expression in J774 macrophage (**Figure 6B**). The results showed that the mRNA levels of inflammatory cytokines and chemokines (including TNFα, IL-6, IL-23, IL-1β, and CCL3, respectively) were increased in response to the stimulation of LPS, but notably decreased by administrating **12f**. Moreover, the expression of nitric oxide synthase 2 (NOS2) is generally up-regulated in the occurrence of various inflammatory diseases.[45-46] Interestingly, NOS2 levels in J774 macrophages showed a markedly negative regulatory response to the administration of **12f**.

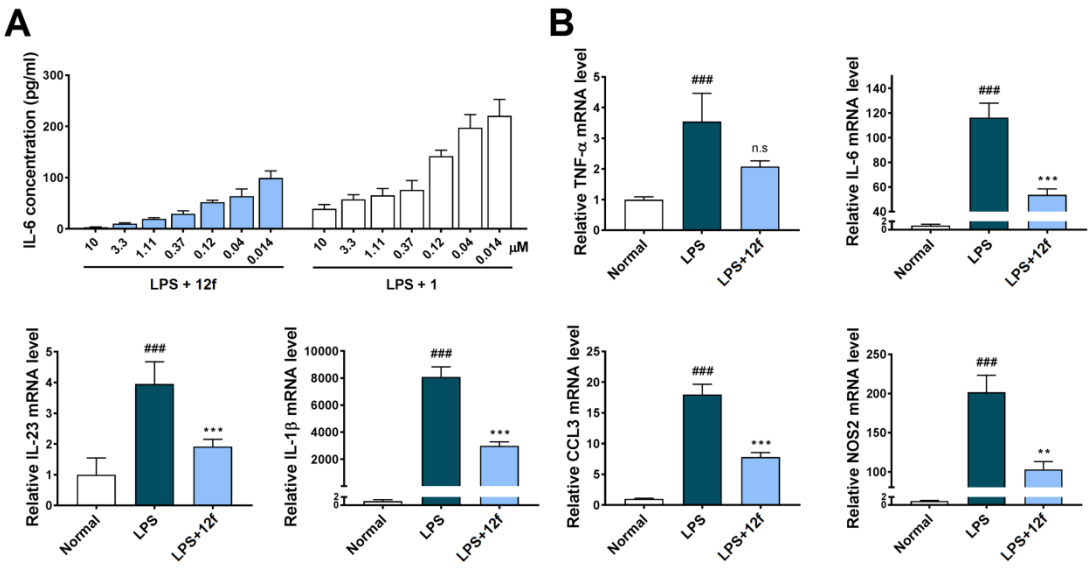


Figure 6. (A) The culture supernatant IL-6 levels in LPS-induced BEAS-2B cells were analyzed and quantified by Elisa. (B) Quantitative PCR analysis of the mRNA levels of exocrine inflammatory factors (TNFα, IL-6, IL-23, IL-1β), chemokine (CCL3), and cytokine (NOS2) in LPS-stimulated J774 cells. P values are determined by two-tailed Student's t-test, between the normal and model group: $^{\#}P<0.05$, $^{\#\#}P<0.005$,$^{\#\#\#}P<0.0005$, between model and **12f** treatment groups: n.s. (not significant), *P<0.05, **P<0.005, ***P<0.0005. Data are represented as mean ± SD from two (A) and three (B) biological replicates.

Treatment of 12f Attenuates Psoriasis-like Phenotype in Imiquimod Model Mice

After obtaining promising in vitro activities and in vivo PK results, we further explored the in vivo pharmacological efficacy of compound **12f** on the IMQ-induced psoriasis model using C57 mice. In order to establish a psoriasis-like dermatitis model, 5% imiquimod cream was evenly applied to the back skin of C57 mice (for seven consecutive days, q.d.). Moreover, **12f** was administered orally on the third day and continued until the end of the experiment (12f-I: 60 mg/kg, 12f-II: 120 mg/kg, i.g./q.d.). The model group of mice exhibited a typical psoriasis-like dermatitis phenotype, including invasive erythema, roughness, swelling, and scales. In contrast, the symptoms of the administration group (12f-I, 12f-II) were significantly attenuated (**Figure 7A**). Hematoxylin and eosin staining revealed the occurrence of epidermal acanthosis, as shown in **Figure 7B**, and the model group had significant morphological and pathological changes in the back skin

compared to the normal and drug-treated groups, such as increased skin thickness and severe scales. Statistical analysis of the back skin thickness of six mice in different experimental groups showed that the skin thickness of the administration group changed little under the induction of IMQ, confirming a dose-dependent enhancement of **12f** treatment efficacy (**Figure 7C**). The spleen index of the **12f** administration group was slightly lower than that of the model group, reflecting that IMQ's activation of systemic immunity was suppressed to a certain extent (**Figure 7D**). Consistently, Elisa detection of TNFα and IL-17A in serum revealed that **12f** could inhibit mouse secretion of both cytokines in a dose-dependent manner (**Figure 7E**).

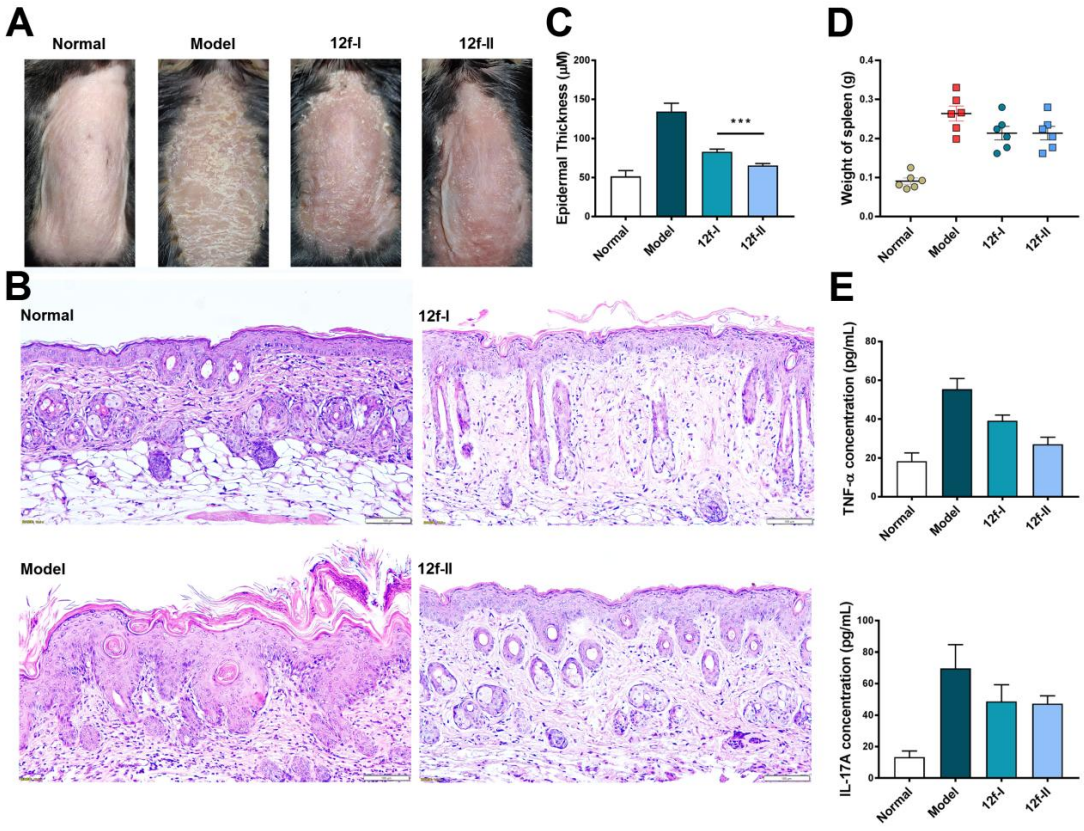


Figure 7. (A) Representative images of dorsal back from wild-type C57 mice untreated or treated with IMQ or **12f** for 7 or 4 days. (B) Hematoxylin and eosin (H&E) staining of skin sections from the dorsal back (normal group: IMQ and **12f** were untreated, model group: only IMQ treatment, 12f-I / 12f-II: at 60 mg/kg or 120 mg/kg doses of **12f** treatment, along with IMQ induction). Scale bar, 50 μm. (C) Epidermal thickness was measured in six randomly chosen fields. (D) The weight of the mouse spleen was counted, and each point represents the value of the spleen weight of a mouse. (E) TNF-α and IL-17A in the mouse serum were

analyzed and quantified by Elisa. Data are shown as the mean ± SEM based on three independent experiments.

Compound 12f Suppressed Epidermal and Dermal Cell Proliferation and Macrophage Infiltration in Mouse Back Skin Tissues through Regulating NF-κB2 Signaling Pathway

Characteristics of psoriasis lesions include excessive cell proliferation in epidermal and dermal tissues and infiltration of immune cells such as macrophages. After orally administering compound **12f** to C57 mice for four days, we quantified the population of $Ki67^+$ cells in psoriatic lesions by immunohistochemistry to reveal the extent of epidermal and dermal cell proliferation in skin tissue. As shown in **Figure 8A**, the population of $Ki67^+$ cells in the back skin of the mice was obviously reduced when treated with a low dose of **12f** (12f-I, 60 mg/kg), compared to the model group mice. Moreover, by comparing the experimental results of the high and low dose groups, we obtained preliminary dose-effect relationship of **12f** on the inhibition of cell proliferation in psoriatic skin tissue: normalized to the model group, the 12f-I (60 mg/kg) and the 12f-II (120 mg/kg) group Ki67 expression percentage decreased by approximately 55% and 83%, respectively (**Figure 8B**). Subsequently, we investigated the effect of compound **12f** on the infiltration of macrophages in the pathogenesis of psoriasis model mice (**Figure 8C**). The results of F4/80 immunofluorescence images showed that the recruitment of macrophages from the back skin tissue of the model group was notably up-regulated following the induction of IMQ. In contrast, treatment with high or low doses of **12f** (12f-I and 12f-II) notably alleviated this symptom.

Consistent with the above data, compound **12f** at different doses exhibited dose-dependent inhibition of the NF-κB2 pathway by targeting the NIK protein. Furthermore, from **Figure 9A** and **9B**, we found that the levels of p52, downstream of NIK, in the model group were obviously higher than that in the administration group, and it decreasing with increased dosing of the **12f** administration. We also found reduced expression of genes associated with the NF-κB2 signaling pathway, such as TNFα, IL-6, IL-10, and IL-12, which in turn validated the critical role of NIK in the development of psoriasis (**Figure 9C**). Related pathological regulation responding to **12f** treatment in psoriasis mice was demonstrated (**Figure 9D**). Together, these results suggest

that **12f** treatment can relieve epidermal and dermal cell proliferation and macrophage infiltration in mouse back skin tissues by targeting NIK to regulate the activation of NF-κB2 signaling pathway.

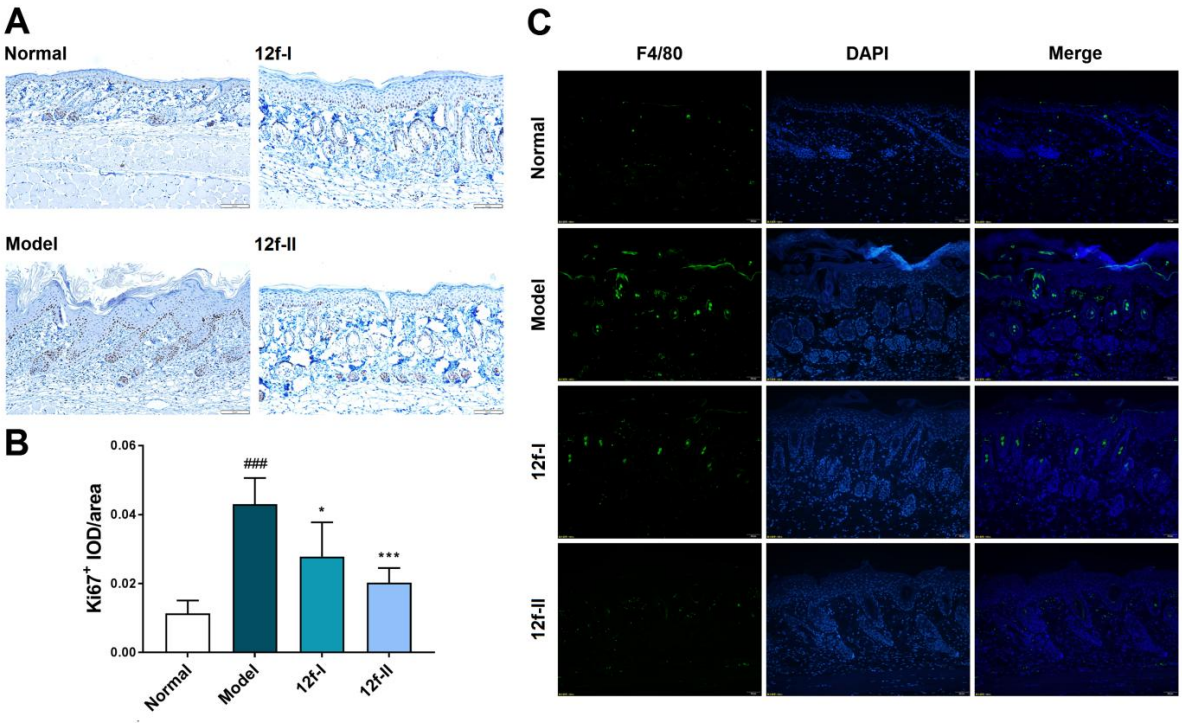


Figure 8. (A) Representative skin sections from the back of the mice, which were stained with Ki67 antibody (normal group: IMQ and **12f** were untreated, model group: only IMQ treatment, 12f-I / 12f-II: at 60 mg/kg or 120 mg/kg doses of **12f** treatment, respectively, along with IMQ induction). Scale bar, 50 μm. (B) The percentage of $Ki67^+$ cells in the total cells was analyzed by Image J software. (C) F4/80 immunofluorescence staining revealed the recruitment of macrophages in the back skin tissue of C57 mice. P values are determined by two-tailed Student's t-test, compared with normal group: $^{\#}P<0.05$, $^{\#\#}P<0.005$, $^{\#\#\#}P<0.0005$, and compared with model group: *P<0.05, **P<0.005, ***P<0.0005. Data are represented as mean ± SD.

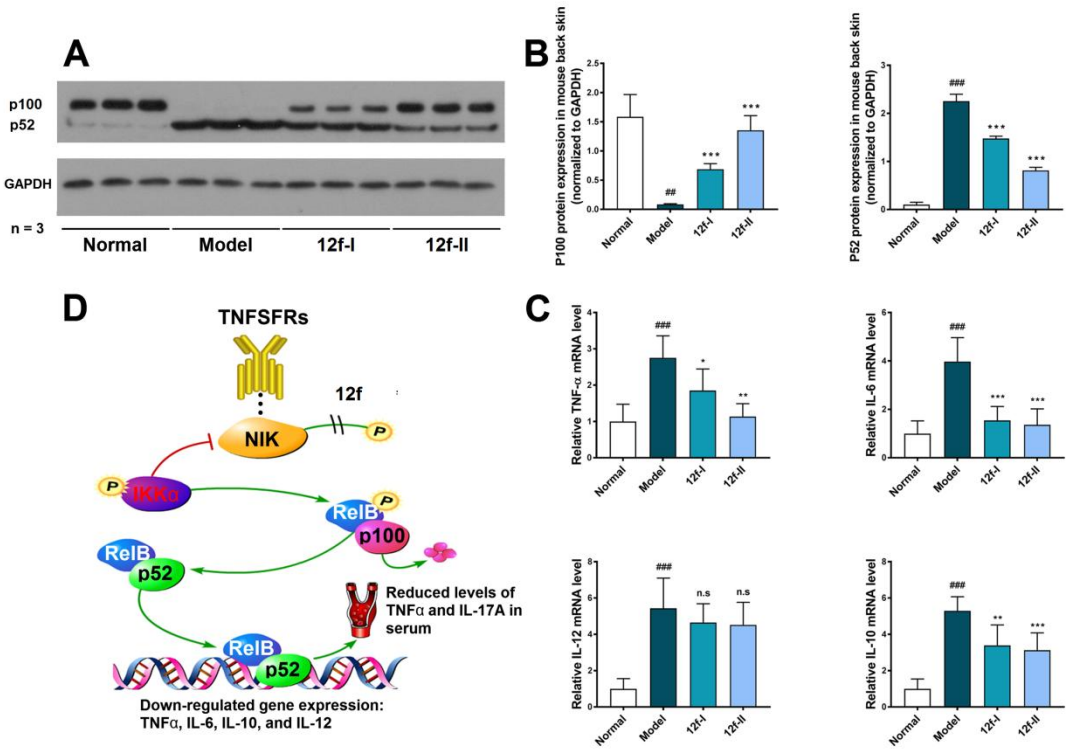


Figure 9. (A) After oral administration of compound **12f** (12f-I: 60 mg/kg, 12f-II: 120 mg/kg), the levels of NF-κB2 signaling pathway-related proteins (p100 and p52) were monitored. Quantitative analysis was normalized to GAPDH. (B) The relative optical densities of specific proteins were recorded. (C) Quantitative analysis of mRNA levels in the skin of the back of mice (TNFα, IL-6, IL-10, IL-12) to investigate the effect of high and low doses of **12f** treatment on the expression of immune regulation-related genes induced by IMQ in mice. (D) Pathological signal response to the treatment of **12f** as the NIK inhibitor was depicted. P values are determined by two-tailed Student's t-test, between normal and model group: $^{\#}P<0.05$, $^{\#\#}P<0.005$, $^{\#\#\#}P<0.0005$, and between model and **12f** treatment groups: n.s. (not significant), $^{*}P<0.05$, $^{**}P<0.005$, $^{***}P<0.0005$. Data are presented as mean ± SD of three independent experiments.

CONCLUSIONS

In this study, we reported the design and synthesis of N-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine derivatives as novel NIK inhibitors. Through structure-activity relationship studies, compound **12f** was identified as a highly potent NIK inhibitor with high selectivity over 268 wild-type kinases. Besides, compound **12f** exhibited excellent NO production-inhibitory and anti-proliferative activities against macrophages (J744)

and splenic T cells, respectively. Compound **12f** performed better than a known NIK inhibitor compound **1** developed by Amgen in PK properties and blocking ability towards the secretion of IL-6 induced by LPS in BEAS-2B cells. Encouragingly, down-regulation of inflammation-related cytokines (including: TNFα, IL-6, IL-10, IL-12, IL-23, IL-1β, and NOS2) and chemokine (CCL3) gene expression in vitro or in vivo, reduced secretion of pro-inflammatory factors (TNFα, IL-17A) in serum, the relieved psoriasis-like phenotype, and the results of hematoxylin and eosin staining, Ki67 immunohistochemistry and F4/80 immunofluorescence staining indicated that treatment of IMQ-induced mice with compound **12f** attenuated the pathological features of psoriasis. Altogether, this work provided a new idea for the design and development of NIK inhibitors, and suggests the possibility of using NIK inhibitors as a new treatment of psoriasis for the first time.

EXPERMENTAL SECTION

Kinase Inhibition Assay

ADP-Glo™ kinase assay: ADP-Glo™ kinase assay was performed by Nanjing Anacon Biotechnology Co., Ltd, which is one of our partners. Reagents: human recombination MAP3K14 protein (Carna, catalogue no. 07-102), NIK ADP-Glo™ assay kit (Promega, catalogue no. V9101). The assay contains two steps: 1. Add an equal volume of ADP-Glo reagent to terminate the kinase reaction and deplete the remaining ATP after the kinase reaction. 2. Add the kinase detection reagents to simultaneously convert ADP to ATP and allow the newly synthesized ATP to be measured using a luciferase/luciferin reaction. Reaction procedure: 1. Preparation of the following buffer solution: (a) General reaction buffer: 2 mM DTT, 2 mM $MgCl_2$, 100 μM Na_3VO_4, 0.02 mg/mL BSA, Tris pH 7.5, 5% DMSO. (b) 2.5 X NIK (5 nM) assay buffer. (c) 2.5 X ATP（25 μM）assay buffers. 2. For 96-well plate, add 1.0 μL of compound in DMSO (final concentration of DMSO: 5%) to the wells of the reaction plate. Positive and negative control wells used buffer only. 3. In the compound and positive control wells, 2 μL 2.5 X NIK and 4 μL 2.5X ATP was delivered. Besides, 2 μL 2.5 X ATP and 2μL assay buffers were utilized for negative control wells. The reaction mixture was incubated for 120 min at room temperature. 4. After addition and incubation of 5 μL ADP-Glo™ reagent for 40 min, deliver the 10 μL of detection reagent

into the wells and incubate for an additional 40 min. 5. Luminescence measurement, using a luminometer. Luminescence can be correlated to ADP concentrations by using an ATP-to-ADP conversion curve. IC_{50} values and curve fits were obtained by Prism (GraphPad Software).

Wild-type kinase screening: To determinate the selectivity of **12f** over different kinases, a wild-type screening was performed by Reaction Biology Corp. (Malvern PA), using Hot-SpotSM kinase assay as described previously.[47] The average % inhibition over 268 kinases were reported (**Supplementary Table S1**).

Griess Reagents Method for Detecting Nitric Oxide Production in J744 Peritoneal Macrophages

Analysis of macrophage nitric oxide production was carried out according to the methods described in the previous literature.[48-49] Peritoneal macrophages (J774) were seeded in a 96-well plate ($2*10^4$ cells/mL, 200 uL per well), with a medium (5% bovine fetal serum, 1% penicillin-streptomycin, 1% nonessential amino acid, and 2% L-glutamine). After 24 h incubation in 5% CO_2 at 37°C, the medium was discarded. Add the supplemented medium 1640 197 µL/well. Subsequently, add 2 µL DMSO with or without test compound (1 µM concentration, 3 replicate wells per sample) to the test drug group or LPS model control group. For the blank group, 2 µL of the medium was spiked into corresponding wells. After 2 h incubation in 5% CO_2 incubator at 37°C, 1 µL of PBS-configured LPS (200 ug/mL) was added to the LPS control group and each test group to a final concentration of 1 ug/mL and cultured for additional 22 h. Then, 100 µL supernatant from each well was transferred to a new 96-well plate. The supernatant was finally treated with 100 µL Griess reagents (1% sulfa, 0.1% naphthalenediamine dihydrochloride, and 2.5% phosphoric acid). Finally, using linear regression analysis of the standard curve (serial double dilutions of sodium nitrite from 200 µmol/L to the eleventh dilution), obtained NO concentrations in the supernatants Ab-sorbance was determined at 540 nm by using the mi-croplate reader.

Anti-Proliferation Assay of Con A-Activated Spleen T Cell

The primary T-cells were isolated from the splenocyte samples using EasySepTM mouse T cell isolation kit (Stemcell Technologies, catalogue no. 19851). T cells were suspended in RPMI with 10% PBS (1%

penicillin/streptomycin, 2% glutamine) and seeded in 96-well culture plates at a density of $2*10^6$/mL cells per well. Con A (Sigma) at 5 μg/mL was used to stimulate the T cell proliferation in the presence or absence of NIK inhibitors (2μM and 0.4μM concentration, 3 replicate wells per sample). Then, incubate the plates for 48 h at 37 °C 5% CO_2. Compound's ability to inhibit Con A dependent primary T cell proliferation were measured by using CCK-8 assay (MedChemExpress) according to the manufacturer's instructions.[50] The toxicity analysis for normal T cells growth was also performed, corresponding data are shown in supplementary **figure S1**.

The Measurement of IL-6 Production in LPS-Induced Human BEAS-2B Cells

Human BEAS-2B Cells line was purchased from the American Type Culture Collection (ATCC, catalogue no. CM-1075). Methods: BEAS-2B cells were resuscitated and cultured (Complete growth medium: 89% DMEM, 10% FBS, 1% penicillin-streptomycin). Then, seed the logarithmic growth phase of BEAS-2B cells into 96-well plates at 5000 cells/well, and incubated under 5% CO_2 for 24 h at 37 °C. After the cells were attached, the test group, the positive control group (dexamethasone), the model group, and the blank control group were set before the treatment of compounds **12f** and **1** (10 μM-0.014 μM, 7 concentrations, 3 replicate-wells). After 8 hours of administration, add 0.1 mL of LPS (20 μg/mL) to each well to stimulate BEAS-2B cells for 24 hours. The IL-6 levels in the supernatant of the culture were determined by using the human IL-6 Elisa kit (MultiSciences, catalogue no. 70-EK1062).

Pharmacokinetic Evaluation of Compound 12f

Metabolic stability in liver microsomes: The metabolic stability of compounds **12f** and **1** on liver microsomes of different species was determined according to the procedures described by 3D BioOptima Co. Ltd., including: RLM (rat liver microsomes), DLM (dog liver microsomes), CLM (monkeys liver microsomes) and HLM (human liver microsomes). To each well (T0, T5, T15, T30, T60, NCF60), add the solution of compound **12f**, **1** or the control working solution. Subsequently, add 80 μL/well to every plate by apricot, incubate the mixture of microsome solution and compound **12f** or **1** at 37°C for 10 min. Add 10 μL 100 mM potassium phosphate buffer/well to NCF60, incubate at 37°C for 1h. After pre-warming, add 10 μL/well to every plate by apricot to start reaction. Reactions were terminated at 5, 15, 30 and 60 min of incubation with a chilled

mixture of Tolbutamide and Labetalol (1:1). The mixture was vortexed for 5 min, centrifuged at 4000 rpm for 20 min at 4°C, and the supernatants were analyzed by LC−MS/MS. The data was analysis by the first order kinetics to calculate $t_{1/2}$ and Cl. Drug elimination rate constant k (min^{-1}), elimination half-life $T_{1/2}$ (min), and in vitro intrinsic clearance C_{Lint}, in vitro ($\mu L \cdot min^{-1} \cdot mg^{-1}$ protein) were calculated according to the following equations: k = - slope, $T_{1/2}$ = 0.693/k, C_{Lint}, in vitro = $k/C_{protein}$, where $C_{protein}$ ($mg \cdot mL^{-1}$) is the microsomal protein concentration in the incubation.[51-52]

Pharmacokinetic (PK) study: To determine oral bioavailability, 3 SD rats (Beijing Charles River Laboratories) per group were treated with **12f** by a single intravenous (IV) bolus or oral gavage administration at the doses of 1 and 10 mg/kg, respectively. Injection formulation of compound **12f** was prepared in solution (DMSO: PEG200: Saline = 20: 20: 60, v/v/v). Suspension of **12f** in normal saline containing 0.5% CMC-Na and 0.2% Tween 80 to obtain the oral formulation and using LC-MS/MS-based methods to quantify drug concentration in plasma. Pharmacokinetic parameters were estimated using Phoenix WinNonlin (version 6.3) from mean plasma concentration-time profiles. The area under the curve (AUC) was calculated from time versus concentration data using the linear trapezoidal rule. The oral bioavailability is calculated as the ratio of AUC for **12f** from PO and IV dosage. The calculation is normalized by relative doses. For **12f** plasma samples: an aliquot of 30 µL of the sample was added with 150 µL ACN which contains verapamil, 5 $ng \cdot mL^{-1}$, and glibenclamide, 50 $ng \cdot mL^{-1}$ for protein precipitation, the mixture was vortexed for 10 min and centrifuged at 3700 rpm for 10 min. Then 70 µL of supernatant was added with 70 µL water and vortexed for 10 min. An aliquot of 15 µL of the mixture was injected into the system and subjected to LC-MS/MS analysis using an Agilent Technologies 6430 Triple Quad LC/MS system. Chromatogram signals were integrated and calibrated using Agilent MassHunter Workstation Software B.06.00. Pharmacokinetic parameters were derived by non-compartmental analysis from individual **12f** plasma concentration versus time profiles (Phoenix WinNonlin, version 6.3).

Animal Models

The IMQ-induced psoriasis-like disease model was performed in a similar way as previously reported in the literature (van der Fits et al., 2009).[53] Otherwise, C57 mice were purchased from the institute of Model Animals, Nanjing University. All animals are housed in specific pathogen-free facilities and used in accordance with the regulations of the Animal Ethics Committee of China Pharmaceutical University. Imiquimod is produced by Sichuan Mingxin Pharmaceutical Co., Ltd. (Chinese Medicine Standard: H20030129). In particular, the mice treated with **12f** (same as normal group and model group: n = 6) were divided into two dose groups (12f-I: 60mg / kg, 12f-II: 120mg / kg). After shaving the spinal hair, the mice received about 65 mg imiquimod cream (5%) topically on the back skin daily, and the normal group was replaced with vaseline for 7 days. Samples (blood and back skin tissue) were taken on the second day after the end of IMQ treatment.

Immunohistochemistry of Back Skin Tissue Sections

Omarin-fixed, paraffin-embedded blocks were selected from the back skin tissue of each group of mice, and 3 micron thick tissue sections were cut out for Hematoxylin-eosin (H&E) staining. On the other side, samples of back skin (3 mm diameter) were dipped into TissueTek (Shanghai Rantai Biotechnology Co., Ltd.), snap-frozen in liquid nitrogen, and stored at 80 ° C until use. Six-micron frozen sections of frozen skin were cut using a cryostat (Jung Frigocut 2800 ELeica). IHC staining was performed in accordance with standard procedures. The following primary antibodies were used: Ki67 (FcMRCS, catalogue no. YM6189), F4/80 (abcam, catalogue no. SP115). Slides were incubated overnight at 4°C, or for 1 h at room temperature. This was followed by in-cubation for 30 min with biotin-linked secondary. After washing with PBS, DAPI was incubated at room temperature for 1 h, and observed with a confocal laser scanning microscope (LSM 700, Zeiss, CA, USA). Histopathological evaluation was performed blindly by two pathologists. Two pathologists conducted the histopathological evaluation using a random, blindly selected manner.

Mouse Serum Elisa Assay.

After the end of the administration, the corresponding blood samples were collected from the mice in each group and left at 4 ° C for 12 hours. Following centrifugation, TNFα (Arigo, catalogue no. ARG80206) and

IL-17A (Immunoway, catalogue no. KE1180) levels in mouse serum were determined by Elisa according to the manufacturer's protocol.

Quantitative PCR Assay of Target Gene

The detection of target gene expression (TNFα, IL-6, IL-10, IL-12, IL-23, IL-1β, CCL3, NOS2) follows the universal SYBR green wuantitative PCR protocol. Special instructions for setting up reaction steps: For NTC reactions, add 4 μL of water to the reaction tube. For experimental reactions, add 4 μL of cDNA solution to the reaction tube. Centrifuge all tubes briefly. Visually confirm that all tubes or wells contain sample at the bottom at the correct volume. Carefully aliquot 16 μL of template master mix into each qPCR tube or plate well. Mix reactions well and spin if needed. Cap tubes or seal the PCR plate and label (according to instrument requirements). (Make sure the labeling does not obscure instrument excitation/detection light path.) Run samples as per instrument manufacturer recommendations. Run the samples following the instrument's operating procedures, and also refer to the instrument manual to analyze the data.

Western Blot

The operation of western blot analysis follows our previous report.[49] The protein extract of the back skin tissue of C57 mice was prepared for western blot analysis of GAPDH, p100, and p52**.** The levels of target proteins were determined using a gel imaging system (ChemiScope 2850, Clinx Science Instruments Co., Ltd., Shanghai, China) and normalized to that of the reference band.

Molecular Modeling

The X-ray co-crystal structure of the NIK protein binding with corresponding small molecules was retrieved from the Protein Data Bank [28, 30] (PDB codes: 4G3E and 6G4Y). Compounds **12f** or **12k** were prepared using the ligand preparation wizard in Maestro with standard settings. Following the standard procedure suggested by Schrodinger, the NIK protein was pre-processed, and then a grid of NIK proteins was generated using Glide (version: 11.5). The conformational ensembles were docked flexibly using Glide with standard settings in both standard and extra precision mode. Only poses with low energy conformations and

good hydrogen bond geometries were considered. Pymol was used to display the small molecule ligand-protein docking results. Finally, the editing of pictures was performed using HprSnap7.

General Procedures.

Unless otherwise specified, reagents were purchased from commercial suppliers and used without further purification. Melting points were determined by a X-4 digital-display micromelting-point apparatus (Beijing Tech Instrument Company, Ltd., Beijing, China). NMR spectras were recorded on a Bruker AVANCE AV-600 spectrometer (400 or 300 MHz for ^{1}H, 100 or 75 MHz for ^{13}C); Mass spectras were obtained on the Agilent 1100 LC / MSD mass spectrometer (Agilent, USA) and Q-tofmicro MS (micromass company). All reactions were monitored by TLC (Merck Kieselgel GF254) and spots were visualized with UV light or iodine. The purity of biologically evaluated compounds was ≥ 95% as determined by HPLC.

The Preparation of 4-chloro-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (6).[54]

To a solution of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (6.14 g, 40 mmol) in dry THF (60 mL) under Ar atmosphere at 0 °C, NaH (60%, 4.80 g, 120 mmol) was added in portions within 5 min under vigorous stirring. The reaction mixture was stirred for a further 30 min after completion of the addition, then TsCl (11.44 g, 60 mmol) dissolved in THF (40 ml) was added dropwise while keeping the temperature between 5–10 °C, the resulting suspension was allowed to reach room temperature and stirred for 4.0 h. The mixture was poured slowly into a vigorous stirred ice-water to quench excessive NaH, the resulting mixture was extracted with EA (150 ml x 3), combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was chromatographed, eluting with a gradient of 10% EA/PE to afford title compund (11.25 g, 91%) as a white solid. $[M + H]^+$: 308.1.

General Procedure A for the Synthesis of Compounds 7, 17a and 17e-17f.

A mixture of Compound **6** or corresponding 4-chloropyrimidine derivatives (5 mmol), 2-fluoro-5-nitroaniline (1.17 g, 7.5 mmol), was treated with AgOTf (1.28 g, 5 mmol) in dioxane (20 mL) and stirred at 130 ^{o}C for about 6 h. The mixture was filtered and the filtrate was concentrated under vacuum. The residue was

chromatographed, eluting with a gradient of 20% to 100% EA/PE to afford title compund (67%–95%) as a yellow solid.

General Procedure B for the Synthesis of Compounds 17b-17d.

A mixture of corresponding 4-bromo-1*H*-pyrrolo[2,3-*b*]pyridine derivatives (10 mmol, 1.0 eq), 2-fluoro-5-nitroaniline (15 mmol, 1.5 eq), $Pd_2(dba)_3$ (0.5 mmol, 5% mmol), X-Phos (1 mmol, 10% mmol), K_2CO_3 (0.15 mmol, 10% mmol) in t-BuOH (60 mL) was stirred at 100 ^oC for 4.0 h under N_2 atmosphere. After completion of reaction, the mixture was evaporated under vacuum. The residue was chromatographed, eluting with a gradient of 4%-5% DCM/MeOH to afford title compounds **17b**-**17d** (yield, 37%-85%) as a yellow solid.

General Procedure C for the Synthesis of Compounds 8b-8n (Method C-I), 8o-8p (Method C-II) and 18a-18f.

Method C-I: To a suspension of corresponding (2-fluoro-5-nitrophenyl)pyrimidine derivatives (3.0 mmol), K_2CO_3 (1.45 g, 10.5 mmol) in DMF (6 mL), the corresponding secondary amine (9 mmol, 3.0 eq) was added, the resulting mixture was stirred at 90 ^oC for about 3 h. After completion of reaction, the mixture was then poured into ice-water. A yellow precipitate formed and was collected by filtration to afford crude title compound. Compounds **8b-8n** or **18a-18f** as yellow solid, which were used without further purification.

Method C-II: To a stirred ice-cooled suspension of NaH (60%, 600 mg, 15 mmol) in DMF (2 mL), the corresponding alcohol (18 mmol, 6.0 eq) in dry DMF (6 mL) was added dropwise under N_2 atmosphere, the reaction mixture was stirred at 0 ^oC for additional 30 min. Subsequently, a solution of compound **7** (1.28 g, 3.0 mmol) in DMF (4 mL) was added dropwise. The mixture was allowed to reach room temperature and stirred for additional 2.0 h. The post-reaction treatment was similar to method **C-I** and afforded the crude title compounds **8o** and **8p**, which was used without further purification.

General Procedure D for the Synthesis of Compounds 9b-9p and 19a-19f.

To a solution of 5-nitrophenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine derivatives (3.0 mmol) in ethanol (21 mL) and water (3 mL), iron (1.68 g, 30 mmol) and ammonium chloride (1.61 g, 30 mmol) were added and refluxed for 1.0 h. The reaction mixture was filtered through celite, washed with MeOH. The organic layer

was dried over Na_2SO_4 and concentrated under vacuum. The residue was chromatographed, eluting with a gradient of 1%-5% DCM/MeOH to afford title compounds **9b-9p** and **19a-19f.** (yield, 21%-88%) as a white solid.

General Procedure E for the Synthesis of Compounds 10b-10p and 20a-20f.

To a solution of compound **9b-9p** or **19a-19f** (1.5 mmol) in CH_3CN (4.5 mL) was added *p*-TsOH (774.9 mg, 4.5 mmol). The thick suspension was cooled to 0 °C, and a solution of sodium nitrite (207.0 mg, 3.0 mmol) and KI (622.5 mg, 3.75 mmol) in water (1.1 mL) was added dropwise while keeping the temperature between 0 - 5 °C. The mixture was allowed to reach room temperature and stirred for 2.0 h. To the mixture was added water (40 mL) and 2 M aq. $Na_2S_2O_3$ (4 mL). The mixture was extracted with CH_2Cl_2 (50 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under vaccum. The residue was chromatographed, eluting with a gradient of 0.5%-4% DCM/MeOH to afford title compounds **10b-10p** and **20a-20f** (yield, 37%-75%) as a yellow or white solid.

General Procedure F for the Synthesis of Compounds 11b-11m and 13.

Compound **10b-10m or 9** (1.0 mmol) was dissolved in EtOH (4.0 mL). The mixture was treated with NaOH (144 mg, 3.0 mmol) and heated at 40 °C for 2.0 h. After completion of reaction, the mixture was evaporated under vacuum. The residue was chromatographed, eluting with a gradient of 4%-10% DCM/MeOH to afford title compounds **11b**-**11m or 13** (yield, 56%-79%) as a yellow solid.

General Procedure G for the Synthesis of Compounds 14a-14c and 15a-15c.

To a stirred ice-cooled solution of **11g** or **13** (0.5 mmol) in dry DMF (1.5 mL, replaced by THF when the synthesis of **15a-15c** were conducted), the corresponding sulfonyl chloride, acetyl chloride or isocyanate (0.55 mmol, 1.1 equiv) in dry DMF or THF (1 mL) was added dropwise under N_2 atmosphere. Subsequently, Et_3N (1.0 mmol, 2.0 equiv) in DMF or THF (1.5 mL) was added dropwise. The mixture was allowed to reach room temperature and stirred for 2.0 h. The mixture was extracted with EA (30 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was chromatographed, eluting with a gradient of 2%-5% DCM/MeOH to afford title compounds **14a**-**14c** or **15a-**

15c (yield, 28%-78%) as a white or yellow solid.

General Procedure H for the Synthesis of Compounds 12a-12u, 16a-16c and 21a-21f.

To a mixture of compounds **11a-11u, 15a-15c** or **20a**-**20f** (1.0 eq), $PdCl_2(PPh_3)_2$ (5% mmol), CuI (10% mmol) in THF/Et_3N = 3:1 (total concentation: 0.2 M), a solution of corresponding terminal alkynes in THF (1 mL) was added dropwise, in some cases, dry DMF (2 mL) was added to the reaction system to keep the starting materials dissolved completely. After completion of reaction, the mixture was evaporated under vacuum. The residue was chromatographed, eluting with a gradient of 4%-11% DCM/MeOH to afford title compounds **12a-12u, 16a-16c** and **21a-21f.** (yield, 45%-91%) as a white or yellow solid.

***N-(2-fluoro-5-nitrophenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine* (7).** Compound **7** was prepared according to general procedure A on a 19.0 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (7.57 g, 17.71 mmol, 93% yield). $[M + H]^+$: 428.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.72 (dd, J = 6.7, 2.8 Hz, 1H), 8.41 (s, 1H), 8.16 – 8.08 (m, 1H), 8.02 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 4.0 Hz, 1H), 7.62 (t, J = 9.5 Hz, 1H), 7.46 (d, J = 8.2 Hz, 2H), 7.13 (d, J = 4.0 Hz, 1H), 2.37 (s, 3H).

N*^{4}*-(2-fluoro-5-nitrophenyl)pyrimidine-2,4-diamine (17a). Compound **17a** was prepared according to general procedure A on a 10.0 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (1.67 g, 6.72 mmol, 67% yield). $[M + H]^+$: 250.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.57 (d, *J* = 2.8 Hz, 1H), 8.40 (s, 1H), 7.93 (dd, *J* = 8.9, 2.8 Hz, 1H), 7.87 (d, *J* = 5.6 Hz, 1H), 7.20 (d, *J* = 8.9 Hz, 1H), 6.27 – 6.11 (m, 3H), 3.74 (t, *J* = 4.4 Hz, 4H), 3.01 (t, *J* = 4.5 Hz, 4H).

N-(2-fluoro-5-nitrophenyl)-1H-pyrrolo[2,3-b]pyridin-4-amine (17b). Compound **17b** was prepared according to general procedure B on a 10 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (2.30 g, 0.275 mmol, 85% yield). $[M + H]^+$: 273.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.53 (s, 1H), 8.92 (s, 1H), 8.13 (dd, *J* = 7.1, 2.9 Hz, 1H), 8.04 – 7.94 (m, 2H), 7.63 – 7.57 (m, 1H), 7.30 – 7.28 (m, 1H), 6.55 – 6.45 (m, 2H).

5-fluoro-N-(2-fluoro-5-nitrophenyl)-1H-pyrrolo[2,3-b]pyridin-4-amine (17c). Compound **17c** was prepared according to general procedure B on a 10 mmol scale. Purification by column chromatography (4%

MeOH/DCM) afforded the title compound (2.36 g, 8.13 mmol, 81% yield). $[M + H]^+$: 291.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.71 (s, 1H), 8.98 (s, 1H), 8.15 (d, *J* = 4.3 Hz, 1H), 7.96 – 7.90 (m, 1H), 7.84 – 7.80 (m, 1H), 7.58 – 7.51 (m, 1H), 7.37 (t, *J* = 3.0 Hz, 1H), 6.22 (dd, *J* = 3.5, 1.9 Hz, 1H).

5-chloro-N-(2-fluoro-5-nitrophenyl)-1H-pyrrolo[2,3-b]pyridin-4-amine (17d). Compound **17d** was prepared according to general procedure B on a 4.0 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (450.8 mg, 1.47 mmol, 37% yield). $[M + H]^+$: 307.7. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.79 (s, 1H), 8.74 (s, 1H), 8.16 (s, 1H), 8.05 – 7.82 (m, 2H), 7.56 (t, *J* = 9.7 Hz, 1H), 7.33 – 7.24 (m, 1H), 5.79 (s, 1H).

N-(2-fluoro-5-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (17e). Compound **17e** was prepared according to general procedure A on a 24.0 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (6.28 g, 22.9 mmol, 95% yield). $[M + H]^+$: 275.2 ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.79 (s, 1H), 10.27 (s, 1H), 8.94 (dd, *J* = 6.7, 2.6 Hz, 1H), 8.41 (d, *J* = 13.0 Hz, 2H), 8.17 – 8.10 (m, 1H), 7.69 – 7.61 (m, 1H).

N-(2-fluoro-5-nitrophenyl)thieno[2,3-d]pyrimidin-4-amine (17f). Compound **17f** was prepared according to general procedure A on a 12.3 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (2.39 g, 8.24mmol, 67% yield). $[M + H]^+$: 291.0. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.68 (dd, *J* = 6.6, 2.9 Hz, 1H), 8.50 (s, 1H), 8.20 – 8.16 (m, 1H), 7.86 (d, *J* = 6.0 Hz, 1H), 7.80 (d, *J* = 6.0 Hz, 1H), 7.65 (t, *J* = 9.5 Hz, 1H).

N^1,N^1-dimethyl-N^2-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,2,4-triamine(9b). Compound **9b** was prepared according to general procedure D on a 4.68 mmol scale. Purification by column chromatography (2.5% MeOH/DCM) afforded the title compound (1.05 g, 2.49 mmol, 53% yield). $[M + H]^+$: 423.2 ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.33 (s, 1H), 8.01 (d, J = 1.8 Hz, 1H), 7.99 (d, J = 1.8 Hz, 1H), 7.64 (d, J = 4.0 Hz, 1H), 7.46 (t, J = 1.2 Hz, 1H), 7.45 – 7.42 (m, 1H), 7.15 (d, J = 2.6 Hz, 1H), 6.92 (d, J = 8.5 Hz, 1H), 6.71 (d, J = 4.0 Hz, 1H), 6.37 (dd, J = 8.4, 2.6 Hz, 1H), 4.88 (s, 2H), 2.48 (s, 6H), 2.36 (s, 3H).

N^1-(methoxymethyl)-N^1-methyl-N^2-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,2,4-triamine (9c).

Compound **9c** was prepared according to general procedure D on a 4.0 mmol scale. Purification by column chromatography (2.5% MeOH/DCM) afforded the title compound (1.13 g, 2.50 mmol, 62% yield). $[M + H]^+$: 453.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (s, 1H), 8.42 (s, 1H), 8.00 (d, J = 8.1 Hz, 2H), 7.74 – 7.69 (m, 2H), 7.45 (d, J = 8.1 Hz, 2H), 7.02 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 4.1 Hz, 1H), 6.31 (dd, J = 8.4, 2.6 Hz, 1H), 5.22 (s, 2H), 3.27 (t, J = 5.2 Hz, 2H), 3.06 (s, 3H), 2.85 (t, J = 5.2 Hz, 2H), 2.59 (s, 3H), 2.36 (s, 3H).

N^1-((dimethylamino)methyl)-N^1-methyl-N^2-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,2,4-triamine (9d). Compound **9d** was prepared according to general procedure D on a 4.0 mmol scale. Purification by column chromatography (8.0% MeOH/DCM) afforded the title compound (1.04 g, 2.24 mmol, 56% yield). $[M + H]^+$: 466.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.31 (s, 1H), 8.06 – 7.94 (m, 2H), 7.65 (d, J = 4.0 Hz, 1H), 7.45 (d, J = 8.2 Hz, 2H), 7.16 – 7.07 (m, 2H), 6.95 (d, J = 8.5 Hz, 1H), 6.39 (dd, J = 8.5, 2.6 Hz, 1H), 4.99 (s, 2H), 3.08 (d, J = 7.3 Hz, 2H), 3.03 (d, J = 7.3 Hz, 3H), 2.59 (s, 5H), 2.44 (s, 3H), 2.37 (s, 3H).

6-(pyrrolidin-1-yl)-N^1-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine(9e). Compound **9e** was prepared according to general procedure D on a 2.98 mmol scale. Purification by column chromatography (2.5% MeOH/DCM) afforded the title compound (828.9 mg, 1.85 mmol, 62% yield). $[M + H]^+$: 449.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (t, *J* = 5.1 Hz, 1H), 8.25 (t, *J* = 5.3 Hz, 1H), 7.96 (d, *J* = 7.9 Hz, 2H), 7.55 (d, *J* = 5.9 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 2H), 6.80 – 6.72 (m, 2H), 6.45 (d, *J* = 24.6 Hz, 2H), 4.73 (s, 2H), 2.89 (s, 4H), 2.34 (d, *J* = 5.6 Hz, 3H), 1.68 (s, 4H).

6-morpholino-N^1-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9f). Compound **9f** was prepared according to general procedure D on a 4.0 mmol scale. Purification by column chromatography (2.5% MeOH/DCM) afforded the title compound (1.35 g, 2.90 mmol, 72% yield). $[M + H]^+$: 465.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.36 (s, 1H), 8.03 – 7.96 (m, 2H), 7.65 (d, *J* = 4.0 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 2.5 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.63 (d, *J* = 4.0 Hz, 1H), 6.35 (dd, *J* = 8.4, 2.6 Hz, 1H), 4.99 (s, 2H), 3.49 (t, *J* = 4.3 Hz, 4H), 2.64 (t, *J* = 4.5 Hz, 4H), 2.36 (s, 3H).

6-(piperazin-1-yl)-N^1-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9g). Compound **9g** was prepared according to general procedure D on a 15.8 mmol scale. Purification by column chromatography (10%

MeOH/DCM) afforded the title compound (3.45 g, 7.45 mmol, 47% yield). $[M + H]^+$: 464.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.37 (s, 1H), 8.03 – 7.97 (m, 2H), 7.65 (d, *J* = 3.9 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 2.5 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.68 (d, *J* = 4.1 Hz, 1H), 6.32 (dd, *J* = 8.4, 2.6 Hz, 1H), 5.01 (s, 2H), 3.25 (s, 4H), 2.60 (t, *J* = 4.9 Hz, 4H), 2.36 (s, 3H), 1.40 (s, 1H).

(R)-6-(3-methylpiperazin-1-yl)-N1-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9h)

Compound **9h** was prepared according to general procedure D on a 4.68 mmol scale. Purification by column chromatography (10% MeOH/DCM) afforded the title compound (1.21 g, 2.53 mmol, 54% yield). $[M + H]^+$: 478.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.58 (s, 1H), 8.28 (s, 1H), 7.73 – 7.67 (m, 3H), 7.44–7.25 (m, 2H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.45 (dd, *J* = 3.5, 1.8 Hz, 1H), 6.26 (dd, *J* = 8.4, 2.6 Hz, 1H), 4.99 (s, 2H), 4.13 (s, 1H), 3.79 (d, *J* = 12.9 Hz, 1H), 3.27 – 3.12 (m, 1H), 2.84 – 2.72 (m, 3H), 2.62 (dd, *J* = 11.6, 8.0 Hz, 2H), 2.41 (s, 3H), 1.25 (d, *J* = 6.7 Hz, 3H).

(S)-6-(3-methylpiperazin-1-yl)-N1-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9i)

Compound **9i** was prepared according to general procedure D on a 3.37 mmol scale. Purification by column chromatography (10% MeOH/DCM) afforded the title compound (1.21 g, 2.54 mmol, 75% yield). $[M + H]^+$: 478.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H), 8.56 (s, 1H), 8.28 (s, 1H), 7.73 – 7.67 (m, 3H), 7.44–7.25 (m, 2H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.44 (dd, *J* = 3.5, 1.8 Hz, 1H), 6.27 (dd, *J* = 8.4, 2.6 Hz, 1H), 4.96 (s, 2H), 4.14 (s, 1H), 3.83 – 3.75 (m, 1H), 3.24 – 3.15 (m, 1H), 2.84 – 2.73 (m, 3H), 2.67 – 2.58 (m, 2H), 2.41 (s, 3H), 1.26 (d, *J* = 6.7 Hz, 3H).

6-(2,5-diazabicyclo[2.2.1]heptan-2-yl)-N1-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9j)

Compound **9j** was prepared according to general procedure D on a 1.58 mmol scale. Purification by column chromatography (10% MeOH/DCM) afforded the title compound (330.3 mg, 0.695 mmol, 44% yield). $[M + H]^+$: 476.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (s, 1H), 8.23 (s, 1H), 7.98 (d, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 4.0 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 6.71 – 6.60 (m, 2H), 6.44 (dd, *J* = 8.6, 2.7 Hz, 2H), 4.71 (s, 2H), 4.15 (d, *J* = 22.4 Hz, 1H), 3.92 (s, 1H), 3.29 – 3.20 (m, 1H), 3.17 (d, *J* = 5.2 Hz, 1H), 3.10 (t, *J* = 7.2 Hz, 1H), 2.86 – 2.73 (m, 1H), 2.36 (s, 3H), 1.66 (s, 2H), 1.30 (s, 1H).

6-(4-methylpiperazin-1-yl)-N^1-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9k). Compound **9k** was prepared according to general procedure D on a 8.47 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (2.33 g, 4.88 mmol, 58% yield). $[M + H]^+$: 478.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.37 (s, 1H), 8.08 – 7.96 (m, 2H), 7.68 (d, *J* = 4.0 Hz, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 2.6 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.75 (d, *J* = 3.9 Hz, 1H), 6.36 (dd, *J* = 8.5, 2.6 Hz, 1H), 5.11 (s, 2H), 3.27 (s, 4H), 3.02 – 2.72 (m, 7H), 2.36 (s, 3H).

6-(4-ethylpiperazin-1-yl)-N^1-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9l). Compound **9l** was prepared according to general procedure D on a 1.82 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (476.5 mg, 0.97 mmol, 53% yield). $[M + H]^+$: 492.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.36 (s, 1H), 8.03 – 7.97 (m, 2H), 7.65 (d, *J* = 4.0 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 2.6 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.51 (s, 1H), 6.35 (dd, *J* = 8.4, 2.6 Hz, 1H), 4.98 (s, 2H), 2.68 (t, *J* = 4.7 Hz, 4H), 2.36 (s, 3H), 2.32 – 2.20 (m, 4H), 1.24 (d, *J* = 3.9 Hz, 2H), 0.97 (t, *J* = 7.1 Hz, 3H).

6-(4-morpholinopiperidin-1-yl)-N^1-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9m). Compound **9m** was prepared according to general procedure D on a 4.72 mmol scale. Purification by column chromatography (1% MeOH/DCM) afforded the title compound (1.16 g, 2.12 mmol, 45% yield). $[M + H]^+$: 548.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.88 (d, *J* = 5.9 Hz, 1H), 8.36 (t, *J* = 5.1 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 2H), 7.66 (q, *J* = 4.0 Hz, 1H), 7.46 (d, *J* = 7.9 Hz, 2H), 7.28 (d, *J* = 6.2 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.52 (s, 1H), 6.35 (d, *J* = 7.9 Hz, 1H), 4.95 (s, 2H), 3.55 (s, 4H), 2.81 (s, 2H), 2.51 (s, 2H), 2.43 – 2.28 (m, 7H), 2.10 (s, 1H), 1.67 (d, *J* = 9.6 Hz, 2H), 1.25 (s, 2H).

6-(4-methyl-1,4-diazepan-1-yl)-N^1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9n). Compound **9n** was prepared according to general procedure D on a 3.14 mmol scale. Purification by column chromatography (10% MeOH/DCM) afforded the title compound (219.2 mg, 0.65 mmol, 21% yield). $[M + H]^+$: 338.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.79 (d, *J* = 6.5 Hz, 1H), 8.82 – 8.50 (m, 1H), 8.23 (d, *J* = 4.3 Hz, 1H), 7.40 (t, *J* = 4.8 Hz, 1H), 7.29 – 7.16 (m, 1H), 6.93 (d, *J* = 8.7 Hz, 1H), 6.71 – 6.59 (m, 1H), 6.31 (d, *J* = 8.5 Hz, 1H),

4.97 (s, 2H), 3.31 – 3.08 (m, 6H), 2.95 – 2.89 (m, 2H), 2.79 – 2.60 (m, 3H), 2.17 – 1.90 (m, 2H).

N^1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-6-((tetrahydro-2H-pyran-4-yl)oxy)benzene-1,3-diamine (9o). Compound **9o** was prepared according to general procedure D on a 2.5 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (565.8 mg, 1.74 mmol, 70% yield). $[M + H]^+$: 326.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.25 (s, 1H), 8.19 (s, 1H), 7.32 (d, *J* = 2.7 Hz, 1H), 7.19 – 7.17 (m, 1H), 6.82 (d, *J* = 8.6 Hz, 1H), 6.46 – 6.45 (m, 1H), 6.31 (dd, *J* = 8.6, 2.7 Hz, 1H), 4.81 (s, 2H), 4.21 – 4.12 (m, 1H), 3.77 – 3.70 (m, 2H), 1.86 – 1.78 (m, 2H), 1.60 – 1.49 (m, 2H), 1.36 – 1.21 (m, 2H).

6-((1-methylpiperidin-4-yl)oxy)-N^1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (9p). Compound **9p** was prepared according to general procedure D on a 2.5 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (544.5 mg, 1.61 mmol, 64% yield). $[M + H]^+$: 339.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.24 (s, 1H), 7.99 (s, 1H), 7.57 (d, *J* = 4.0 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 6.64 – 6.51 (m, 3H), 6.45 (dd, *J* = 8.4, 2.4 Hz, 1H), 3.24 (s, 1H), 3.07 (s, 2H), 2.69 – 2.66 (m, 2H), 2.36 (s, 3H), 1.89 (d, *J* = 9.5 Hz, 2H), 1.59 – 1.42 (m, 2H).

N^4-(5-amino-2-morpholinophenyl)pyrimidine-2,4-diamine (19a). Compound **19a** was prepared according to general procedure D on a 5.3 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (1.20 g, 4.18 mmol, 79% yield). $[M + H]^+$: 287.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.15 (s, 1H), 7.81 (d, *J* = 6.7 Hz, 1H), 7.31 (s, 2H), 7.06 (d, *J* = 2.6 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.39 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.25 (d, *J* = 6.6 Hz, 1H), 4.97 (s, 2H), 3.72 – 3.57 (m, 4H), 2.68 (t, *J* = 4.5 Hz, 4H).

6-morpholino-N^1-(1H-pyrrolo[2,3-b]pyridin-4-yl)benzene-1,3-diamine (19b). Compound **19b** was prepared according to general procedure D on a 3.0 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (745.2 mg, 2.41 mmol, 80% yield). $[M + H]^+$: 310.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.37 (s, 1H), 7.95 – 7.83 (m, 2H), 7.54 (s, 1H), 7.36 (d, *J* = 8.3 Hz, 1H), 7.22 – 7.20 (m, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.45 (d, *J* = 5.4 Hz, 1H), 6.35 (d, *J* = 3.4 Hz, 1H), 4.96 (s, 2H), 3.53 (s, 4H), 2.81 (s, 4H).

N^1-(5-fluoro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-morpholinobenzene-1,3-diamine (19c). Compound **19c** was prepared according to general procedure D on a 3.0 mmol scale. Purification by column chromatography (4%

MeOH/DCM) afforded the title compound (863.5 mg, 2.64 mmol, 88% yield). $[M + H]^+$: 328.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.39 (s, 1H), 8.03 (d, *J* = 4.2 Hz, 1H), 7.48 (d, *J* = 1.9 Hz, 1H), 7.20 (t, *J* = 3.0 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 1H), 6.36 – 6.22 (m, 2H), 5.96 (dd, *J* = 3.5, 2.0 Hz, 1H), 4.84 (s, 2H), 3.44 (s, 4H), 2.70 (s, 4H).

N1-(5-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-morpholinobenzene-1,3-diamine (19d). Compound **19d** was prepared according to general procedure D on a 1.3 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (322.5 mg, 0.935 mmol, 72% yield). $[M + H]^+$: 345.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.49 (s, 1H), 8.06 (s, 1H), 7.51 (s, 1H), 7.16 (s, 1H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.42 (s, 1H), 6.34 (d, *J* = 8.3 Hz, 1H), 5.75 (s, 1H), 4.87 (s, 2H), 3.45 (s, 4H), 2.69 (s, 4H).

6-morpholino-N^1-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)benzene-1,3-diamine (19e). Compound **19e** was prepared according to general procedure D on a 3.0 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (462.0 mg, 1.48 mmol, 49% yield). $[M + H]^+$: 312.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.66 – 13.41 (m, 1H), 9.19 (s, 1H), 8.33 (s, 1H), 7.81 (s, 1H), 7.28 (s, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.42 (dd, *J* = 8.4, 2.6 Hz, 1H), 5.02 (s, 2H), 3.58 (s, *J* = 4.3 Hz, 4H), 2.70 (t, *J* = 4.5 Hz, 4H).

6-morpholino-N^1-(thieno[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (19f). Compound **19f** was prepared according to general procedure D on a 3.0 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (448.1 mg, 1.37 mmol, 46% yield). $[M + H]^+$: 328.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.50 (s, 1H), 7.74 (d, *J* = 6.0 Hz, 1H), 7.58 (d, *J* = 2.6 Hz, 1H), 7.55 (d, *J* = 6.0 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 1H), 6.35 (dd, *J* = 8.4, 2.6 Hz, 1H), 5.04 (s, 2H), 3.71 – 3.63 (m, 4H), 2.75 – 2.68 (m, 4H).

4-iodo-N^1,N^1-dimethyl-N^2-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,2-diamine(10b). Compound **10b** was prepared according to general procedure E on a 2.4 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (559.7 mg, 1.05 mmol, 44% yield). $[M + H]^+$: 534.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.17 (s, 1H), 8.33 (s, 1H), 8.02 (d, *J* = 8.3 Hz, 2H), 7.92 (d, *J* = 2.0 Hz, 1H), 7.67 (d, *J* = 4.0 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 3H), 6.93 – 6.88 (m, 2H), 2.60 (s, 6H), 2.37 (s, 3H).

4-iodo-N^1-(2-methoxyethyl)-N^1-methyl-N^2-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,2-diamine (10c). Compound **10c** was prepared according to general procedure E on a 0.45 mmol scale. Purification by

column chromatography (2% MeOH/DCM) afforded the title compound (179.5 mg, 0.311 mmol, 69% yield). $[M + H]^+$: 578.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.43 (s, 1H), 8.39 (d, *J* = 2.1 Hz, 1H), 8.03 – 7.98 (m, 2H), 7.74 (d, *J* = 4.0 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 2H), 7.42 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.03 (d, *J* = 8.5 Hz, 1H), 6.84 (d, *J* = 4.0 Hz, 1H), 3.35 (t, *J* = 5.5 Hz, 2H), 3.05 (s, 3H), 2.90 (t, *J* = 5.5 Hz, 2H), 2.68 (s, 3H), 2.36 (s, 3H).

N^1-(2-(dimethylamino)ethyl)-4-iodo-N^1-methyl-N^2-(7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,2-diamine (10d). Compound **10d** was prepared according to general procedure E on a 0.70 mmol scale. Purification by column chromatography (6% MeOH/DCM) afforded the title compound (309.8 mg, 0.525 mmol, 75% yield). $[M + H]^+$: 591.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.47 (s, 1H), 8.35 (s, 1H), 8.09 – 7.93 (m, 3H), 7.71 (d, *J* = 4.0 Hz, 1H), 7.50 – 7.39 (m, 3H), 7.23 (s, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 2.76 (t, *J* = 6.0 Hz, 2H), 2.57 (s, 3H), 2.37 (s, 3H), 2.24 (t, *J* = 6.0 Hz, 2H), 1.98 (s, 6H).

N-(5-iodo-2-(pyrrolidin-1-yl)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10e). Compound **10e** was prepared according to general procedure E on a 0.56 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (219.7 mg, 0.393 mmol, 70% yield). $[M + H]^+$: 560.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.33 (d, *J* = 18.4 Hz, 1H), 8.22 (d, *J* = 10.8 Hz, 1H), 8.02 – 7.98 (m, 2H), 7.61 – 7.54 (m, 1H), 7.46 (s, 1H), 7.43 (s, 1H), 7.41 – 7.38 (m, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 6.85 – 6.70 (m, 1H), 6.63 (d, *J* = 9.3 Hz, 1H), 3.16 – 3.11 (m, 4H), 2.37 (s, 3H), 1.78 – 1.65 (m, 4H).

N-(5-iodo-2-morpholinophenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10f). Compound **10f** was prepared according to general procedure E on a 1.60 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (571.1 mg, 0.993 mmol, 62% yield). $[M + H]^+$: 576.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (s, 1H), 8.39 (s, 1H), 8.18 (d, *J* = 2.1 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 2H), 7.68 (d, *J* = 4.0 Hz, 1H), 7.57 – 7.40 (m, 3H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J* = 4.0 Hz, 1H), 3.50 (t, *J* = 4.3 Hz, 4H), 2.77 (t, *J* = 4.3 Hz, 4H), 2.36 (s, 3H).

N-(5-iodo-2-(piperazin-1-yl)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10g). Compound **10g** was prepared according to general procedure E on a 7.44 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (2.67 g, 4.65 mmol, 63% yield). $[M + H]^+$: 575.1. ^{1}H NMR (300 MHz,

DMSO-d_6) δ 8.98 (s, 1H), 8.41 (s, 1H), 8.28 (d, *J* = 2.1 Hz, 1H), 8.05 – 7.97 (m, 2H), 7.69 (d, *J* = 4.0 Hz, 1H), 7.48 – 7.43 (m, 3H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 4.1 Hz, 1H), 3.27 (s, 4H), 2.74 (d, *J* = 5.4 Hz, 4H), 2.36 (s, 3H), 1.39 (s, 1H).

(R)-N-(5-iodo-2-(3-methylpiperazin-1-yl)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10h). Compound **10h** was prepared according to general procedure E on a 0.92 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (282.3 mg, 0.48 mmol, 52% yield). $[M + H]^+$: 589.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ10.04 (s, 1H), 8.60 – 8.53 (m, 2H), 8.30 (s, 1H), 7.53 – 7.41 (m, 2H), 7.27 – 7.15 (m, 3H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.52 (dd, *J* = 3.5, 1.8 Hz, 1H), 4.12 – 4.08 (m, 2H), 3.81 (d, *J* = 13.1 Hz, 1H), 3.17 (t, *J* = 12.1 Hz, 1H), 2.98 (d, *J* = 11.1 Hz, 1H), 2.81 (d, *J* = 11.7 Hz, 1H), 2.71 (dd, *J* = 11.7, 3.6 Hz, 2H), 2.44 (s, 3H), 1.21 (d, *J* = 6.8 Hz, 3H).

(S)-N-(5-iodo-2-(3-methylpiperazin-1-yl)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10i). Compound **10i** was prepared according to general procedure E on a 0.82 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (217.6 mg, 0.37 mmol, 45% yield). $[M + H]^+$: 589.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.58 (d, *J* = 2.1 Hz, 2H), 8.30 (s, 1H), 7.53 – 7.41 (m, 2H), 7.27 – 7.15 (m, 3H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.52 (dd, *J* = 3.5, 1.8 Hz, 1H), 4.12 – 4.08 (m, 2H), 3.80 (d, *J* = 12.9 Hz, 1H), 3.17 (t, *J* = 12.4 Hz, 1H), 2.98 (d, *J* = 11.3 Hz, 1H), 2.81 (d, *J* = 11.7 Hz, 1H), 2.76 – 2.66 (m, 2H), 2.44 (s, 3H), 1.23 – 1.19 (m, 3H).

N-(2-(2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-iodophenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10j). Compound **10j** was prepared according to general procedure E on a 1.16 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (451.5 mg, 4 0.77 mmol, 66% yield). $[M + H]^+$: 587.4. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.12 (s, 1H), 7.98 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 2.3 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 2H), 6.71 – 6.60 (m, 2H), 6.70 (dd, *J* = 8.6, 2.7 Hz, 2H), 4.35 (d, *J* = 16.4 Hz, 1H), 4.21 (d, *J* = 30.3 Hz, 1H), 3.54 (d, *J* = 9.5 Hz, 1H), 3.38 (d, *J* = 10.3 Hz, 1H), 3.24 – 3.14 (m, 1H), 2.88 (d, *J* = 9.2 Hz, 1H), 2.39 (s, 3H), 1.80 (t, *J* = 7.0 Hz, 1H), 1.73 (t, *J* = 7.9 Hz, 2H).

N-(5-iodo-2-(4-methylpiperazin-1-yl)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10k). Compound **10k** was prepared according to general procedure E on a 4.40 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (1.35 g, 2.29 mmol, 52% yield). $[M + H]^+$: 589.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.38 (s, 1H), 8.12 (d, *J* = 2.1 Hz, 1H), 8.05 – 7.97 (m, 2H), 7.67 (d, *J* = 4.0 Hz, 1H), 7.51 – 7.41 (m, 3H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.60 (s, 1H), 2.75 (t, *J* = 4.8 Hz, 4H), 2.36 (s, 3H), 2.13 (s, 4H), 2.03 (s, 3H).

N-(2-(4-ethylpiperazin-1-yl)-5-iodophenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10l). Compound **10l** was prepared according to general procedure E on a 1.05 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (273.9 mg, 0.44 mmol, 42% yield). $[M + Na]^+$: 625.5. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.40 (s, 1H), 8.22 – 8.14 (m, 1H), 8.05 – 7.98 (m, 2H), 7.70 (d, *J* = 4.0 Hz, 1H), 7.53 – 7.41 (m, 3H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.74 (s, 1H), 3.93 – 3.05 (m, 6H), 2.87 (s, 4H), 2.36 (s, 3H), 1.01 (t, *J* = 7.2 Hz, 3H).

N-(5-iodo-2-(4-morpholinopiperidin-1-yl)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10m). Compound **10m** was prepared according to general procedure E on a 2.24 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (769.9 mg, 1.17 mmol, 52% yield). $[M + H]^+$: 659.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.82 (d, *J* = 5.9 Hz, 1H), 8.66 (t, *J* = 5.0 Hz, 1H), 8.46 (d, *J* = 8.2 Hz, 2H), 7.39 (s, 1H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.03 (d, *J* =6.1 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.52 – 6.35 (m, 2H), 3.57 (s, 4H), 3.11 (s, 2H), 3.86 – 2.69 (m, 7H), 2.60 (s, 2H), 2.52 – 2.34 (m, 1H), 1.85 – 1.82 (m, 2H), 1.63 (s, 2H).

N-(5-iodo-2-(4-methyl-1,4-diazepan-1-yl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10n). Compound **10n** was prepared according to general procedure E on a 0.65 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (128.6 mg, 0.287 mmol, 44% yield). $[M + H]^+$: 449.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 8.26 (s, 1H), 7.52 (d, *J* = 7.7 Hz, 1H), 7.46 – 7.41 (m, 1H), 7.27 (t, *J* = 2.9 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.57 (d, *J* = 3.4 Hz, 1H), 3.25 – 3.17 (m, 4H), 3.15 – 3.03 (m, 3H), 2.71 (s, 3H), 2.30 (s, 1H), 2.06 – 1.94 (m, 2H).

N-(5-iodo-2-((tetrahydro-2H-pyran-4-yl)oxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10o). Compound **10o** was prepared according to general procedure E on a 0.56 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (162.2 mg, 0.372 mmol, 67% yield). $[M + H]^+$: 437.0. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 9.15 (d, *J* = 2.2 Hz, 1H), 8.54 (s, 1H), 8.41 (s, 1H), 7.60 (d, *J* = 2.4 Hz, 1H), 7.33 – 7.31 (m, 2H), 7.05 (d, *J* = 8.7 Hz, 1H), 4.75 – 4.68 (m, 1H), 3.90 (s, 2H), 3.48 – 3.43 (m, 2H), 2.06 – 2.03 (m, 2H), 1.84 – 1.74 (m, 2H).

N-(5-iodo-2-((1-methylpiperidin-4-yl)oxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (10p). Compound **10p** was prepared according to general procedure D on a 0.68 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (170.2 mg, 0.379 mmol, 56% yield). $[M + H]^+$: 450.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.47 (s, 1H), 8.49 (s, 1H), 8.12 (d, *J* = 8.3 Hz, 2H), 7.67 (d, *J* = 2.4 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 2H), 6.62 – 6.59 (m, 1H), 3.35 (s, 1H), 3.22 – 3.18 (m, 2H), 2.72 (s, 2H), 2.41 (s, 3H), 1.95 - 1.89 (m, 2H), 1.71 – 1.64 (m, 2H).

N^4-(5-iodo-2-morpholinophenyl)pyrimidine-2,4-diamine (20a). Compound **20a** was prepared according to general procedure E on a 1.20 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (293.8 mg, 0.740 mmol, 62% yield). $[M + H]^+$: 398.0. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, *J* = 2.1 Hz, 1H), 8.11 (s, 1H), 7.83 (d, *J* = 5.7 Hz, 1H), 7.35 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.22 (s, 2H), 6.15 (d, *J* = 5.7 Hz, 1H), 3.82 – 3.66 (m, 4H), 2.88 – 2.72 (m, 4H).

N-(5-iodo-2-morpholinophenyl)-1H-pyrrolo[2,3-b]pyridin-4-amine (20b). Compound **20b** was prepared according to general procedure E on a 1.77 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (399.5 mg, 0.951 mmol, 54% yield). $[M + H]^+$: 421.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.41 (s, 1H), 7.99 – 7.87 (m, 2H), 7.54 (d, *J* = 2.0 Hz, 1H), 7.40 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.22 – 7.20 (m, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.50 (d, *J* = 5.4 Hz, 1H), 6.45 (d, *J* = 3.4 Hz, 1H), 3.56 (t, *J* = 4.4 Hz, 4H), 2.85 (t, *J* = 4.5 Hz, 4H).

5-fluoro-N-(5-iodo-2-morpholinophenyl)-1H-pyrrolo[2,3-b]pyridin-4-amine (20c). Compound **20c** was prepared according to general procedure E on a 1.28 mmol scale. Purification by column chromatography (5%

MeOH/DCM) afforded the title compound (306.2 mg, 0.699 mmol, 55% yield). $[M + H]^+$: 439.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.48 (s, 1H), 8.05 (d, *J* = 4.3 Hz, 1H), 7.95 (d, *J* = 1.7 Hz, 1H), 7.37 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.32 – 7.20 (m, 2H), 6.85 (d, *J* = 8.4 Hz, 1H), 5.93 – 5.91 (m, 1H), 3.30 (t, *J* = 4.3 Hz, 4H), 2.79 (t, *J* = 4.5 Hz, 4H).

5-chloro-N-(5-iodo-2-morpholinophenyl)-1H-pyrrolo[2,3-b]pyridin-4-amine (20d). Compound **20d** was prepared according to general procedure E on a 0.79 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (195.2 mg, 0.43 mmol, 54% yield). $[M + H]^+$: 455.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.56 (s, 1H), 8.07 (s, 1H), 7.83 (s, 1H), 7.43 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.18 (t, *J* = 3.0 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 5.53 (dd, *J* = 3.5, 2.0 Hz, 1H), 3.27 (t, *J* = 4.5 Hz, 4H), 2.77 (t, *J* = 4.5 Hz, 4H).

N-(5-iodo-2-morpholinophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (20e). Compound **20e** was prepared according to general procedure E on a 1.18 mmol scale. Purification by column chromatography (6% MeOH/DCM) afforded the title compound (227 mg, 0.538 mmol, 46% yield). $[M + H]^+$: 423.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.62 (s, 1H), 9.35 (s, 1H), 8.36 (s, 1H), 8.20 (s, 1H), 8.02 – 7.99 (m, 1H), 7.54 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 1H), 3.67 – 3.50 (m, 4H), 2.83 (t, *J* = 4.5 Hz, 4H).

N-(5-iodo-2-morpholinophenyl)thieno[2,3-d]pyrimidin-4-amine (20f). Compound **20f** was prepared according to general procedure E on a 2.0 mmol scale. Purification by column chromatography (6% MeOH/DCM) afforded the title compound (326.1 mg, 0.744 mmol, 37% yield). $[M + Na]^+$: 461.3. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.52 (s, 1H), 8.38 (d, *J* = 2.1 Hz, 1H), 7.75 (d, *J* = 6.0 Hz, 1H), 7.68 (d, *J* = 6.1 Hz, 1H), 7.50 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 3.69 – 3.59 (m, 4H), 2.8 – 2.78 (m, 4H).

4-iodo-N^1,N^1-dimethyl-N^2-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,2-diamine (11b). Compound **11b** was prepared according to general procedure F on a 1.0 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (299.4 mg, 0.790 mmol, 79% yield). $[M + H]^+$: 380.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.81 (s, 1H), 8.58 (s, 1H), 8.41 (d, *J* = 2.1 Hz, 1H), 8.28 (s, 1H), 7.39 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.26–7.24 (m, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.62–6.60 (m, 1H), 2.65 (s, 6H).

4-iodo-N^1-(2-methoxyethyl)-N^1-methyl-N^2-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,2-diamine (11c). Compound **11c** was prepared according to general procedure F on a 0.28 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (72.8 mg, 0.172 mmol, 61% yield). $[M + H]^+$: 424.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.88 (s, 1H), 8.93 (d, *J* = 2.1 Hz, 1H), 8.82 (s, 1H), 8.37 (s, 1H), 7.39 – 7.29 (m, 2H), 7.10 (d, *J* = 8.3 Hz, 1H), 6.54 (dd, *J* = 3.5, 1.9 Hz, 1H), 3.42 (t, *J* = 5.2 Hz, 2H), 3.19 (s, 3H), 2.94 (t, *J* = 5.3 Hz, 2H), 2.70 (s, 3H).

N1-(2-(dimethylamino)ethyl)-4-iodo-N^1-methyl-N^2-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,2-diamine (11d). Compound **11d** was prepared according to general procedure F on a 0.46 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (126.0 mg, 0.289 mmol, 63% yield). $[M + H]^+$: 437.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.85 (s, 1H), 9.27 (s, 1H), 8.91 (d, *J* = 2.1 Hz, 1H), 8.34 (s, 1H), 7.35 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.30 (dd, *J* = 3.4, 2.4 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 6.52 (dd, *J* = 3.5, 1.8 Hz, 1H), 2.89 (t, *J* = 6.0 Hz, 2H), 2.68 (s, 3H), 2.27 (t, *J* = 6.0 Hz, 2H), 2.03 (s, 6H).

N-(5-iodo-2-(pyrrolidin-1-yl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11e). Compound **11e** was prepared according to general procedure F on a 0.45 mmol scale. Purification by column chromatography (3% MeOH/DCM) afforded the title compound (129.3 mg, 0.32 mmol, 71% yield). $[M + Na]^+$: 428.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.60 (s, 1H), 8.83 (s, 1H), 8.09 (d, *J* = 9.0 Hz, 1H), 7.54 – 7.35 (m, 1H), 7.29 – 7.02 (m, 2H), 6.88 – 6.73 (m, 1H), 6.27 (d, *J* = 42.9 Hz, 1H), 3.19 (d, *J* = 3.7 Hz, 4H), 1.82 – 1.68 (m, 4H).

N-(5-iodo-2-morpholinophenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11f). Compound **11f** was prepared according to general procedure F on a 0.88 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (234.9 mg, 0.558 mmol, 62% yield). $[M + H]^+$: 422.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.88 (s, 1H), 8.73 (d, *J* = 2.0 Hz, 1H), 8.56 (s, 1H), 8.33 (s, 1H), 7.40 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.33 – 7.28 (m, 1H), 7.03 (d, *J* = 8.3 Hz, 1H), 6.60 – 6.58 (m, 1H), 3.79 – 3.69 (m, 4H), 2.88 – 2.79 (m, 4H).

N-(5-iodo-2-(piperazin-1-yl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11g). Compound **11g** was prepared according to general procedure F on a 3.55 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (1.1 g, 2.62 mmol, 74% yield). $[M + Na]^+$: 443.3. ^{1}H NMR (300 MHz,

DMSO-d_6) δ 11.91 (s, 1H), 8.75 (d, *J* = 2.1 Hz, 1H), 8.52 (s, 1H), 8.34 (s, 1H), 7.39 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.33 – 7.31 (m, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 6.60 – 6.58 (m, 1H), 5.28 (s, 1H), 3.05 – 2.92 (m, 4H), 2.90 – 2.76 (m, 4H).

(R)-N-(5-iodo-2-(3-methylpiperazin-1-yl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11h) Compound **11h** was prepared according to general procedure F on a 0.88 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (247.6 mg, 0.57 mmol, 65% yield). $[M + H]^+$: 435.3. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.73 (d, *J* = 2.0 Hz, 1H), 8.53 (s, 1H), 8.34 (s, 1H), 7.41 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.32 (dd, *J* = 3.5, 2.1 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.61 (dd, *J* = 3.5, 1.6 Hz, 1H), 3.25 – 3.16 (m, 2H), 3.07 (dd, *J* = 11.2, 3.1 Hz, 1H), 3.02 – 2.97 (m, 2H), 2.83 – 2.76 (m, 1H), 2.61 – 2.56 (m, 1H), 1.91 (s, 1H), 1.10 (d, *J* = 6.5 Hz, 3H).

(S)-N-(5-iodo-2-(3-methylpiperazin-1-yl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11i) Compound **11i** was prepared according to general procedure F on a 0.94 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (312.7 mg, 0.72 mmol, 76% yield). $[M + H]^+$: 435.3. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.74 (d, *J* = 2.1 Hz, 1H), 8.53 (s, 1H), 8.34 (s, 1H), 7.40 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.31 (dd, *J* = 3.5, 2.0 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 6.59 (dd, *J* = 3.5, 1.5 Hz, 1H), 3.22 – 3.09 (m, 2H), 3.07 – 2.90 (m, 3H), 2.82 – 2.76 (m, 1H), 2.55 (s, 1H), 1.91 (s, 1H), 1.08 (d, *J* = 6.3 Hz, 3H).

N-(2-(2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-iodophenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11j) Compound **11j** was prepared according to general procedure F on a 1.43 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (436.6 mg, 1.01 mmol, 71% yield). $[M + H]^+$: 433.3. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1H), 8.77 (s, 1H), 8.11 (s, 1H), 7.54 (d, *J* = 2.2 Hz, 1H), 7.37 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.11 (dd, *J* = 3.5, 1.8 Hz, 1H), 6.65 (d, *J* = 8.7 Hz, 1H), 6.42 (s, 1H), 4.21 (s, 1H), 3.58 – 3.49 (m, 2H), 3.17 (s, 1H), 3.01 (d, *J* = 10.0 Hz, 1H), 2.87 – 2.76 (m, 2H), 1.69 (d, *J* = 9.0 Hz, 1H), 1.59 (d, *J* = 9.8 Hz, 1H).

N-(5-iodo-2-(4-methylpiperazin-1-yl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11k). Compound **11k** was prepared according to general procedure F on a 1.59 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (419.5 mg, 0.966 mmol, 61% yield). $[M + H]^+$: 435.1. ^{1}H NMR (300

MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.79 (d, *J* = 2.1 Hz, 1H), 8.48 (s, 1H), 8.35 (s, 1H), 7.41 – 7.30 (m, 2H), 7.02 (d, *J* = 8.3 Hz, 1H), 6.52 (dd, *J* = 3.5, 1.8 Hz, 1H), 3.30 (s, 4H) 2.84 (t, *J* = 4.7 Hz, 4H), 2.24 (s, 3H).

N-(2-(4-ethylpiperazin-1-yl)-5-iodophenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11l). Compound **11l** was prepared according to general procedure F on a 0.44 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (109.6 mg, 0.245 mmol, 56% yield). $[M + H]^+$: 449.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.79 (d, *J* = 2.0 Hz, 1H), 8.50 (s, 1H), 8.35 (s, 1H), 7.38 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.33 (dd, *J* = 3.5, 2.3 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.51 (dd, *J* = 3.5, 1.8 Hz, 1H), 2.85 (t, *J* = 4.7 Hz, 4H), 2.54 (d, *J* = 5.0 Hz, 4H), 2.45 – 2.34 (m, 2H), 1.02 (t, *J* = 7.1 Hz, 3H).

N-(5-iodo-2-(4-morpholinopiperidin-1-yl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (11m). Compound **11m** was prepared according to general procedure F on a 0.60 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (180.4 mg, 0.357 mmol, 60% yield). $[M + H]^+$: 505.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.90 (s, 1H), 8.72 (d, *J* = 2.1 Hz, 1H), 8.46 (s, 1H), 8.35 (s, 1H), 7.41 – 7.30 (m, 2H), 6.99 (d, *J* = 8.3 Hz, 1H), 6.53 (d, *J* = 3.4 Hz, 1H), 3.59 (s, 4H), 3.29 (s, 4H), 3.11 – 3.02 (m, 2H), 2.65 (t, *J* = 11.4 Hz, 2H), 2.27 – 2.21 (m, 1H), 1.90 – 1.85 (m, 2H), 1.62 – 1.50 (m, 2H).

6-morpholino-N1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)benzene-1,3-diamine (13). Compound **13** was prepared according to general procedure F on a 2.0 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (452.9 mg, 1.460 mmol, 73% yield). $[M + H]^+$: 311.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.83 (s, 1H), 8.66 (s, 1H), 8.30 (s, 1H), 7.82 (d, *J* = 2.5 Hz, 1H), 7.28 (t, *J* = 2.9 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.52 – 6.50 (m, 1H), 6.25 (dd, *J* = 8.4, 2.6 Hz, 1H), 4.98 (s, 2H), 3.75 (t, *J* = 4.3 Hz, 4H), 2.73 (t, *J* = 4.5 Hz, 4H).

N-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)cyclopentanecarboxamide (14a). Compound **14a** was prepared according to general procedure G on a 0.25 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (48.7 mg, 0.14 mmol, 28% yield). White solid; mp 284-287 ^{o}C. HPLC analysis: retention time, 4.318 min; peak area, 96.80%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.84 (s, 1H), 9.87 (s, 1H), 8.61 (s, 1H), 8.39 (s, 1H), 8.29 (s, 1H), 7.50 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.28 (t, *J* = 2.8 Hz,

1H), 7.14 (d, *J* = 8.7 Hz, 1H), 6.54 (d, *J* = 3.3 Hz, 1H), 3.71 (d, *J* = 4.8 Hz, 4H), 2.82 – 2.78 (m, 4H), 1.88 – 1.79 (m, 2H), 1.77 – 1.60 (m, 5H), 1.55 (s, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.63, 153.79, 151.40, 151.26, 138.67, 136.39, 134.34, 123.25, 120.65, 114.71, 113.38, 104.17, 98.02, 67.22, 52.46, 45.62, 30.59, 26.21; HRMSEI m/z $[M + H]^+$ calcd for C22H26N6O2: 407.2194, found: 407.2189.

1-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)-3-cyclopentylurea (14b). Compound **14b** was prepared according to general procedure G on a 0.5 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (109.5 mg, 0.26 mmol, 52% yield). White solid; mp 252-254 ^{o}C. HPLC analysis: retention time, 4.183 min; peak area, 95.48%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.87 (d, *J* = 7.5 Hz, 1H), 8.63 (d, *J* = 7.0 Hz, 1H), 8.26 (d, *J* = 18.5 Hz, 3H), 7.30 (s, 2H), 7.13 (d, *J* = 11.1 Hz, 1H), 6.56 (d, *J* = 6.9 Hz, 1H), 6.10 (s, 1H), 4.00 – 3.85 (m, 1H), 3.74 (s, 4H), 2.78 (s, 4H), 1.82 (s, 2H), 1.58 (d, *J* = 23.5 Hz, 4H), 1.35 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 155.33, 153.73, 151.36, 151.26, 137.73, 136.57, 134.78, 123.33, 121.09, 112.70, 110.99, 104.27, 97.83, 67.38, 52.75, 51.32, 33.39, 23.61; HRMSEI m/z $[M + H]^+$ calcd for C22H27N7O2:422.2290 , found: 422.2298.

N-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)-2-hydroxy-2-(thiazol-2-yl)propanamide (14c). Compound **14c** was prepared according to general procedure G on a 0.5 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (79.1 mg, 0.17 mmol, 34% yield). White solid; mp 292-295 ^{o}C. HPLC analysis: retention time, 3.188 min; peak area, 95.05%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.80 (s, 1H), 9.77 (s, 1H), 8.60 (s, 1H), 8.53 (d, *J* = 2.2 Hz, 1H), 8.28 (s, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.70 (d, *J* = 3.2 Hz, 1H), 7.45 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.27 – 7.24 (m, 1H), 7.15 (d, *J* = 9.2 Hz, 2H), 6.53 (s, 1H), 3.70 (s, 4H), 2.80 (s, 4H), 1.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.62, 170.71, 153.89, 151.42, 151.27, 142.63, 139.85, 134.94, 134.17, 123.16, 121.26, 120.47, 115.81, 114.99, 104.13, 98.13, 76.99, 67.14, 52.29, 40.45, 27.04; HRMSEI m/z $[M + H]^+$ calcd for C22H23N7O3S: 466.1647, found: 466.1654.

1-(4-(2-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-iodophenyl)piperazin-1-yl)ethan-1-one (15a). Compound **15a** was prepared according to general procedure G on a 0.4 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (129.8 mg, 0.281 mmol, 70% yield). $[M + H]^+$:

463.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.89 (s, 1H), 8.76 (d, *J* = 2.1 Hz, 1H), 8.57 (s, 1H), 8.35 (s, 1H), 7.39 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.31 – 7.29 (m, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 6.64 – 6.63 (m, 1H), 3.59 (q, *J* = 5.3 Hz, 4H), 2.84–2.81 (m, 4H), 2.03 (s, 3H).

N-(2-(4-(ethylsulfonyl)piperazin-1-yl)-5-iodophenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (15b). Compound **15b** was prepared according to general procedure G on a 0.4 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (160.3 mg, 0.312 mmol, 78% yield). [M + H]$^{+}$: 513.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.79 – 8.69 (m, 1H), 8.50 (s, 1H), 8.34 (s, 1H), 7.41 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.34 – 7.27 (m, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.70 – 6.59 (m, 1H), 3.33 (s, 4H), 3.13 (q, *J* = 7.3 Hz, 2H), 2.98 – 2.80 (m, 4H), 1.25 (t, *J* = 7.3 Hz, 3H).

N-(2-(4-(cyclopropylsulfonyl)piperazin-1-yl)-5-iodophenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (15c). Compound **15c** was prepared according to general procedure G on a 0.4 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (143.9 mg, 0.275 mmol, 69% yield). [M + H]$^{+}$: 525.0. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.88 (s, 1H), 8.67 (d, *J* = 2.1 Hz, 1H), 8.57 (s, 1H), 8.34 (s, 1H), 7.42 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.34 – 7.28 (m, 1H), 7.05 (d, *J* = 8.3 Hz, 1H), 6.59 – 6.58 (m, 1H), 3.37 – 3.34 (m, 4H) 2.95 – 2.92 (m, 4H), 2.68 – 2.62 (m, 1H), 1.07 – 1.00 (m, 2H), 0.99–0.94 (m, 2H).

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12a). Compound **12a** was prepared according to general procedure H on a 0.4 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (121.1 mg, 0.335 mmol, 84% yield). White solid; mp 121-123 ^{o}C. HPLC analysis: retention time, 3.560 min; peak area, 97.99%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.82 (s, 1H), 9.36 (s, 1H), 8.33 (s, 1H), 8.08 – 8.06 (m, 1H), 7.96 (d, *J* = 8.6, 1H), 7.79 (d, *J* = 3.2 Hz, 1H), 7.70 (d, *J* = 3.3 Hz, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 7.27 (t, *J* = 2.8 Hz, 1H), 7.11 – 7.00 (m, 2H), 6.81 – 6.80 (m, 1H), 1.89 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.48, 153.70, 151.35, 151.14, 143.03, 141.23, 129.45, 125.09, 122.96, 122.73, 122.28, 120.92, 120.74, 104.33, 99.14, 92.50, 83.70, 68.37, 31.99; HRMSEI m/z [M + H]$^{+}$ calcd for C19H15N5OS:362.1071, found: 362.1068.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(dimethylamino)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12b).

Compound **12b** was prepared according to general procedure H on a 0.4 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (86.9 mg, 0.215 mmol, 54% yield). White solid; mp 129-131 ^{o}C. HPLC analysis: retention time, 4.135 min; peak area, 95.62%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.77 (s, 1H), 8.59 (s, 1H), 8.24 (s, 1H), 7.93 (d, *J* = 1.9 Hz, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.68 (d, *J* = 3.2 Hz, 1H), 7.22–7.20 (m, 1H), 7.13 (d, *J* = 2.0 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 7.01 (s, 1H), 2.68 (s, 6H), 1.86 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.63, 154.27, 151.51, 151.40, 147.18, 142.98, 132.30, 127.94, 127.90, 122.81, 120.85, 119.26, 115.31, 103.95, 98.78, 91.72, 83.79, 68.36, 43.41, 32.05; HRMSEI m/z $[M + H]^+$ calcd for C21H20N6OS:405.1493, found: 405.1488.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-((2-methoxyethyl)(methyl)amino)phenyl)-2-(thiazol-2yl)but-3-yn-2-ol (12c). Compound **12c** was prepared according to general procedure H on a 0.13 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (36.0 mg, 0.08 mmol, 62% yield). White solid; mp 112-115 ^{o}C. HPLC analysis: retention time, 4.409 min; peak area, 96.82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.76 (s, 1H), 8.45 – 8.44 (m, 1H), 8.35 (d, *J* = 1.0 Hz, 1H), 7.78 (d, *J* = 3.2 Hz, 1H), 7.68 (d, *J* = 3.2 Hz, 1H), 7.29 – 7.28 (m, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 7.09 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.00 (s, 1H), 6.54 – 6.52 (m, 1H), 3.43 (t, *J* = 5.3 Hz, 2H), 3.18 (s, 3H), 2.98 (t, *J* = 5.3 Hz, 2H), 2.73 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.59, 153.68, 151.38, 151.35, 144.43, 143.01, 134.55, 126.49, 124.13, 123.19, 121.64, 120.89, 117.13, 104.39, 98.30, 91.86, 83.90, 69.85, 68.37, 58.48, 56.52, 41.07, 32.04; HRMSEI m/z $[M + H]^+$ calcd for C23H24N6O2S: 449.1745, found: 449.1752.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-((2(dimethylamino)ethyl)(methyl)amino)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12d). Compound **12d** was prepared according to general procedure H on a 0.16 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (53.1 mg, 0.115 mmol, 72% yield). White solid; mp 109-112 ^{o}C. HPLC analysis: retention time, 4.078 min; peak area, 96.14%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.80 (s, 1H), 9.12 (s, 1H), 8.41 (d, *J* = 2.0 Hz, 1H), 8.33 (s, 1H), 7.78 (d, *J* = 3.3 Hz, 1H), 7.68 (d, *J* = 3.2 Hz, 1H), 7.30 – 7.22 (m, 2H), 7.10 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.01 (s, 1H), 6.53 (d, 1H), 2.93 (t, *J* = 6.2 Hz, 2H), 2.70 (s, 3H), 2.31 (t, *J* = 6.2 Hz, 2H), 2.03 (s, 6H), 1.89 (s, 3H); ^{13}C NMR (75 MHz, DMSO-

d_6) δ 176.58, 153.86, 151.41, 151.33, 144.72, 143.00, 135.24, 126.63, 124.68, 123.05, 122.05, 120.89, 117.27, 104.36, 98.38, 91.89, 83.89, 68.37, 57.21, 54.91, 45.57, 42.08, 32.03. HRMSEI m/z $[M + H]^+$ calcd for C24H27N7OS:462.2061, found: 462.2069.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(pyrrolidin-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12e). Compound **12e** was prepared according to general procedure H on a 0.16 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (62.3 mg, 0.144 mmol, 90% yield). White solid; mp 143-147 ^{o}C. HPLC analysis: retention time, 3.286 min; peak area, 95.11%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.61 (s, 1H), 8.86 (s, 1H), 8.11 (s, 1H), 7.76 (d, *J* = 3.2 Hz, 1H), 7.66 (d, *J* = 3.2 Hz, 1H), 7.21 (d, *J* = 2.0 Hz, 1H), 7.18 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.11 – 7.07 (m, 1H), 6.90 (s, 1H), 6.77 (d, *J* = 8.6 Hz, 1H), 6.28 (s, 1H), 3.28 (t, *J* = 6.3 Hz, 4H), 1.85 (s, 3H), 1.78 (t, *J* = 6.4 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.85, 156.05, 151.81, 151.39, 147.04, 142.92, 134.41, 130.43, 125.43, 121.95, 120.76, 115.37, 109.89, 103.19, 99.40, 68.34, 50.05, 32.06, 25.52; HRMSEI m/z $[M + H]^+$ calcd for C23H22N6OS: 431.1639, found: 431.1646.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12f). Compound **12f** was prepared according to general procedure H on a 0.4 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (137.1 mg, 0.307 mmol, 77% yield). White solid; mp 207-210 ^{o}C. HPLC analysis: retention time, 3.220 min; peak area, 98.59%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.83 (s, 1H), 8.53 (s, 1H), 8.33 – 8.32 (m, 1H), 8.31 – 8.27 (m, 1H), 7.78 (d, *J* = 3.2 Hz, 1H), 7.68 (d, *J* = 3.3 Hz, 1H), 7.29 – 7.27 (m, 1H), 7.21 – 7.11 (m, 2H), 7.01 (s, 1H), 6.59 – 6.57 (m, 1H), 3.82 – 3.63 (m, 4H), 2.96 – 2.78 (m, 4H), 1.89 (s, 3H) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.56, 153.62, 151.41, 151.37, 144.29, 143.01, 133.89, 127.18, 125.42, 123.40, 120.90, 120.67, 117.32, 104.21, 98.18, 92.09, 83.69, 68.38, 66.91, 51.75, 32.04; HRMSEI m/z $[M + H]^+$ calcd for C23H22N6O2S:447.1598 , found: 447.1604.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(piperazin-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12g). Compound **12g** was prepared according to general procedure H on a 0.4 mmol scale. Purification by column chromatography (9% MeOH/DCM) afforded the title compound (138.6 mg, 0.311 mmol, 78% yield). White solid; mp 223-226 ^{o}C. HPLC analysis: retention time, 3.385min; peak area, 96.89%. ^{1}H NMR (300 MHz, DMSO-

d_6) δ 11.89 (s, 1H), 8.49 (s, 1H), 8.41 – 8.28 (m, 2H), 7.78 (d, *J* = 3.2 Hz, 1H), 7.69 (d, *J* = 3.3 Hz, 1H), 7.30 – 7.29 (m, 1H), 7.19 – 7.10 (m, 2H), 7.04 (s, 1H), 6.55 – 6.54 (m, 1H), 2.90 – 2.83 (m, 4H), 2.79 – 2.75 (m, 4H), 1.87 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 176.59, 153.48, 151.46, 151.30, 144.64, 143.02, 133.96, 126.90, 124.50, 123.51, 120.90, 120.67, 117.14, 104.20, 97.83, 91.97, 83.81, 68.38, 52.77, 46.50, 32.06. HRMSEI m/z $[M + H]^+$ calcd for C23H23N7OS: 446.1748, found: 446.1757.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-((R)-3-methylpiperazin-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12h) Compound **12h** was prepared according to general procedure H on a 0.25 mmol scale. Purification by column chromatography (9% MeOH/DCM) afforded the title compound (57.7 mg, 0.126 mmol, 50% yield). White solid; mp 130-132 °C. HPLC analysis: retention time, 3.522 min; peak area, 97.79%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.90 (s, 1H), 8.49 (s, 1H), 8.36 (d, *J* = 13.1 Hz, 2H), 7.78 (d, *J* = 3.3 Hz, 1H), 7.69 (d, *J* = 3.3 Hz, 1H), 7.30 (d, *J* = 3.5 Hz, 1H), 7.22 – 6.99 (m, 3H), 6.51 (d, *J* = 3.5 Hz, 1H), 2.87 (d, *J* = 8.9 Hz, 5H), 2.62 (d, *J* = 8.2 Hz, 1H), 2.30 (t, *J* = 10.5 Hz, 1H), 2.18 (s, 1H), 1.88 (s, 3H), 0.93 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.58, 153.46, 151.44, 151.26, 144.32, 143.00, 133.99, 126.86, 124.33, 123.52, 120.89, 120.76, 117.14, 104.19, 97.73, 91.94, 83.81, 68.37, 59.16, 52.05, 51.19, 46.26, 32.05, 19.75. HRMSEI m/z $[M + H]^+$ calcd for C24H25N7OS: 460.1909, found: 460.1916.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-((S)-3-methylpiperazin-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12i) Compound **12i** was prepared according to general procedure H on a 0.23 mmol scale. Purification by column chromatography (9% MeOH/DCM) afforded the title compound (61.4 mg, 0.134 mmol, 58% yield). White solid; mp 130-133 °C. HPLC analysis: retention time, 3.529 min; peak area, 97.54%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (s, 1H), 8.49 (s, 1H), 8.41 – 8.31 (m, 2H), 7.78 (d, *J* = 3.3 Hz, 1H), 7.69 (d, *J* = 3.3 Hz, 1H), 7.30 (d, *J* = 3.5 Hz, 1H), 7.18 – 7.01 (m, 3H), 6.51 (d, *J* = 3.5 Hz, 1H), 2.95 – 2.82 (m, 5H), 2.63 – 2.58 (m, 1H), 2.30 (t, *J* = 10.8 Hz, 1H), 2.19 (s, 1H), 1.88 (s, 3H), 0.93 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.57, 153.47, 151.45, 151.27, 144.32, 143.01, 133.98, 126.89, 124.40, 123.51, 120.89, 120.77, 117.15, 104.19, 97.76, 91.95, 83.80, 68.37, 59.05, 51.96, 51.19, 46.19, 32.05, 19.66. HRMSEI m/z $[M + H]^+$ calcd for C24H25N7OS: 460.1909, found: 460.1917.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(2,5-diazabicyclo[2.2.1]heptan-2-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12j) Compound **12j** was prepared according to general procedure H on a 0.2 mmol scale. Purification by column chromatography (9% MeOH/DCM) afforded the title compound (56.3 mg, 0.123 mmol, 61% yield). White solid; mp 152-154 ^{o}C. HPLC analysis: retention time, 2.873 min; peak area, 96.19%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1H), 8.73 (s, 1H), 8.09 (s, 1H), 7.74 (d, *J* = 3.2 Hz, 1H), 7.64 (d, *J* = 3.2 Hz, 1H), 7.20 (s, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 7.08 (d, *J* = 3.5 Hz, 1H), 6.92 (s, 1H), 6.73 (d, *J* = 8.6 Hz, 1H), 6.35 (s, 1H), 4.26 (s, 1H), 3.58 (d, *J* = 8.8 Hz, 1H), 3.39 (s, 1H), 3.17 (s, 1H), 2.92 (d, *J* = 9.3 Hz, 1H), 2.77 (d, *J* = 8.2 Hz, 2H), 1.83 (s, 3H), 1.63 (d, *J* = 7.8 Hz, 1H), 1.52 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.88, 155.64, 151.74, 151.32, 146.15, 142.91, 134.27, 130.00, 125.91, 121.97, 120.75, 115.98, 109.86, 103.27, 99.39, 90.99, 84.05, 68.33, 61.14, 58.99, 56.31, 49.22, 37.03, 32.06; HRMSEI m/z $[M + H]^+$ calcd for C24H25N7OS: 458.1738, found: 458.1745.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(4-methylpiperazin-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12k). Compound **12k** was prepared according to general procedure H on a 0.4 mmol scale. Purification by column chromatography (9% MeOH/DCM) afforded the title compound (121.8 mg, 0.265 mmol, 66% yield). White solid; mp 223-225 ^{o}C. HPLC analysis: retention time, 3.911 min; peak area, 97.00%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (s, 1H), 8.45 (s, 1H), 8.35 – 8.33 (m, 2H), 7.78 (d, *J* = 3.2 Hz, 1H), 7.69 (d, *J* = 3.2 Hz, 1H), 7.31 – 7.30 (m, 1H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.13 – 7.10 (m, 1H), 7.05 (s, 1H), 6.54 – 6.52 (m, 1H), 2.87 (t, *J* = 4.3 Hz, 4H), 2.50 – 2.44 (m, 4H), 2.23 (s, 3H), 1.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.56, 153.47, 151.45, 151.32, 144.07, 143.01, 133.94, 126.89, 124.62, 123.54, 120.89, 120.69, 117.27, 104.20, 97.81, 92.01, 83.76, 68.38, 55.54, 51.29, 46.21, 32.06; HRMSEI m/z $[M + H]^+$ calcd for C24H25N7OS: 460.1915, found: 460.1921.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(4-ethylpiperazin-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12l). Compound **12l** was prepared according to general procedure H on a 0.4 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (114.1 mg, 0.241 mmol, 60% yield). White solid; mp 213-215 ^{o}C. HPLC analysis: retention time, 4.097 min; peak area, 96.54%. ^{1}H NMR (300 MHz,

DMSO-d_6) δ 11.87 (s, 1H), 8.46 (s, 1H), 8.36 – 8.32 (m, 2H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.68 (d, *J* = 3.2 Hz, 1H), 7.30 (dd, *J* = 3.5, 2.3 Hz, 1H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.14 – 7.09 (m, 1H), 7.04 (s, 1H), 6.52 (dd, *J* = 3.5, 1.8 Hz, 1H), 2.87 (t, *J* = 4.7 Hz, 4H), 2.52 (d, *J* = 3.8 Hz, 4H), 2.38 (q, *J* = 7.2 Hz, 2H), 1.87 (s, 3H), 1.01 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.55, 153.48, 151.44, 151.30, 144.13, 143.00, 133.92, 126.88, 124.63, 123.53, 120.89, 120.64, 117.24, 104.18, 97.80, 91.99, 83.76, 68.37, 53.28, 52.10, 51.43, 32.05, 12.50; HRMSEI m/z $[M + H]^+$ calcd for C25H27N7OS: 474.2081, found: 474.2086.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(4-morpholinopiperidin-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12m). Compound **12m** was prepared according to general procedure H on a 0.33 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (118.1 mg, 0.223 mmol, 68% yield). White solid; mp 152-156 ^{o}C. HPLC analysis: retention time, 4.127 min; peak area, 95.19%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.87 (s, 1H), 8.42 – 8.29 (m, 3H), 7.77 (d, *J* = 3.1 Hz, 1H), 7.68 (d, *J* = 3.2 Hz, 1H), 7.30 (d, *J* = 3.2 Hz, 1H), 7.16 – 7.09 (m, 2H), 7.03 (s, 1H), 6.55 (s, 1H), 3.58 (s, 4H), 3.09 (d, *J* = 10.8 Hz, 2H), 2.65 (t, *J* = 11.4 Hz, 2H), 2.24 (s, 1H), 1.87 – 1.84 (m, 5H), 1.61 – 1.50 (m, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.58, 153.50, 144.77, 143.00, 133.76, 126.99, 124.98, 123.49, 120.91, 120.50, 116.93, 97.97, 91.93, 83.79, 79.64, 68.37, 61.41, 51.16, 49.96, 32.04, 28.87; HRMSEI m/z $[M + H]^+$ calcd for C28H31N7O2S:530.2333, found: 530.2335.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(4-methyl-1,4-diazepan-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12n). Compound **12n** was prepared according to general procedure H on a 0.2 mmol scale. Purification by column chromatography (9% MeOH/DCM) afforded the title compound (49.3 mg, 0.104 mmol, 52% yield). White solid; mp 169-172 ^{o}C. HPLC analysis: retention time, 3.454 min; peak area, 95.09%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.29 (s, 1H), 8.42 – 7.90 (m, 2H), 7.88 (d, *J* = 1.7 Hz, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.67 (d, *J* = 3.2 Hz, 1H), 7.57 – 7.41 (m, 2H), 6.98 (s, 2H), 3.79 (t, *J* = 6.8 Hz, 2H), 3.17 (s, 3H), 2.50 – 2.44 (m, 2H), 2.23 (s, 6H), 1.88 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.62, 157.23, 148.67, 142.99, 140.46, 132.05, 128.88, 127.24, 120.98, 120.84, 117.76, 114.54, 109.49, 92.03, 84.20, 68.38, 56.90, 47.42, 45.88, 36.62, 32.03; HRMSEI m/z $[M + H]^+$ calcd for C25H27N7OS: 474.1953, found: 474.1961.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-((tetrahydro-2H-pyran-4-yl)oxy)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12o). Compound **12o** was prepared according to general procedure H on a 0.23 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (84.9 mg, 0.184 mmol, 80% yield). White solid; mp 184-187 ^{o}C. HPLC analysis: retention time, 3.533 min; peak area, 97.96%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.43 (s, 1H), 8.27 (d, *J* = 1.2 Hz, 1H), 8.14 (s, 1H), 7.77 (dd, *J* = 3.2, 1.2 Hz, 1H), 7.68 (dd, *J* = 3.3, 1.2 Hz, 1H), 7.24 (d, *J* = 3.0 Hz, 1H), 7.16 (s, 2H), 6.99 (s, 1H), 6.59 – 6.52 (m, 1H), 4.67 (dt, *J* = 8.3, 4.4 Hz, 1H), 3.80 – 3.67 (m, 2H), 3.44 (t, *J* = 8.7 Hz, 2H), 3.22 – 3.00 (m, 1H), 1.87 (s, 3H), 1.64 (qd, *J* = 8.9, 6.0, 4.3 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 1H) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.64, 154.00, 151.28, 149.52, 143.00, 129.82, 128.06, 126.87, 123.17, 120.88, 114.60, 114.16, 103.97, 98.73, 91.42, 83.63, 72.82, 68.37, 64.56, 46.11, 32.05, 31.78, 9.05. HRMSEI m/z $[M + H]^+$ calcd for C24H23N5O3S: 462.1584, found: 462.1595.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-((1-methylpiperidin-4-yl)oxy)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (12p). Compound **12p** was prepared according to general procedure H on a 0.25 mmol scale. Purification by column chromatography (8% MeOH/DCM) afforded the title compound (70.2 mg, 0.148 mmol, 59% yield). White solid; mp 211-215 ^{o}C. HPLC analysis: retention time, 3.577 min; peak area, 96.55%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.80 (s, 1H), 8.28 (s, 2H), 8.22 (s, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.68 (d, *J* = 3.2 Hz, 1H), 7.26 – 7.25 (m, 1H), 7.12 (s, 2H), 7.00 (s, 1H), 6.53 – 6.52 (m, 1H), 4.51 (s, 1H), 2.40 (s, 2H), 2.22 – 2.17 (m, 2H), 2.08 (s, 3H), 1.91 – 1.83 (m, 5H), 1.75 – 1.66 (m, 2H) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.66, 154.11, 151.38, 149.24, 143.00, 130.13, 127.75, 126.30, 123.13, 120.88, 114.50, 114.06, 98.38, 91.34, 83.72, 68.37, 52.15, 46.26, 32.06, 30.51. HRMSEI m/z $[M + H]^+$ calcd for C25H26N6O2S: 475.1901, found: 475.1910.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)-2-methylbut-3-yn-2-ol (12q). Compound **12q** was prepared according to general procedure H on a 0.28 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (50.2 mg, 0.133 mmol, 48% yield). White solid; mp 253-255 ^{o}C. HPLC analysis: retention time, 4.059 min; peak area, 96.57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.86 (s, 1H), 8.54 (s, 1H), 8.35 – 8.23 (m, 2H), 7.29 – 7.28 (m, 1H), 7.17 (d, *J* = 8.2 Hz, 1H), 7.11 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.59 – 6.58 (m, 1H), 5.76 (s, 1H), 5.47 (s, 1H), 3.74 – 3.72 (m, 4H), 2.89 – 2.83 (m, 4H), 1.47 (s,

6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.63, 151.40, 151.34, 143.65, 133.92, 126.92, 125.15, 123.38, 120.61, 118.31, 104.21, 98.12, 95.61, 81.05, 66.96, 64.10, 51.84, 32.16; HRMSEI m/z $[M + H]^+$ calcd for C21H23N5O2: 378.1915, found: 378.1929.

1-((3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)ethynyl)cyclopentan-1-ol (12r). Compound **12r** was prepared according to general procedure H on a 0.24 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (45.1 mg, 0.112 mmol, 47% yield). White solid; mp 212-214 ^{o}C. HPLC analysis: retention time, 4.876 min; peak area, 96.14%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.85 (s, 1H), 8.53 (s, 1H), 8.32 – 8.28 (m, 2H), 7.29 – 7.26 (m, 1H), 7.17 (d, *J* = 8.2 Hz, 1H), 7.11 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.59 – 6.57 (m, 1H), 5.32 (s, 1H), 3.75 – 3.72 (m, 4H), 2.87 – 2.84 (m, 4H), 1.93 – 1.84 (m, 4H), 1.76 – 1.65 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.63, 151.40, 151.33, 143.59, 133.94, 126.87, 125.10, 123.38, 120.62, 118.45, 104.22, 98.11, 94.65, 82.02, 79.64, 73.27, 66.96, 51.84, 42.51, 23.54; HRMSEI m/z $[M + H]^+$ calcd for C23H25N5O2: 404.2082, found: 404.2082.

1-((3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)ethynyl)cyclohexan-1-ol (12s). Compound **12s** was prepared according to general procedure H on a 0.24 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (52.2 mg, 0.125 mmol, 52% yield). White solid; mp 211-213 ^{o}C. HPLC analysis: retention time, 5.367 min; peak area, 96.11%.^{1}H NMR (300 MHz, DMSO-d_6) δ 11.85 (s, 1H), 8.54 (s, 1H), 8.31 (s, 1H), 8.27 (d, *J* = 1.7 Hz, 1H), 7.29 – 7.27 (m, 1H), 7.18 – 7.10 (m, 2H), 6.59 – 6.57 (m, 1H), 5.44 (s, 1H), 3.73 (t, *J* = 4.5 Hz, 4H), 2.86 (t, *J* = 4.5 Hz, 4H), 1.87 – 1.79 (m, 2H), 1.66 – 1.63 (m, 2H), 1.59 – 1.47 (m, 5H), 1.26 – 1.23 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.64, 151.34, 143.76, 133.91, 127.07, 125.25, 123.39, 120.64, 118.44, 104.21, 98.17, 94.54, 83.33, 67.42, 66.96, 51.84, 25.42, 23.28; HRMSEI m/z $[M + H]^+$ calcd for C24H27N5O2: 418.2228, found: 418.2235.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)-2-cyclopentylbut-3-yn-2ol (12t). Compound **12t** was prepared according to general procedure H on a 0.24 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (46.2 mg, 0.107 mmol, 45% yield). White solid; mp 185-187 ^{o}C. HPLC analysis: retention time, 5.931 min; peak area, 97.08%. ^{1}H NMR (300 MHz, DMSO-

d_6) δ 11.82 (s, 1H), 8.52 (s, 1H), 8.29 (d, *J* = 6.9 Hz, 2H), 7.27 (s, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 6.57 (s, 1H), 5.24 (s, 1H), 3.73 – 3.71 (m, 4H), 2.86 – 2.84 (m, 4H), 2.12 – 2.02 (m, 1H), 1.71 – 1.68 (m, 2H), 1.66 – 1.56 (m, 3H), 1.55 – 1.50 (m, 3H), 1.42 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 153.69, 151.42, 151.31, 143.67, 134.06, 126.92, 125.22, 123.36, 120.69, 118.58, 104.27, 98.10, 93.93, 82.61, 70.03, 67.00, 51.89, 51.49, 29.71, 28.76, 28.11, 26.27, 26.21; HRMSEI m/z $[M + H]^+$ calcd for C25H29N5O2: 432.2395, found: 432.2398.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)-2-(thiophen-2-yl)but-3-yn-2-ol (12u). Compound **12u** was prepared according to general procedure H on a 0.24 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (54.8 mg, 0.123 mmol, 51% yield). White solid; mp 213-215 ^{o}C. HPLC analysis: retention time, 3.880 min; peak area, 97.14%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.85 (s, 1H), 8.55 (s, 1H), 8.31 (s, 2H), 7.43 (dd, *J* = 5.1, 1.3 Hz, 1H), 7.29 – 7.27 (m, 1H), 7.18 – 7.15 (m, 3H), 6.98 (dd, *J* = 5.1, 3.5 Hz, 1H), 6.60 – 6.58 (m, 1H), 6.53 (s, 1H), 3.73 (t, *J* = 4.5 Hz, 4H), 2.87 (t, *J* = 4.5 Hz, 4H), 1.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.65, 152.44, 151.40, 151.36, 144.18, 133.93, 127.13, 127.00, 125.41, 125.26, 123.63, 123.38, 120.66, 117.61, 104.22, 98.17, 93.39, 83.12, 66.93, 66.51, 51.77, 34.32; HRMSEI m/z $[M + H]^+$ calcd for C24H23N5O2S: 446.1636, found: 446.1643.

1-(4-(2-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(3-hydroxy-3-(thiazol-2-yl)but-1-yn-1yl)phenyl)piperazin-1-yl)ethan-1-one (16a). Compound **16a** was prepared according to general procedure H on a 0.23 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (60.9 mg, 0.125 mmol, 54% yield). White solid; mp 244-247 ^{o}C. HPLC analysis: retention time, 3.259 min; peak area, 98.89%.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 8.54 (s, 1H), 8.33 – 8.32 (m, 2H), 7.78 (d, *J* = 3.3 Hz, 1H), 7.68 (d, *J* = 3.2 Hz, 1H), 7.30 – 7.27 (m, 1H), 7.18 – 7.12 (m, 2H), 7.01 (s, 1H), 6.63 – 6.62 (m, 1H), 3.61 – 3.56 (m, 4H), 2.88 – 2.81 (m, 4H), 2.02 (s, 3H), 1.89 (s, 3H) ; ^{13}C NMR (100 MHz, DMSO-d6) δ 176.53, 168.89, 153.61, 151.40, 151.36, 144.09, 143.01, 133.93, 127.08, 125.31, 123.39, 120.90, 117.45, 104.22, 98.29, 92.11, 83.66, 68.38, 51.69, 51.14, 46.47, 41.60, 32.04, 21.70; HRMSEI m/z $[M + H]^+$ calcd for C25H25N7O2S: 488.1854, found: 488.1863.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(4-(ethylsulfonyl)piperazin-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (16b). Compound **16b** was prepared according to general procedure H on a 0.27 mmol scale. Purification by column chromatography (5% MeOH/DCM) afforded the title compound (65.1 mg, 0.121 mmol, 45% yield). White solid; mp 137-139 ^{o}C. HPLC analysis: retention time, 3.379 min; peak area, 96.70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.84 (s, 1H), 8.47 (s, 1H), 8.33–8.31 (m, 2H), 7.78 (d, *J* = 3.2 Hz, 1H), 7.68 (d, *J* = 3.3 Hz, 1H), 7.29 – 7.28 (m, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.14 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.01 (s, 1H), 6.63 – 6.61 (m, 1H), 3.12 (q, *J* = 7.4 Hz, 2H), 2.96 – 2.93 (m, 4H), 1.89 (s, 3H), 1.27 – 1.23 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d6) δ 176.53, 153.60, 151.38, 143.92, 143.01, 133.93, 127.12, 125.44, 123.41, 121.05, 120.91, 117.55, 98.38, 92.15, 83.62, 68.38, 51.19, 45.97, 43.39, 32.04, 8.06; HRMSEI m/z [M + H]$^{+}$ calcd for C25H27N7O3S2: 538.1678, found: 538.1691.

4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-(4-(cyclopropylsulfonyl)piperazin-1-yl)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (16c). Compound **16c** was prepared according to general procedure H on a 0.24 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (73.1 mg, 0.133 mmol, 55% yield). White solid; mp 132-134 ^{o}C. HPLC analysis: retention time, 3.427 min; peak area, 96.25%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 8.52 (s, 1H), 8.33 (s, 1H), 8.25 (d, *J* = 2.0 Hz, 1H), 7.78 – 7.77 (m, 1H), 7.69 – 7.68 (m, 1H), 7.29 – 7.28 (m, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 7.17 – 7.14 (m, 1H), 7.01 (s, 1H), 6.57 – 6.56 (m, 1H), 2.98 – 2.95 (m, 4H), 2.67 – 2.60 (m, 1H), 1.89 (s, 3H), 1.06 – 1.03 (m, 2H), 0.96 – 0.94 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d6) δ 176.53, 153.70, 151.43, 151.39, 144.15, 143.01, 133.91, 127.25, 125.78, 123.34, 121.03, 120.91, 117.49, 104.27, 98.34, 92.16, 83.60, 68.37, 50.84, 46.53, 32.04, 25.49, 4.42; HRMSEI m/z [M + H]$^{+}$ calcd for C26H27N7O3S2: 550.1680, found: 550.1689.

4-(3-((2-aminopyrimidin-4-yl)amino)-4-morpholinophenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (21a). Compound **21a** was prepared according to general procedure H on a 0.25 mmol scale. Purification by column chromatography (2% MeOH/DCM) afforded the title compound (63.7 mg, 0.151 mmol, 60% yield). White solid; mp 188-190 ^{o}C. HPLC analysis: retention time, 3.633 min; peak area, 96.56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.84 (d, *J* = 5.7 Hz, 1H), 7.79 – 7.78 (m, 2H), 7.69 (d, *J* = 3.3 Hz, 1H), 7.12 (dd, *J* = 8.2, 1.9 Hz,

1H), 7.07 (d, *J* = 8.3 Hz, 1H), 6.96 (s, 1H), 6.14 – 6.11 (m, 3H), 3.78 – 3.69 (m, 4H), 2.90 – 2.82 (m, 4H), 1.89 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.37, 152.49, 145.01, 133.38, 127.52, 126.99, 126.60, 125.22, 123.67, 120.06, 117.03, 93.33, 83.08, 66.61, 66.50, 51.38, 34.33; HRMSEI m/z $[M + H]^+$ calcd for C21H22N6O2S: 423.1598, found: 423.1596.

4-(3-((1H-pyrrolo[2,3-b]pyridin-4-yl)amino)-4-morpholinophenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (21b). Compound **21b** was prepared according to general procedure H on a 0.24 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (61.8 mg, 0.139 mmol, 58% yield). White solid; mp 198-201 ^{o}C. HPLC analysis: retention time, 4.318 min; peak area, 96.80%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.35 (s, 1H), 7.91 – 7.89 (m, 2H), 7.75 (d, *J* = 3.2 Hz, 1H), 7.66 (d, *J* = 3.2 Hz, 1H), 7.24 (d, *J* = 1.9 Hz, 1H), 7.20 – 7.13 (m, 2H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.95 (s, 1H), 6.49 – 6.39 (m, 2H), 3.55 (t, *J* = 4.5 Hz, 4H), 2.90 (t, *J* = 4.5 Hz, 4H), 1.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.53, 150.04, 146.17, 144.15, 143.64, 142.97, 134.32, 127.86, 126.54, 122.82, 120.86, 120.02, 116.23, 109.22, 100.00, 98.23, 92.12, 83.38, 68.33, 66.60, 50.77, 31.98; HRMSEI m/z $[M + H]^+$ calcd for C24H23N5O2S: 446.1656, found: 446.1644.

4-(3-((5-fluoro-1H-pyrrolo[2,3-b]pyridin-4-yl)amino)-4-morpholinophenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (21c). Compound **21c** was prepared according to general procedure H on a 0.25 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (105.4 mg, 0.227 mmol, 91% yield). White solid; mp 128-131 ^{o}C. HPLC analysis: retention time, 4.077 min; peak area, 97.21%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.42 (s, 1H), 8.03 (d, *J* = 4.3 Hz, 1H), 7.93 (s, 1H), 7.75 (d, *J* = 3.2 Hz, 1H), 7.65 (d, *J* = 3.2 Hz, 1H), 7.21 – 7.17 (m, 1H), 7.13 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.02 – 7.00 (m, 2H), 6.93 (s, 1H), 5.84 – 5.82 (m, 1H), 3.31 – 3.28 (m, 4H), 2.86 – 2.83 (m, 4H), 1.83 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.53, 146.77, 146.01, 142.96, 135.16, 131.44, 131.18, 130.83, 130.72, 127.59, 126.15, 125.13, 120.84, 119.75, 116.05, 99.31, 91.98, 83.42, 68.30, 66.43, 50.56, 31.96; HRMSEI m/z $[M + H]^+$ calcd for C24H22FN5O2S: 464.1552, found: 464.1548.

4-(3-((5-chloro-1H-pyrrolo[2,3-b]pyridin-4-yl)amino)-4-morpholinophenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (21d) Compound **21d** was prepared according to general procedure H on a 0.20 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (74.2 mg, 0.154 mmol, 76% yield). White

solid; mp 128-130 °C. HPLC analysis: retention time, 4.752 min; peak area, 96.23%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.51 (s, 1H), 8.05 (s, 1H), 7.83 (s, 1H), 7.75 (d, *J* = 3.2 Hz, 1H), 7.66 (d, *J* = 3.3 Hz, 1H), 7.19 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.11 (dd, *J* = 3.7, 2.3 Hz, 2H), 7.01 (d, *J* = 8.3 Hz, 1H), 6.96 (s, 1H), 5.39 (dd, *J* = 3.5, 2.0 Hz, 1H), 3.23 (t, *J* = 4.5 Hz, 4H), 2.81 (t, *J* = 4.7 Hz, 4H), 1.83 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.51, 148.63, 146.62, 142.99, 142.28, 139.77, 134.72, 128.40, 127.50, 124.22, 120.88, 119.97, 116.06, 110.73, 110.30, 99.26, 92.14, 83.31, 68.31, 66.54, 50.50, 31.96; HRMSEI m/z $[M + H]^+$ calcd for C22H21N7O2S: 480.1216, found: 448.1558.

4-(3-((1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (21e). Compound **21e** was prepared according to general procedure H on a 0.25 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (86.8 mg, 0.194 mmol, 78% yield). White solid; mp 236-238 °C. HPLC analysis: retention time, 3.287 min; peak area, 98.61%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.59 (s, 1H), 9.37 (s, 1H), 8.35 (s, 1H), 7.94 (s, 1H), 7.80 – 7.76 (m, 2H), 7.68 (d, *J* = 3.3 Hz, 1H), 7.27 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.02 (s, 1H), 3.58 (t, *J* = 4.4 Hz, 4H), 2.87 (t, *J* = 4.6 Hz, 4H), 1.86 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.51, 155.76, 155.37, 146.91, 143.00, 132.03, 129.58, 129.46, 120.89, 120.24, 116.41, 100.99, 92.31, 83.25, 68.36, 66.55, 51.29, 32.00; HRMSEI m/z $[M + H]^+$ calcd for C22H21N7O2S: 448.1551, found: 448.1558.

4-(4-morpholino-3-(thieno[2,3-d]pyrimidin-4-ylamino)phenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (21f). Compound **21f** was prepared according to general procedure H on a 0.25 mmol scale. Purification by column chromatography (4% MeOH/DCM) afforded the title compound (101.5 mg, 0.219 mmol, 88% yield). White solid; mp 229-231 °C. HPLC analysis: retention time, 4.197 min; peak area, 98.36%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.50 (s, 1H), 7.97 (d, *J* = 2.0 Hz, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.73 (d, *J* = 6.0 Hz, 1H), 7.71 – 7.66 (m, 2H), 7.24 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 7.03 (s, 1H), 3.64 (dd, *J* = 5.8, 3.2 Hz, 4H), 2.86 (t, *J* = 4.5 Hz, 4H), 1.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.53, 167.02, 155.34, 153.82, 146.05, 143.01, 132.58, 128.86, 127.98, 124.75, 120.89, 120.56, 119.26, 117.43, 116.88, 92.29, 83.39, 68.39, 66.75, 51.52, 32.02; HRMSEI m/z $[M + H]^+$ calcd for C23H21N5O2S2: 464.1220, found: 464.1212.

(R)-4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (22). Compound **22** was resolved from **12f** on a 0.3 mmol scale. (64.6 mg, 0.139 mmol, 46% yield). White solid; mp 195-197 ^{o}C. HPLC analysis: retention time, 3.725 min; peak area, 99.43%. ^{1}H NMR (300 MHz, DMSO-d6) δ 11.87 (s, 1H), 8.54 (s, 1H), 8.33 – 8.29 (m, 2H), 7.78 – 7.68 (m, 2H), 7.29 – 7.07 (m, 4H), 6.60 (s, 1H), 3.73 (s, 4H), 2.86 (s, 4H), 1.88 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 176.55, 153.62, 151.42, 144.29, 143.02, 133.85, 127.19, 125.44, 123.40, 120.90, 120.64, 117.27, 104.21, 98.19, 92.06, 83.67, 68.38, 66.90, 56.51, 51.72, 32.04, 19.02; HRMSEI m/z $[M + H]^{+}$ calcd for C23H22N6O2S:447.1598 , found: 447.1608.

(S)-4-(3-((7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-4-morpholinophenyl)-2-(thiazol-2-yl)but-3-yn-2-ol (23). Compound **23** was resolved from **12f** on a 0.3 mmol scale. (63.2 mg, 0.136 mmol, 45% yield). White solid; mp 132-135 ^{o}C. HPLC analysis: retention time, 3.738 min; peak area, 98.56%. 1H NMR (300 MHz, DMSO-d6) δ 11.87 (s, 1H), 8.54 (s, 1H), 8.30 (d, J = 10.7 Hz, 2H), 7.78 – 7.68 (m, 2H), 7.35 – 7.01 (m, 4H), 6.65 – 6.55 (m, 1H), 3.95 – 3.65 (m, 4H), 2.86 (t, J = 4.4 Hz, 4H), 1.88 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 176.54, 153.61, 151.41, 151.33, 144.30, 143.02, 133.84, 127.19, 125.45, 123.39, 120.91, 120.64, 117.26, 104.19, 98.18, 92.05, 83.66, 68.37, 66.89, 56.50, 51.72, 32.04, 19.03; HRMSEI m/z $[M + H]^{+}$ calcd for C23H22N6O2S:447.1598 , found: 447.1607.

ASSOCIATED CONTENT

The following files are available free of charge.

The % Ctrl of compound **12f** against the 268 wild-type kinases. IC_{50} of compound **12f** against the selected kianses. The reagents and antibodies used for biological evaluation in vitro or vivo. Toxicity screening of selected target compounds on normal T cells. ^{1}H NMR and ^{13}C NMR spectra for target compounds. The HPLC spectra for target compounds. (.pdf)

Molecular Formula Strings (.CSV)

Molecular modeling information (.pdb)

Authors will release the atomic coordinates and experimental data upon article publication.

AUTHOR INFORMATION

Corresponding Authors

*(Tao Lu) Tel: +86-25-86185180; Fax: +86-25-86185179; E-mail: lutao@cpu.edu.cn

*(Yadong Chen) Tel: +86-25-86185153; Fax: +86-25-861851170; E-mail: ydchen@cpu.edu.cn

Author Contributions

⊥Yuqin Zhu and Yuxiang Ma contributed equally to this work. The authors declare no competing financial interest. The manuscript was written through contributions of all authors.

ACKNOWLEDGMENT

This study was supported by grants from the National Natural Science Foundation of China (Grant No. 81973182, 81673301) ; "Double First-Class" University project from China Pharmaceutical University (Program No. CPU2018GF02). We thank Prof. Lei Qiang, China Pharmaceutical University, for technical assistance of pharmacology.

ABBREVIATIONS

NIK, Nuclear factor kappa-B inducing kinase; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; TNFSFR, Tumor necrosis factor superfamily receptor; CD40, Cluster of differentiation 40; CD40L, Cluster of differentiation 40 ligand; TRAF3, Tumor necrosis factor receptor-associated factor 3; BAFF, B-cell activating factor; BAFF-R, B-cell activating factor receptor; Fn14 (TWEAK-R or TNFRSF12A), Tumor necrosis factor receptor superfamily member 12A; TRAF2-cIAPs, Tumor necrosis factor receptor (TNFR)-associated factor 2-Cellular Inhibitor of apoptosis proteins; IKKα, IκB kinase α; IMQ, Imiquimod.

REFERENCES

(1) Parisi, R.; Symmons, D. P.; Griffiths, C. E.; Ashcroft, D. M. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. *J. Invest. Dermatol.* **2013**, *133*, 377-385.

(2) Gaffen, S. L.; Jain, R.; Garg, A. V.; Cua, D. J. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat. Rev. Immunol.* **2014**, *14*, 585-600.

(3) Elder, J. T. Genome-wide association scan yields new insights into the immunopathogenesis of psoriasis. *Genes Immun.* **2009**, *10*, 201-209.

(4) Myers, W. A.; Gottlieb, A. B.; Mease, P. Psoriasis and psoriatic arthritis: clinical features and disease mechanisms. *Clin. Dermatol.* **2006**, *24*, 438-447.

(5) Gudjonsson, J. E.; Johnston, A.; Stoll, S. W.; Riblett, M. B.; Xing, X.; Kochkodan, J. J.; Ding, J.; Nair, R. P.; Aphale, A.; Voorhees, J. J.; Elder, J. T. Evidence for altered Wnt signaling in psoriatic skin. *J. Invest. Dermatol.* **2010**, *130*, 1849-1859.

(6) Lowes, M. A.; Suarez-Farinas, M.; Krueger, J. G. Immunology of psoriasis. *Annu. Rev. Immunol.* **2014**, *32*, 227-255.

(7) Guttman-Yassky, E.; Krueger, J. G.; Lebwohl, M. G. Systemic immune mechanisms in atopic dermatitis and psoriasis with implications for treatment. *Exp. Dermatol.* **2018**, *27*, 409-417.

(8) Furumoto, Y.; Gadina, M. The arrival of JAK inhibitors: advancing the treatment of immune and hematologic disorders. *Biodrugs* **2013**, *27*, 431-438.

(9) Cohen, P.; Alessi, D. R. Kinase drug discovery--what's next in the field? *ACS Chem. Biol.* **2013**, *8*, 96-104.

(10) Mócsai, A.; Kovács, L.; Gergely, P. What is the future of targeted therapy in rheumatology: biologics or small molecules? *BMC Med.* **2014**. DOI: 10.1186/1741-7015-12-43.

(11) Ritchlin, C. T.; Krueger, J. G. New therapies for psoriasis and psoriatic arthritis. *Curr. Opin. Rheumatol.*

2016, *28*, 204-210.

(12) Behrens, F.; Koehm, M.; Schwaneck, E. C.; Schmalzing, M.; Gnann, H.; Greger, G.; Tony, H. P.; Burkhardt, H. Minimal disease activity is a stable measure of therapeutic response in psoriatic arthritis patients receiving treatment with adalimumab. *Rheumatology (Oxford)* **2018**, *57*, 1938-1946.

(13) Mease, P.; van der Heijde, D.; Landewe, R.; Mpofu, S.; Rahman, P.; Tahir, H.; Singhal, A.; Boettcher, E.; Navarra, S.; Meiser, K.; Readie, A.; Pricop, L.; Abrams, K. Secukinumab improves active psoriatic arthritis symptoms and inhibits radiographic progression: primary results from the randomised, double-blind, phase III future 5 study. *Ann. Rheum. Dis.* **2018**, *77*, 890-897.

(14) Schwartz, D. M.; Bonelli, M.; Gadina, M.; O'Shea, J. J. Type I/II cytokines, JAKs, and new strategies for treating autoimmune diseases. *Nat. Rev. Rheumatol.* **2016**, *12*, 25-36.

(15) Bryan, M. C.; Rajapaksa, N. S. Kinase inhibitors for the treatment of immunological disorders: recent advances. *J. Med. Chem.* **2018**, *61*, 9030-9058.

(16) Meyer, S. C.; Levine, R. L. Molecular pathways: molecular basis for sensitivity and resistance to JAK kinase inhibitors. *Clin. Cancer Res.* **2014**, *20*, 2051-2059.

(17) Harrison, C.; Vannucchi, A. M. Ruxolitinib: a potent and selective janus kinase 1 and 2 inhibitor in patients with myelofibrosis. an update for clinicians. *Ther. Adv. Hematol.* **2012**, *3*, 341-354.

(18) Papp, K. A.; Menter, M. A.; Raman, M.; Disch, D.; Schlichting, D. E.; Gaich, C.; Macias, W.; Zhang, X.; Janes, J. M. A randomized phase 2b trial of baricitinib, an oral janus kinase (JAK) 1/JAK2 inhibitor, in patients with moderate-to-severe psoriasis. *Br. J. Dermatol.* **2016**, *174*, 1266-1276.

(19) Schwartz, D. M.; Kanno, Y.; Villarino, A.; Ward, M.; Gadina, M.; O'Shea, J. J. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat. Rev. Drug Discov.* **2017**, *16*, 843-862.

(20) Wernig, G.; Kharas, M. G.; Okabe, R.; Moore, S. A.; Leeman, D. S.; Cullen, D. E.; Gozo, M.; McDowell, E. P.; Levine, R. L.; Doukas, J.; Mak, C. C.; Noronha, G.; Martin, M.; Ko, Y. D.; Lee, B. H.; Soll, R. M.; Tefferi, A.; Hood, J. D.; Gilliland, D. G. Efficacy of TG101348, a selective JAK2 inhibitor, in treatment of a murine model of

JAK2V617F-induced polycythemia vera. *Cancer Cell* **2008**, *13*, 311-320.

(21) Papp, K.; Pariser, D.; Catlin, M.; Wierz, G.; Ball, G.; Akinlade, B.; Zeiher, B.; Krueger, J. G. A phase 2a randomized, double-blind, placebo-controlled, sequential dose-escalation study to evaluate the efficacy and safety of ASP015K, a novel janus kinase inhibitor, in patients with moderate-to-severe psoriasis. *Br. J. Dermatol.* **2015**, *173*, 767-776.

(22) Wrobleski, S. T.; Moslin, R.; Lin, S.; Zhang, Y.; Spergel, S.; Kempson, J.; Tokarski, J. S.; Strnad, J.; Zupa-Fernandez, A.; Cheng, L.; Shuster, D.; Gillooly, K.; Yang, X.; Heimrich, E.; McIntyre, K. W.; Chaudhry, C.; Khan, J.; Ruzanov, M.; Tredup, J.; Mulligan, D.; Xie, D.; Sun, H.; Huang, C.; D'Arienzo, C.; Aranibar, N.; Chiney, M.; Chimalakonda, A.; Pitts, W. J.; Lombardo, L.; Carter, P. H.; Burke, J. R.; Weinstein, D. S. Highly selective inhibition of tyrosine kinase 2 (TYK2) for the treatment of autoimmune diseases: discovery of the allosteric inhibitor BMS-986165. *J. Med. Chem.* **2019**, *62*, 8973−8995.

(23) Musumeci, F.; Sanna, M.; Greco, C.; Giacchello, I.; Fallacara, A. L.; Amato, R.; Schenone, S. Pyrrolo[2,3-d]pyrimidines active as BTK inhibitors. *Expert Opin. Ther. Patents.* **2017**, *27*, 1305-1318.

(24) Lou, Y.; Han, X.; Kuglstatter, A.; Kondru, R. K.; Sweeney, Z. K.; Soth, M.; McIntosh, J.; Litman, R.; Suh, J.; Kocer, B.; Davis, D.; Park, J.; Frauchiger, S.; Dewdney, N.; Zecic, H.; Taygerly, J. P.; Sarma, K.; Hong, J.; Hill, R. J.; Gabriel, T.; Goldstein, D. M.; Owens, T. D. Structure-based drug design of RN486, a potent and selective bruton's tyrosine kinase (BTK) inhibitor, for the treatment of rheumatoid arthritis. *J. Med. Chem.* **2015**, *58*, 512-516.

(25) Geahlen, R. L. Getting SYK: spleen tyrosine kinase as a therapeutic target. *Trends Pharmacol. Sci.* **2014**, *35*, 414-422.

(26) Smith, A.; Manoli, H.; Jaw, S.; Frutoz, K.; Epstein, A. L.; Khawli, L. A.; Theil, F. P. Unraveling the effect of immunogenicity on the PK/PD, efficacy, and safety of therapeutic proteins. *J. Immunol. Res.* **2016**, *2016*, 1-9.

(27) Liu, J.; Huang, X.; Hao, S.; Wang, Y.; Liu, M.; Xu, J.; Zhang, X.; Yu, T.; Gan, S.; Dai, D.; Luo, X.; Lu, Q.;

Mao, C.; Zhang, Y.; Shen, N.; Li, B.; Huang, M.; Zhu, X.; Jin, J.; Cheng, X.; Sun, S. C.; Xiao, Y. Peli1 negatively regulates noncanonical NF-kappaB signaling to restrain systemic lupus erythematosus. *Nat. Commun.* **2018**, *9*, 1136.

(28) Brightbill, H. D.; Suto, E.; Blaquiere, N.; Ramamoorthi, N.; Sujatha-Bhaskar, S.; Gogol, E. B.; Castanedo, G. M.; Jackson, B. T.; Kwon, Y. C.; Haller, S.; Lesch, J.; Bents, K.; Everett, C.; Kohli, P. B.; Linge, S.; Christian, L.; Barrett, K.; Jaochico, A.; Berezhkovskiy, L. M.; Fan, P. W.; Modrusan, Z.; Veliz, K.; Townsend, M. J.; DeVoss, J.; Johnson, A. R.; Godemann, R.; Lee, W. P.; Austin, C. D.; McKenzie, B. S.; Hackney, J. A.; Crawford, J. J.; Staben, S. T.; Alaoui Ismaili, M. H.; Wu, L. C.; Ghilardi, N. NF-kappaB inducing kinase is a therapeutic target for systemic lupus erythematosus. *Nat. Commun.* **2018**, *9*, 179.

(29) Castanedo, G. M.; Blaquiere, N.; Beresini, M.; Bravo, B.; Brightbill, H.; Chen, J.; Cui, H. F.; Eigenbrot, C.; Everett, C.; Feng, J.; Godemann, R.; Gogol, E.; Hymowitz, S.; Johnson, A.; Kayagaki, N.; Kohli, P. B.; Knuppel, K.; Kraemer, J.; Kruger, S.; Loke, P.; McEwan, P.; Montalbetti, C.; Roberts, D. A.; Smith, M.; Steinbacher, S.; Sujatha-Bhaskar, S.; Takahashi, R.; Wang, X.; Wu, L. C.; Zhang, Y.; Staben, S. T. Structure-based design of tricyclic NF-kappaB inducing kinase (NIK) inhibitors that have high selectivity over phosphoinositide-3-kinase (PI3K). *J. Med. Chem.* **2017**, *60*, 627-640.

(30) Blaquiere, N.; Castanedo, G. M.; Burch, J. D.; Berezhkovskiy, L. M.; Brightbill, H.; Brown, S.; Chan, C.; Chiang, P. C.; Crawford, J. J.; Dong, T.; Fan, P.; Feng, J.; Ghilardi, N.; Godemann, R.; Gogol, E.; Grabbe, A.; Hole, A. J.; Hu, B.; Hymowitz, S. G.; Alaoui Ismaili, M. H.; Le, H.; Lee, P.; Lee, W.; Lin, X.; Liu, N.; McEwan, P. A.; McKenzie, B.; Silvestre, H. L.; Suto, E.; Sujatha-Bhaskar, S.; Wu, G.; Wu, L. C.; Zhang, Y.; Zhong, Z.; Staben, S. T. Scaffold-hopping approach to discover potent, selective, and efficacious inhibitors of NF-kappaB inducing kinase. *J. Med. Chem.* **2018**, *61*, 6801-6813.

(31) Willmann, K. L.; Sacco, R.; Martins, R.; Garncarz, W.; Krolo, A.; Knapp, S.; Bennett, K. L.; Boztug, K. Expanding the interactome of the noncanonical NF-kappaB signaling pathway. *J. Proteome Res.* **2016**, *15*, 2900-2909.

(32) Noort, A. R.; van Zoest, K. P.; Weijers, E. M.; Koolwijk, P.; Maracle, C. X.; Novack, D. V.; Siemerink, M. J.; Schlingemann, R. O.; Tak, P. P.; Tas, S. W. NF-kappaB-inducing kinase is a key regulator of inflammation-induced and tumour-associated angiogenesis. *J. Pathol.* **2014**, *234*, 375-385.

(33) Janda, A.; Rizzi, M. Humoral primary immunodeficiency and autoimmune and inflammatory manifestations. *Humoral Primary Immunodeficiencies*, D'Elios, M. M.; Rizzi, M., Eds. Springer international publishing: cham. **2019**, *pp* 325-346.

(34) Valino-Rivas, L.; Vaquero, J. J.; Sucunza, D.; Gutierrez, S.; Sanz, A. B.; Fresno, M.; Ortiz, A.; Sanchez-Nino, M. D. NIK as a druggable mediator of tissue injury. *Trends Mol. Med.* **2019**, *25*, 341-360.

(35) Shen, H.; Sheng, L.; Chen, Z.; Jiang, L.; Su, H.; Yin, L.; Omary, M. B.; Rui, L. Mouse hepatocyte overexpression of NF-kappaB-inducing kinase (NIK) triggers fatal macrophage-dependent liver injury and fibrosis. *Hepatology* **2014**, *60*, 2065-2076.

(36) Ren, X.; Li, X.; Jia, L.; Chen, D.; Hou, H.; Rui, L.; Zhao, Y.; Chen, Z. A small-molecule inhibitor of NF-kappaB-inducing kinase (NIK) protects liver from toxin-induced inflammation, oxidative stress, and injury. *Faseb J.* **2017**, *31*, 711-718.

(37) Etemadi, N.; Chopin, M.; Anderton, H.; Tanzer, M. C.; Rickard, J. A.; Abeysekera, W.; Hall, C.; Spall, S. K.; Wang, B.; Xiong, Y.; Hla, T.; Pitson, S. M.; Bonder, C. S.; Wong, W. W.; Ernst, M.; Smyth, G. K.; Vaux, D. L.; Nutt, S. L.; Nachbur, U.; Silke, J. TRAF2 regulates TNF and NF-kappaB signalling to suppress apoptosis and skin inflammation independently of sphingosine kinase 1. *Elife* **2015**, *4*, e10592.

(38) Mair, F.; Joller, S.; Hoeppli, R.; Onder, L.; Hahn, M.; Ludewig, B.; Waisman, A.; Becher, B. The NF-kappaB-inducing kinase is essential for the developmental programming of skin-resident and IL-17-producing gammadelta T cells. *Elife* **2015**, *4*, e10087.

(39) Eshima, K.; Okabe, M.; Kajiura, S.; Noma, H.; Shinohara, N.; Iwabuchi, K. Significant involvement of nuclear factor-kappaB-inducing kinase in proper differentiation of alphabeta and gammadelta T cells. *Immunology* **2014**, *141*, 222-232.

(40) El Malki, K.; Karbach, S. H.; Huppert, J.; Zayoud, M.; Reissig, S.; Schuler, R.; Nikolaev, A.; Karram, K.; Munzel, T.; Kuhlmann, C. R.; Luhmann, H. J.; von Stebut, E.; Wortge, S.; Kurschus, F. C.; Waisman, A. An alternative pathway of imiquimod-induced psoriasis-like skin inflammation in the absence of interleukin-17 receptor a signaling. *J. Invest. Dermatol.* **2013**, *133*, 441-451.

(41) Lin, W. J.; Su, Y. W.; Lu, Y. C.; Hao, Z.; Chio, II; Chen, N. J.; Brustle, A.; Li, W. Y.; Mak, T. W. Crucial role for TNF receptor-associated factor 2 (TRAF2) in regulating NFkappaB2 signaling that contributes to autoimmunity. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 18354-18359.

(42) Li, Y.; Zhang, G.; Chen, M.; Tong, M.; Zhao, M.; Tang, F.; Xiao, R.; Wen, H. Rutaecarpine inhibited imiquimod-induced psoriasis-like dermatitis via inhibiting the NF-kappaB and TLR7 pathways in mice. *Biomed. Pharmacother.* **2019**, *109*, 1876-1883.

(43) Li, K.; McGee, L. R.; Fisher, B.; Sudom, A.; Liu, J.; Rubenstein, S. M.; Anwer, M. K.; Cushing, T. D.; Shin, Y.; Ayres, M.; Lee, F.; Eksterowicz, J.; Faulder, P.; Waszkowycz, B.; Plotnikova, O.; Farrelly, E.; Xiao, S. H.; Chen, G.; Wang, Z. Inhibiting NF-kappaB-inducing kinase (NIK): discovery, structure-based design, synthesis, structure-activity relationship, and co-crystal structures. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1238-1244.

(44) Wang, M.; Zhang, S.; Zheng, G.; Huang, J.; Songyang, Z.; Zhao, X.; Lin, X. Gain-of-function mutation of card14 leads to spontaneous psoriasis-like skin inflammation through enhanced keratinocyte response to IL-17A. *Immunity* **2018**, *49*, 66-79 e5.

(45) Klimov, E.; Tretiakov, A.; Gapanovich, E.; Kokaeva, Z.; Soboleva, A.; Sakaniya, L.; Korsunskaya, I.; Sobolev, V. Assessment of the role of NO synthase genes polymorphisms in the pathogenesis of psoriasis. *J. Adv. Med. Med. Res.* **2018**, *26*, 1-6.

(46) Zhong, J.; Scholz, T.; Yau, A. C. Y.; Guerard, S.; Hüffmeier, U.; Burkhardt, H.; Holmdahl, R. Mannan-induced NOS2 in macrophages enhances IL-17–driven psoriatic arthritis by innate lymphocytes. *Sci. Adv.* **2018**, *4*, eaas9864.

(47) Yang, W.; Chen, Y.; Zhou, X.; Gu, Y.; Qian, W.; Zhang, F.; Han, W.; Lu, T.; Tang, W. Design, synthesis

and biological evaluation of bis-aryl ureas and amides based on 2-amino-3-purinylpyridine scaffold as DFG-out B-Raf kinase inhibitors. *Eur. J. Med. Chem.* **2015**, *89*, 581-596.

(48) Toledo, C. E. P.; Souza, M. A.; Fraga, M. R.; Ribeiro, L. C.; Ferreira, A. P.; Vitral, R. W. F. Cellular viability and nitric oxide (NO) production by J774 macrophages in the presence of orthodontic archwires. *J. Biomed. Sci. Eng.* **2012**, *5*, 255-262.

(49) Yao, D.; Vlessidis, A. G.; Evmiridis, N. P. Determination of nitric oxide in biological samples. *Microchim. Acta.* **2004**, *147*, 1-21.

(50) Huang, Z. B.; Zheng, Y. X.; Li, N.; Cai, S. L.; Huang, Y.; Wang, J.; Hu, X. W.; Wang, Y.; Wu, J.; Fan, X. G. Protective effects of specific cannabinoid receptor 2 agonist GW405833 on concanavalin A-induced acute liver injury in mice. *Acta. Pharmacol. Sin.* **2019**, *40*, 1404-1411.

(51) Wang, Y.; Zhi, Y.; Jin, Q.; Lu, S.; Lin, G.; Yuan, H.; Yang, T.; Wang, Z.; Yao, C.; Ling, J.; Guo, H.; Li, T.; Jin, J.; Li, B.; Zhang, L.; Chen, Y.; Lu, T. Discovery of 4-((7H-Pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-1H-pyrazole-3-carboxamide (FN-1501), an FLT3- and CDK-kinase inhibitor with potentially high efficiency against acute myelocytic leukemia. *J. Med. Chem.* **2018**, *61*, 1499-1518.

(52) Jiang, F.; Hu, Q.; Zhang, Z.; Li, H.; Li, H.; Zhang, D.; Li, H.; Ma, Y.; Xu, J.; Chen, H.; Cui, Y.; Zhi, Y.; Zhang, Y.; Xu, J.; Zhu, J.; Lu, T.; Chen, Y. Discovery of benzo[cd]indol-2(1H)-ones and pyrrolo[4,3,2-de]quinolin-2(1H)-ones as bromodomain and extra-terminal domain (BET) inhibitors with selectivity for the first bromodomain with potential high efficiency against acute gouty arthritis. *J. Med. Chem.* **2019**, *62*, 11080-11107.

(53) Van der Fits, L.; Mourits, S.; Voerman, J. S.; Kant, M.; Boon, L.; Laman, J. D.; Cornelissen, F.; Mus, A. M.; Florencia, E.; Prens, E. P.; Lubberts, E. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J. Immunol.* **2009**, *182*, 5836-5845.

(54) Zhu, Y. Q.; Dong, L. One-pot synthesis of polysubstituted spirofluorene-indene via Ru(II)-catalyzed [3 + 2] annulation and intramolecular friedel-crafts cyclization. *J. Org. Chem.* **2015**, *80*, 9973-9979.

Table of Contents Graphic