学位論文（博士）

三重変異 EGFR に対する新規阻害薬の創製研究

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目次

論文リスト

本論文には学術雑誌に掲載された次の報文を基礎とするものである。第 1 章には 1)、第 2 章には 2)の論文を用いた。

1) Synthesis, activity, and their relationships of 2,4-diaminonicotinamide derivatives as EGFR inhibitors targeting C797S mutation, Hideaki Kageji, Takayuki Momose, Yasuhito Nagamoto, Noriko Togashi, Isao Yasumatsu, Yosuke Nishikawa, Kawori Kihara, Kumiko Hiramoto, Megumi Minami, Naomi Kasanuki, Takeshi Isoyama, Hiroyuki Naito, Bioorg Med Chem Lett. 2024;98:129575.

2) Discovery of a potent, selective, and orally available EGFR C797S mutant inhibitor (DS06652923) with *in vivo* antitumor activity, Hideaki Kageji, Takayuki Momose, Masayuki Ebisawa, Yusuke Nakazawa, Hiroyuki Okada, Noriko Togashi, Yasuhito Nagamoto, Wataru Obuchi, Isao Yasumatsu, Kawori Kihara, Kumiko Hiramoto, Megumi Minami, Naomi Kasanuki, Takeshi Isoyama, Hiroyuki Naito, Naoki Tanaka, Bioorg Med Chem. 2024;111:117862.

略語リスト

本論文では、以下の略語を用いた。

ADME	absorption, distribution, metabolism, and excretion,
ATP	adenosine triphosphate
Boc	*tert*-butoxycarbonyl
CMBP	cyanomethylenetributylphosphorane
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMA	*N*,*N*-dimethylacetamide
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DMT-MM	4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EtOAc	ethyl acetate
Et_2O	diethyl ether
GI_{50}	50% growth inhibitory concentration
Hex	*n*-hexane
HOAt	3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-ol
IC_{50}	50% inhibitory concentration
MeCN	acetonitrile
MeOH	methanol
NMR	nuclear magnetic resonance (e.g. ^{13}C-NMR, ^{1}H-NMR)
NSCLC	non-small cell lung cancer
$Pd_2(dba)_3$	tris(dibenzylideneacetone)dipalladium(0)
$Pd(dppf)Cl_2$·DCM	[1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane
p-TsCl	*p*-toluenesulfonyl chloride

quant.	quantitative yield
Select-fluor®	1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)
SBDD	structure-based drug design
SEM	2-(trimethylsilyl)ethoxymethyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyranyl
tert-BuOH	*tert*-butylalcohol
WSCI	1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide
Xphos	2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl

序論

がんは、本邦における令和 4 年の死因のおよそ四分の一を占め、中でも肺がんによる死亡者数が最も多い[1)]。肺がん患者の 3 割以上は初回診断時に既に遠隔への転移が見られ[2)]、外科的切除または放射線治療のみでは根治が望めないため[3)]、薬物療法の需要は非常に高い。

肺がんに対する薬物療法は、非選択的な細胞毒性を有する「細胞傷害性抗がん薬」を用いた治療が長らく主流であったが、副作用の強さが課題であった。近年では分子生物学の発展により、がん細胞の増殖や転移に関わる因子に選択的に作用する「分子標的薬」の開発が進んでいる。肺がんに対する分子標的薬の代表的な例として、上皮成長因子受容体(Epidermal Growth Factor Receptor; EGFR）阻害薬が挙げられる。EGFR 阻害薬は、EGFR 活性化変異を有する肺がんに対して高い治療効果を示し、細胞傷害性抗がん薬やがん免疫治療薬よりもその使用が推奨されている [3)]。

一方で、EGFR 阻害薬を用いた治療に対しては、ほぼ必ず耐性が生じて再増悪することが知られている。EGFR 変異陽性肺がんが耐性を獲得する機構は多様であるが、代表的な例として耐性変異の発生が挙げられる。例えば、第 1 世代 EGFR 阻害薬であるゲフィチニブやエルロチニブに対しては、T790M 耐性変異が生じ、9~14 か月で再増悪してしまう[4)]。また、第 2 世代、第 3 世代の阻害薬は EGFR の Cys 残基と共有結合を形成することで不可逆的に EGFR の機能を阻害するが、こうした不可逆型阻害薬に対しては C797S 変異による耐性化が報告されている[5)]。共有結合を形成する Cys 残基が Ser 残基へと変異することで、薬剤が EGFR と共有結合を形成できなくなり、阻害活性が著しく低下するのである。

これまでに、T790M 変異と C797S 変異のそれぞれに対して有効な薬剤は見出されているものの、その両方の耐性変異を有する EGFR に対して有効な薬剤は未だ承認されていない。すなわち、初発の活性化変異に加えて、T790M と C797S の両方の耐性変異が生じた三重変異 EGFR 陽性肺がんに対する有効な治療法は存在せず、新規阻害薬の開発が望まれている。

本論文では、未だ有効な治療薬の無い三重変異 EGFR に対する新規阻害薬の発見と、その構造最適化について報告する。第 1 章では、4-アミノニコチンアミド骨格を有する新規 EGFR 阻害薬の獲得とその構造最適化ついて述べる。第 2 章では、4-アミノニコチンアミ

ド骨格からのスキャフォールドホッピングによる新規骨格の獲得と、構造最適化による高活性化およびキナーゼ選択性の改善について述べる。本研究は構造生物学的手法を駆使して推進されており、Structure-Based Drug Design（SBDD）の好例となった。また、本研究で得られた DS06652923 は三重変異 EGFR 陽性肺がんモデルにおいて、一日一回経口投与で強力な抗腫瘍効果を示し、EGFR 阻害薬としての高い有効性を示した。

本論

第1章　4-アミノニコチンアミド骨格を有する新規 EGFR 阻害薬の構造活性相関研究

1-1 背景

EGFR は ErbB ファミリーに属する受容体型チロシンキナーゼであり、細胞の増殖や成長を調節する機能を持つ。EGFR は細胞外のリガンド結合領域、膜貫通領域、細胞内のキナーゼ領域から成り、他の ErbB ファミリーに属するタンパク質と共通した立体構造を有している。EGFR のリガンド結合領域に上皮成長因子（EGF）等のリガンドが結合すると、EGFR はホモ二量体もしくは他の ErbB ファミリーの受容体とのヘテロ二量体を形成する。その結果、キナーゼ領域が活性化され、ATP を利用した Tyr 残基の自己リン酸化が促進される。リン酸化された Tyr 残基が種々の細胞内タンパク質の結合部位として働くことで下流のシグナルが活性化され、細胞の増殖、生存、分化、接着、浸潤等、様々な機能が発現する[6)]。

EGFR の変異や過剰発現は多くのがんと密接に関係している。非小細胞肺がん （Non-Small Cell Lung Cancer; NSCLC）では 12～47%の患者で EGFR 遺伝子の変異が確認されており、リガンド非依存性の EGFR シグナル活性化と、それに伴うがん細胞の異常な増殖が引き起こされている[7)]。NSCLC で最も頻繁に見られる EGFR 活性化変異はエクソン 19 フレーム欠損変異 （del19） とエクソン 21 点変異（L858R）の 2 つであるが[8)]、これらの活性化変異を有する EGFR 変異陽性肺がんに対して有効な薬剤として見出されたのが第 1 世代（ゲフィチニブ、エルロチニブ）および第 2 世代（アファチニブ）の EGFR 阻害薬である。これらの EGFR 阻害薬はキナーゼ領域の ATP 結合ポケットに結合することで ATP 拮抗的なリン酸化活性阻害を示し、がん細胞の増殖を抑制する。しかしながら、これらの EGFR 阻害薬による治療に対しては 9～14 ヶ月で耐性が生じることが知られており [4)]、最も頻度の多い耐性機構が T790M 変異である。T790M 変異により、薬剤との親和性が低下するだけでなく、ATP との親和性が向上することで、EGFR は耐性を獲得する[9)]。

近年、第 3 世代 EGFR 阻害薬であるオシメルチニブ（**1**）が T790M 耐性変異陽性 NSCLC に対して有効性を示すことが報告された[10)]。オシメルチニブは第 1 世代、第 2 世代の EGFR 阻害薬と同様、ATP 結合ポケットに結合するが、Cys797 との共有結合による不可逆的阻

害と、変異した残基である Met790 との高い親和性により、T790M 耐性変異を克服したと考えられている[11]。

しかし、オシメルチニブに対しても耐性が生じることが既に報告されており[12]、臨床上の課題として認識されている。肺がんがオシメルチニブへの耐性を獲得する機構は多様であるが、その代表的な例として C797S 変異が挙げられる[5]。オシメルチニブと共有結合を形成する Cys 残基が Ser 残基へと変異することで共有結合の形成を形成できなくなり、薬効が著しく低下するのである。C797S 耐性変異に対しては可逆型の第 1 世代 EGFR 阻害薬が有効であるものの、これらの薬剤では T790M 耐性変異には対応できない。従って、T790M と C797S の両方の耐性変異に有効な阻害薬、すなわち、初発の活性化変異（del19 および L858R）に加えて 2 つの耐性変異が生じた三重変異（del19/T790M/C797S および L858R/T790M/C797S）EGFR に対して有効な阻害薬の開発が求められている。これまでに三重変異 EGFR を標的とした阻害薬（**2**, **3**, **4**）は報告されている[13)-16)](Figure 1-1）ものの、未だ承認に至った薬剤は無く、依然として高いアンメットニーズが存在している。以上の背景から、三重変異 EGFR に対して有効な新規阻害薬の獲得に着手した。

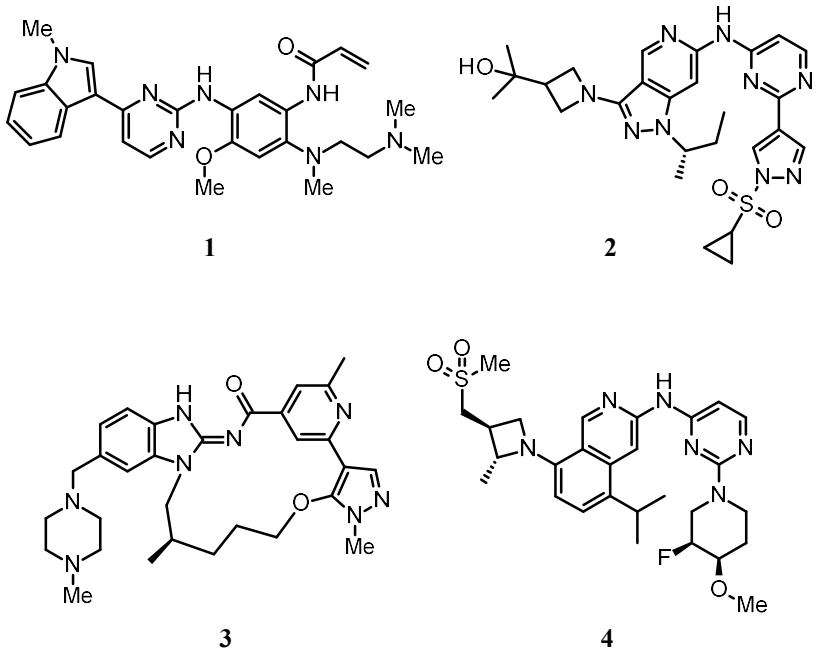


Figure 1-1. Chemical structures of osimertinib (**1**) and selected examples of reversible inhibitors (**2**)[14], (**3**)[15], and (**4**)[16] against EGFR mutant.

1-2 新規骨格を有する EGFR 阻害薬の設計

三重変異 EGFR に対する新規阻害薬を獲得するため、まず既知の可逆型 EGFR 阻害薬 **2**

の共結晶構造を精査した（Figure 1-2A）[14]。報告されている構造情報は L858R/T790M 二重変異体との共結晶構造であり、C797S 変異は含まれていないものの、Cys797 と化合物 **2** との間には相互作用が見られないことから、三重変異 EGFR 阻害薬の設計にも有用であると考えた。この構造情報によると、化合物 **2** は他のほとんどの EGFR 阻害薬と同様、ATP 結合ポケットに結合していた。また、化合物 **2** のアミノピリジン部位はヒンジ領域の Gln791 および Met793 と、スルホニルピラゾール部位は Thr854 および Lys745 とそれぞれ強固な水素結合を形成しており、これらの部位の変換は難しいことが示唆された。以上の解析から、タンパク質との相互作用の少ないアザインダゾール部位に着目した。すなわち、分子内水素結合によりアザインダゾールの環構造を模倣した 4-アミノニコチンアミド骨格を設計し、本骨格を有する誘導体を合成・評価した（Figure 1-2B）。

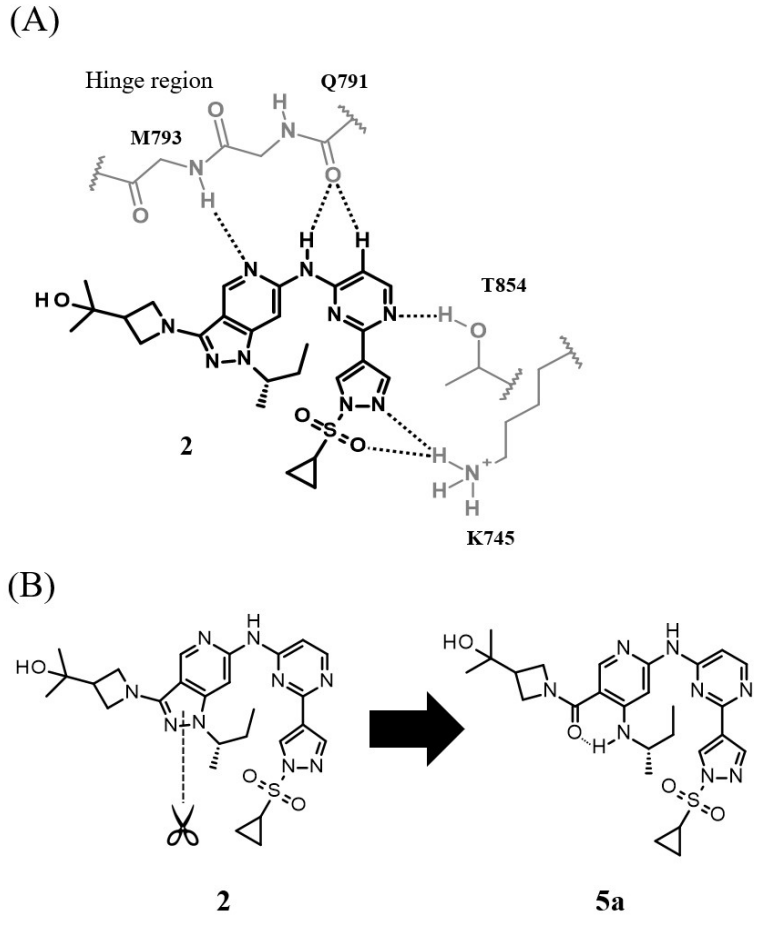
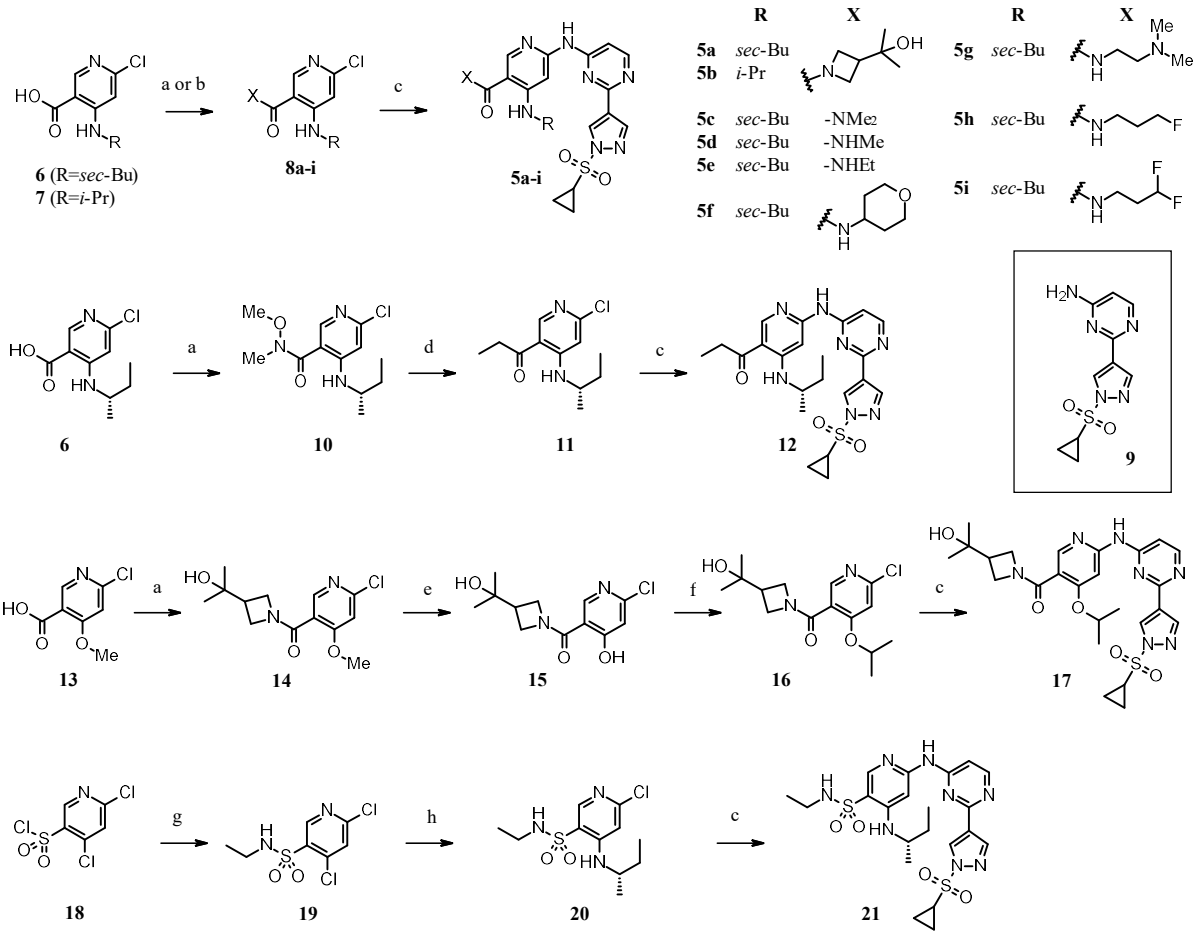


Figure 1-2. (A) Key hydrogen bonds between EGFR L858R/T790M and compound **2**[14]. The side chains of Q791-M793 are omitted. (B) Design of 4-aminonicotinamide scaffold.

1-3 新規骨格を有する誘導体の合成

Scheme 1-1 にニコチンアミド誘導体の合成法を示す。化合物 **5a-i** はニコチン酸誘導体

6 又は **7** より合成した。まず、対応するアミンとのアミド縮合反応により中間体 **8a-i** を得た後、化合物 **9** とバックワルドアミノ化反応を実施することにより化合物 **5a-i** を得た。プロピオニル基を有する化合物 **12** は、**6** から得られたワインレブアミド **10** に対してエチルマグネシウムブロミドを求核付加した後、**9** とバックワルドアミノ化反応を行うことにより得られた。4 位にイソプロピルオキシ基を有する誘導体 **17** は、既知のカルボン酸 **13** から、アミド縮合反応、脱メチル化反応、アルキル化反応、そして、バックワルドアミノ化反応の 4 工程にて合成した。スルホンアミド誘導体 **21** はスルホニルクロリド **18** より合成した。エチルアミンの求核付加反応によりスルホンアミド **19** を得た後、*sec*-ブチルアミンとの芳香族求核置換反応により化合物 **20** を得た。最後に、化合物 **9** とのバックワルドアミノ化反応によりスルホンアミド誘導体 **21** を得た。

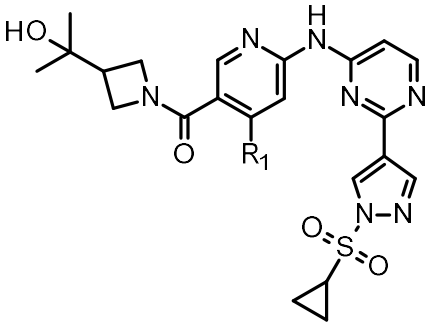


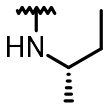
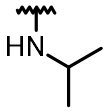
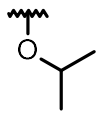
Scheme 1-1. Preparation of 4- and 5-position variants. (a) X-H or *N,O*-dimethylhydroxylamine･HCl, DMT-MM, DIPEA, DMF, rt, 74%–quant.; (b) X-H, HOAt, WSCI･HCl, DIPEA, DMF, rt, 59%–97%; (c) **9**, XPhos, $Pd_2(dba)_3$, Cs_2CO_3 1,4-dioxane or *tert*-BuOH, reflux, 23%–76%; (d) EtMgBr in Et_2O, THF, 0°C, 81%; (e) BBr_3, DCM, rt, 17%; (f) *i*-PrI, K_2CO_3, DMF, rt, 99%; (g) $EtNH_2$･HCl, DIPEA, DCM, rt, quant.; (h) (2*S*)-butan-2-amine･HCl, EtOH, 50°C, 68%.

1-4 新規骨格を有する誘導体の評価

化合物 **5a** およびその類縁化合物 **5b**、**17** の無細胞系における EGFR リン酸化阻害活性を Table 1-1 に示す。化合物 **5a** の del19/T790M/C797S および L858R/T790M/C797S 変異 EGFR に対する IC_{50} はそれぞれ 30 nmol/L、22 nmol/L であり、化合物 **2** には劣るものの明確な EGFR リン酸化阻害活性を有することがわかった。また、野生型 EGFR との選択性は化合物 **2** と同程度であった。野生型 EGFR に対する阻害作用は発疹や下痢といった副作用を示すことが知られているため[17]、変異型 EGFR に対する選択的な阻害は EGFR 阻害薬にとって重要な要素の一つである。化合物 **5a** の *sec*-ブチルアミノ基をイソプロピルアミノ基へと変換した化合物 **5b** は化合物 **5a** と同程度の阻害活性と選択性を示した。一方で、イソプロピルオキシ基へと変換した化合物 **17** では活性が大きく減弱した。この結果から、仮説通り、ピリジン環 4 位と 5 位の置換基間の分子内水素結合によりアザインダゾール環を模倣することが活性発現に重要であることが示唆された。

Table 1-1. Structure-activity relationship of 4-position transformation[a]



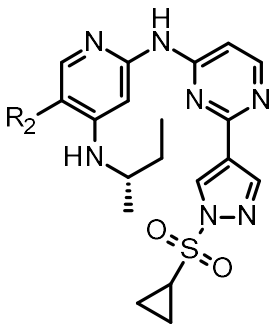
Compd.	R_1	Del19/T790M/C797S IC_{50} (nmol/L)	L858R/T790M/C797S IC_{50} (nmol/L)	WT IC_{50} (nmol/L)
2	-	3.1	2.1	25
5a		30	22	235
5b		72	51	552
17		>5000	>5000	>5000

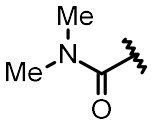
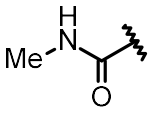
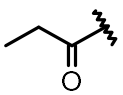
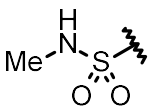
[a] The IC_{50} values are for the enzymatic assay, the method of which is described in the experimental section.

1-5 ピリジン環 5 位の置換基効果

次いで、ピリジン環 5 位に種々の水素結合受容体を導入し、その EGFR リン酸化阻害活性を調べた（Table 1-2)。ジメチルアミド基を導入した化合物 **5c** は化合物 **5a** と比べて 10 倍以上の活性減弱を示した。また、スルホンアミド基を導入した化合物 **21** ではさらに大きく活性が減弱した。プロピオニル基へと変換した化合物 **12** は、化合物 **5a** よりも分子量が小さいにも関わらず、より強いリン酸化阻害活性を示した。さらに、化合物 **12** のメチレンを窒素原子（-NH-）へと変換したメチルアミド **5d** は、野生型 EGFR に対する選択性を維持しつつ、およそ 4 倍の活性向上を示した。以上の結果から、ピリジン環 5 位の構造としては第二級アミドが好ましいことが判った。

Table 1-2. Structure-activity relationship of 5-position transformation[a]



Compd.	R_2	Del19/T790M/C797S IC_{50} (nmol/L)	L858R/T790M/C797S IC_{50} (nmol/L)	WT IC_{50} (nmol/L)
5c		450	300	>5000
5d		3.6	3.6	45
12		13	11	212
21		>5000	4875	>5000

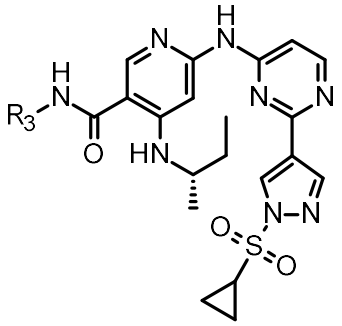
[a] The IC_{50} values are for the enzymatic assay, the method of which is described in the experimental section.

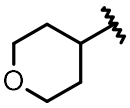
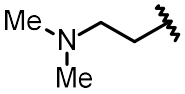
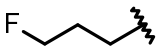
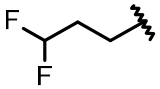
1-6 アミド窒素上置換基の最適化

ピリジン環 5 位の構造を第二級アミドに固定し、アミド窒素上の置換基について精査した（Table 1-3)。無細胞系のリン酸化阻害活性に加えて、EGFR シグナル依存的な増殖を示す Ba/F3 細胞に対する増殖抑制活性も評価した。化合物 **5d** のメチル基をエチル基へと変換したところ、活性の変化は見られなかった（**5e**)。嵩高いテトラヒドロピラニル基（**5f**）や塩基性の *N*,*N*-ジメチルアミノエチル基 （**5g**） は活性の減弱を示した。一方で、疎水性のモノフルオロプロピル基を有する化合物 **5h** およびジフルオロプロピル基を有する化合物 **5i** は、無細胞系での活性については化合物 **5d** と同程度であったものの、増殖抑制活性については向上した。

化合物 **5i** の薬理活性をオシメルチニブ（**1**）と比較したところ、化合物 **5i** は三重変異 EGFR に対してはオシメルチニブよりも優れたリン酸化阻害作用と増殖抑制作用を示した。一方、野生型 EGFR に対する阻害作用はオシメルチニブよりも弱く、化合物 **5i** は三重変異 EGFR 阻害薬としての高い有効性を示した。

Table 1-3. Optimization of mono-substituted amide moiety[a,b]



Compd.	R_3	D19 [c] IC_{50} (nmol/L)	LR [d] IC_{50} (nmol/L)	WT IC_{50} (nmol/L)	D19 [c] GI_{50} (nmol/L)	LR [d] GI_{50} (nmol/L)	WT GI_{50} (nmol/L)
5d		3.6	3.6	45	68	163	927
5e		6.0	5.6	77	72	191	1266
5f		13	14	283	156	452	1655
5g		11	10	265	193	446	1220
5h		4.5	3.7	55	56	129	1047
5i		6.5	5.6	60	35	81	1078
Osimertinib **(1)**		2158	3524	3.8	2065	1288	107

[a] The IC_{50} values are for the enzymatic assay, the method of which is described in the experimental section. [b] The GI_{50} values are for Ba/F3 proliferation assays, the method of which is described in the experimental section. [c] del19/T790M/C797S. [d] L858R/T790M/C797S.

1-7 化合物 **5i** の共結晶構造解析

EGFR L858R/T790M/C797S と化合物 **5i** の複合体の相互作用の様式を分析するために、X 線結晶構造解析を実施した （Figure 1-3)。基本的な結合様式は化合物 **2** と同じであり、化合物 **5i** においても重要な水素結合は維持されていた。一方で、化合物 **5i** においては、アミド基の水素原子と Met793 のカルボニル基の間に新たな水素結合が形成されていた。この新たな水素結合により、化合物 **5i** の阻害活性は化合物 **5c** に比べて向上したと推察さ

れた。また、化合物 **5i** のジフルオロプロピル基は Leu718 および Leu792 の側鎖と疎水性相互作用を形成しており、この相互作用も活性向上に寄与したことが示唆された（Figure 1-4)。

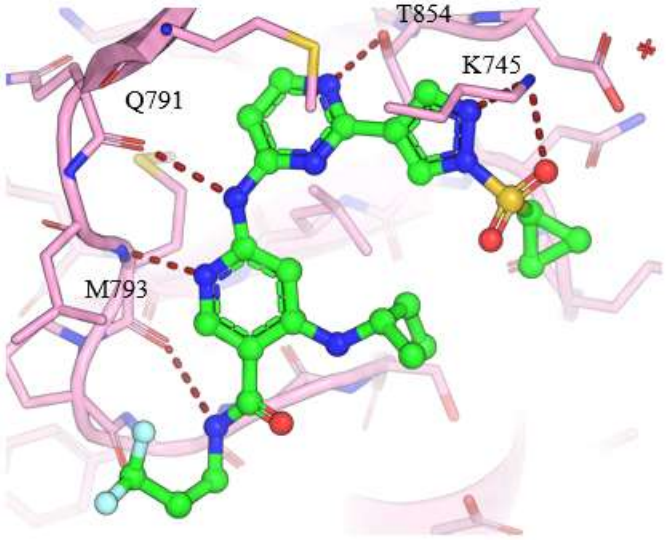


Figure 1-3. X-ray analysis of the EGFR (L858R/T790M/C797S) –compound **5i** complex refined at 2.55 Å resolution (PDB ID: 8WD4).

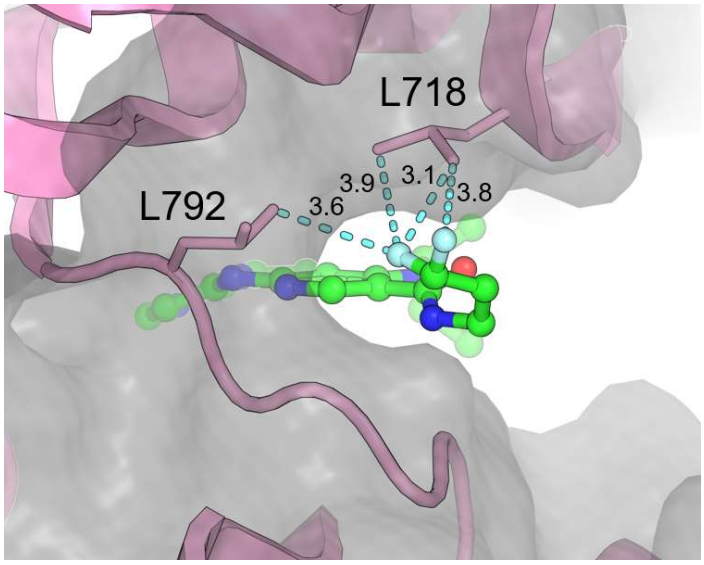


Figure 1-4. Hydrophobic interaction of difluoropropyl group. Structural model figures were generated using PyMOL (Version 2.4.0, Schrödinger, LLC). The protein heavy atoms that were located within 3.95 Å from the fluorine atoms were extracted and the distances are shown in Figure 1-4 with a dashed line. The distances between the fluorine atoms and the three Cδ atoms in L718/L792 (3.1–3.9 Å) were consistent with the typical distances of aliphatic carbon and fluorine interactions (3.3–3.9 Å).[18)]

1-8 小括

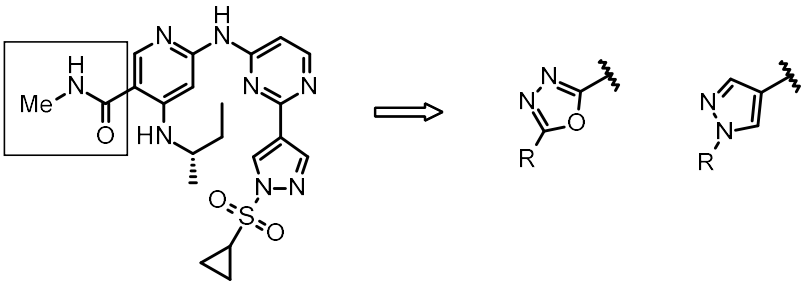
既知の EGFR 阻害薬 **2** と EGFR L858R/T790M との共結晶構造を解析することにより、新規 4-アミノニコチンアミド骨格を有する EGFR 阻害薬 **5a** を獲得した。また、本骨格の構造活性相関研究の結果、ピリジン環 4 位と 5 位の間の分子内水素結合が活性発現に重要であること、4 位の構造としては第二級アミドが好適であること、およびアミド窒素上の置換基としては疎水性の高い置換基が好適であることを見出した。構造最適化の結果、得られた化合物 **5i** は、EGFR del19/T790M/C797S 変異および L858R/T790M/C797S 変異に対して強いリン酸化阻害活性を示した。また、化合物 **5i** は野生型 EGFR に対しては弱い阻害作用を示し、高い変異型 EGFR 選択性を示した。化合物 **5i** と EGFR タンパク質との共結晶構造解析により、化合物 **5i** の強力な阻害活性は Met793 との水素結合および Leu718 および Leu792 との疎水性相互作用に起因することが示唆された。

第 2 章　ビアリール骨格を有する新規 EGFR 阻害薬の構造活性相関研究

2-1 背景

第 1 章にて、三重変異 EGFR 阻害薬である新規 4-アミノニコチンアミド誘導体について報告した。種々の 4-アミノニコチンアミド誘導体を合成、評価したものの、化合物 **5i** よりも阻害活性の優れた化合物を獲得することはできず、本骨格においてこれ以上の活性向上は見込めないと判断した。そこで、更なる活性の向上を目的とし、4-アミノニコチンアミド誘導体を起点とした新規骨格の探索に着手した。

新規骨格を獲得するため、4-アミノニコチンアミド骨格のアミド構造に着目した。アミドは医薬品の部分構造として頻繁に使用される構造であり、その生物学的等価体については多くの研究が報告されている[19]。アミドの生物学的等価体の一つであるヘテロ芳香環に着目し、ピラゾールや 1,3,4-オキサジアゾールを含むビアリール骨格への変換を試みた。



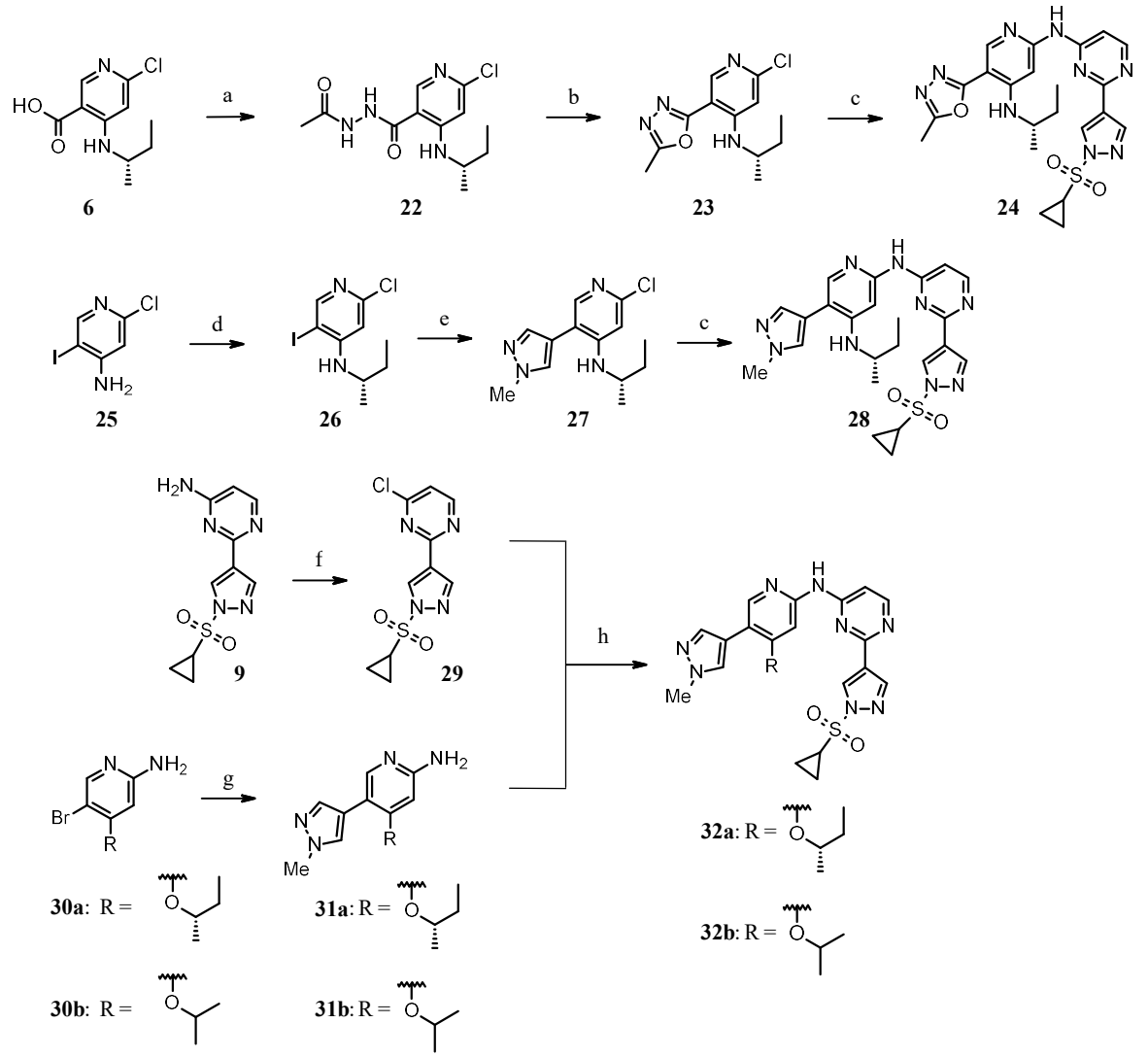
5d

Figure 2-1. 4-アミノニコチンアミド誘導体を起点とした新規骨格のデザイン

2-2 ビアリール骨格を有する誘導体の合成

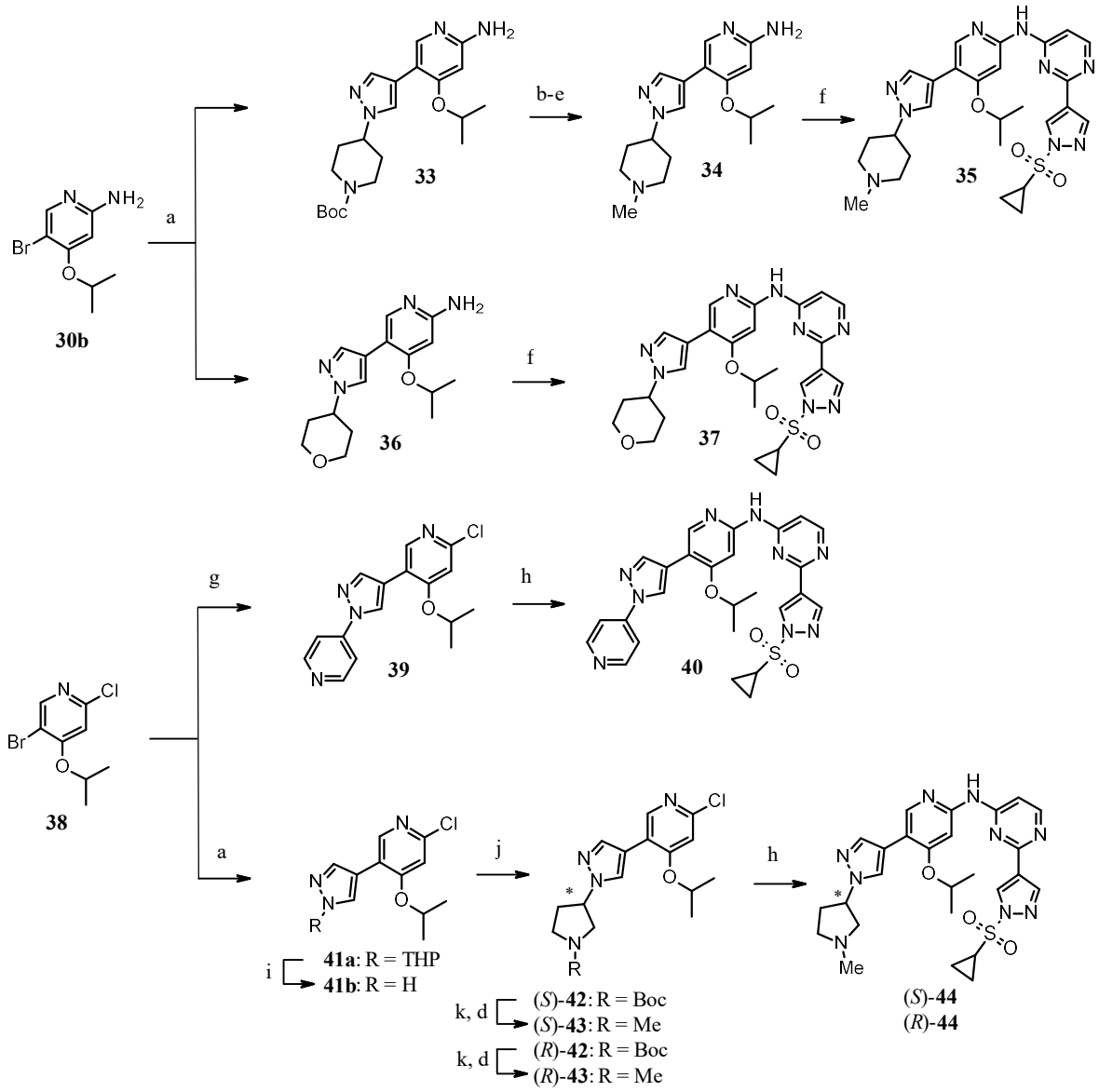
ビアリール骨格を有する誘導体 **24**、**28**、**32a**、そして **32b** は Scheme 2-1 に示した方法にて合成した。1,3,4-オキサジアゾール環を有する化合物 **24** はニコチン酸誘導体 **6** より合成された。アセトヒドラジドとの縮合反応により化合物 **22** を得た後、*p*-トルエンスルホン酸塩化物を用いた脱水反応により環化体 **23** を得た。最後に、**9** とのバックワルドアミノ化反応により化合物 **24** を得た。ピラゾール誘導体 **28** は 4-アミノピリジン誘導体 **25** より三工程にて合成した。まず、メタンスルホン酸エステルを用いたアミノ基のアルキル化反応により化合物 **26** を得た後、鈴木カップリング反応により化合物 **27** を得た。さらに、化合物 **9** とのバックワルドアミノ化反応により化合物 **28** を得た。4-アルコキシピリジン誘導体である **32a** および **32b** は対応する 2-アミノピリジン中間体 **31a** および **31b** とクロロピリミジ

ン中間体 **29** とのバックワルドアミノ化反応により得られた。中間体 **29** は化合物 **9** に対するザンドマイヤー反応により、中間体 **31a** および **31b** は化合物 **30a** および **30b** に対する鈴木カップリング反応により、それぞれ得られた。



Scheme 2-1. Synthesis of compounds **24**, **28**, **32a**, and **32b**. Reagents and conditions: (a) 3*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ol, acetohydrazide, WSCI·HCl, DMF, rt; (b) Et_3N, *p*-TsCl, DCM, 0°C– rt, 94% in 2 steps; (c) **9**, Xphos, $Pd_2(dba)_3$, Cs_2CO_3, 1,4-dioxane or *tert*-BuOH, reflux, 18–34%; (d) (2*R*)-butan-2-yl 4-methylbenzenesulfonate, NaH, DMF, 80°C, quant.; (e) 1-methylpyrazole-4-boronic acid pinacol ester, K_2CO_3, Pd(dppf)Cl_2·DCM, DMF, 100°C, 98%; (f) $CuCl_2$, $MgSO_4$, *tert*-butyl nitrite, MeCN, 60°C, 34%; (g) 1-methylpyrazole-4-boronic acid pinacol ester, Xphos, $Pd_2(dba)_3$, aq. K_2CO_3, 1,4-dioxane, 85°C, 64–84%; (h) Xphos or Xantphos, $Pd_2(dba)_3$, Cs_2CO_3, 1,4-dioxane, reflux, 40–49%.

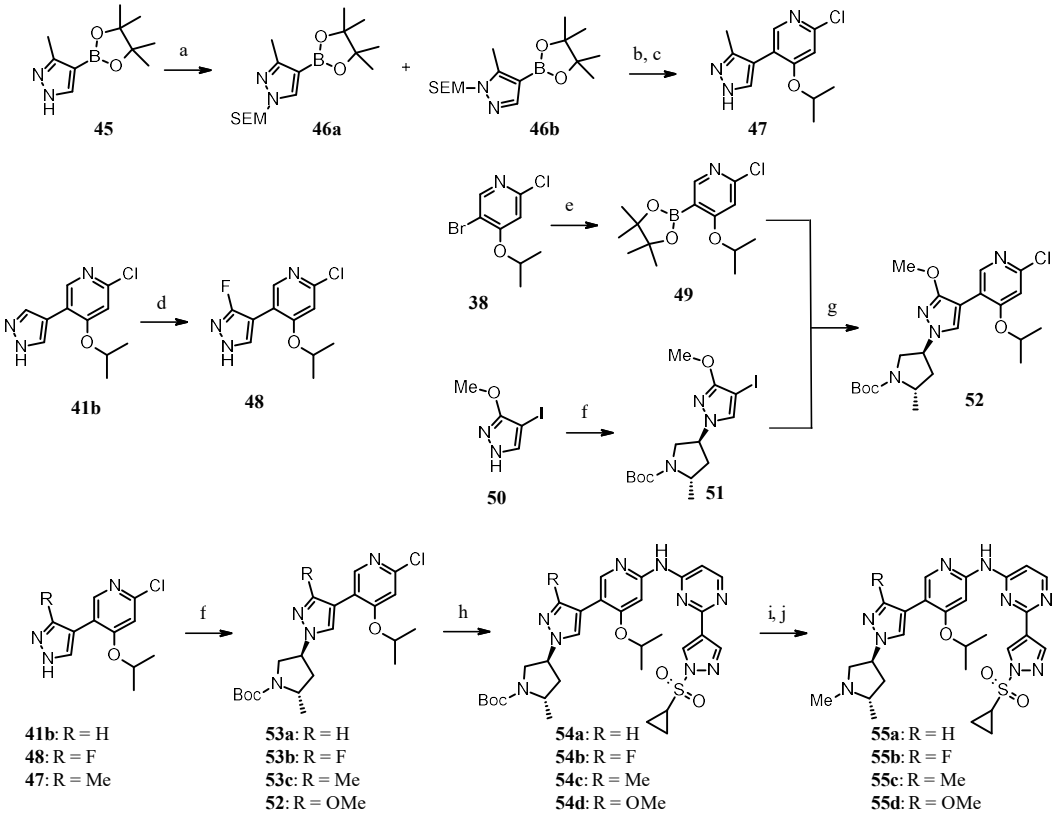
化合物 **35**、**37**、**40** および **44** の合成経路を Scheme 2-2 に示した。化合物 **35** は 5-ブロモピリジン誘導体 **30b** より合成された。まず、鈴木カップリング反応によりピペリジニルピラゾール部位を導入し、得られた化合物 **33** に対してアセチル化、Boc 基の除去、ホルムアルデヒドとの還元的アミノ化およびアセチル基の除去を行うことで化合物 **34** を得た。得られた **34** と 4-クロロピリミジン誘導体 **29** とのバックワルドアミノ化反応により、化合物 **35** を得た。テトラヒドロピラン誘導体 **37** は、**30b** に対して鈴木カップリングによりテトラヒドロピラニルピラゾール部位を導入した後、化合物 **29** とバックワルドアミノ化反応を行うことで得られた。ピリジン誘導体 **40** は、5-ブロモピリジン誘導体 **38** に対して鈴木カップリング反応および化合物 **9** とのバックワルドアミノ化反応を実施することにより合成した。*N*-メチルピロリジン誘導体 **44** については、THP 基により保護されたピラゾールボロン酸エステルと化合物 **38** との鈴木カップリングにより化合物 **41a** を得た後、THP 基を除去し、その後、メシル酸エステルとのアルキル化反応によりピロリジン部分を導入し、化合物 **42** を得た。Boc 基を除去した後、還元的アミノ化反応によりメチル基を導入することで化合物 **43** を得た。最後に、化合物 **9** とバックワルドアミノ化反応を行うことにより、目的の化合物 **44** を得た。



Scheme 2-2. Synthesis of compounds **35**, **37**, **40**, and **44**. Reagents and conditions: (a) The corresponding boronic acid pinacol ester, Xphos, $Pd_2(dba)_3$, aq. K_2CO_3, 1,4-dioxane, 85°C–95°C, 35%–70%; (b) AcCl, pyridine, DCM, 96%; (c) HCl, 1,4-dioxane, DCM, quant.; (d) aq. HCHO, $NaBH(OAc)_3$, DIPEA, MeOH, 64%–94%; (e) HCl, MeOH, DCM, 60°C, quant.; (f) **29**, Xphos, $Pd_2(dba)_3$, Cs_2CO_3, *tert*-BuOH or 1,4-dioxane, reflux, 60%–70%; (g) 4-[4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-1-yl]pyridine, $Pd(dppf)Cl_2$ ·DCM, aq. K_2CO_3, 1,4-dioxane, 90°C, 66%; (h) **9**, Xphos, $Pd_2(dba)_3$, Cs_2CO_3, *tert*-BuOH, reflux, 19%–26%; (i) TFA, Et_3SiH, DCM, 77%; (j) the corresponding mesylate, Cs_2CO_3, DMA, 100°C, 77%–qunat.; (k) HCl, 1,4-dioxane, quant..

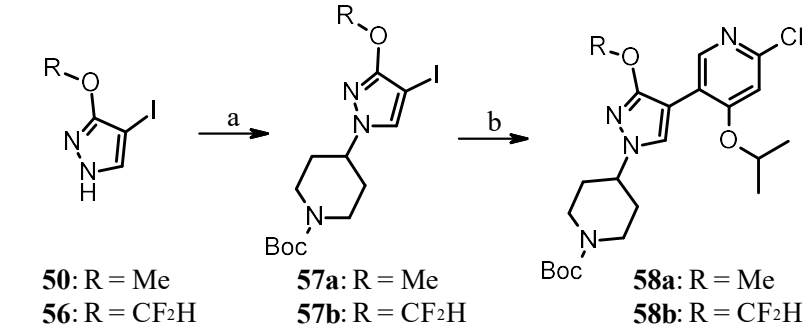
ピラゾール環の 3 位に多様な置換基を有する 1,5-ジメチルピロリジン誘導体 **55a-d** は Scheme 2-3 に示す方法で合成された。ピラゾール環の 3 位にメチル基を有する中間体 **47**

は、ボロン酸エステル **45** から合成された。まず、ピラゾールを SEM 基により保護することで、位置異性体 **46a** および **46b** の 1:1 混合物を得た。これらの混合物に対して鈴木カップリングによりピリジン部位を導入した後、SEM 基を脱保護することで中間体 **47** を得た。ピラゾール 3 位にフルオロ基を有する中間体 **48** は、Select-fluor®を用いて化合物 **41b** をフッ素化することにより得られた。ピラゾール 3 位にメトキシ基を有する中間体 **52** は、ボロン酸エステル **49** とヨードピラゾール **51** の鈴木カップリング反応により合成された。化合物 **49** および **51** は、臭化物 **38** のボリル化反応および化合物 **50** の光延反応によりそれぞれ得られた。得られた中間体 **41b**、**47** および **48** に対して、光延反応によりピロリジン部位を導入した後、化合物 **52** および **53a-c** に対して、バックワルドアミノ化反応、Boc 基の除去、並びに還元的アミノ化反応を実施することで対応する最終体 **55a-d** を得た。



Scheme 2-3. Synthesis of compounds **55a–d**. Reagents and conditions: (a) SEMCl, DIPEA, DCM, 0°C–rt, 99%; (b) **38**, Pd(dppf)Cl_2·DCM, aq. Na_2CO_3, 1,4-dioxane, 90°C, 69%; (c) TFA, DCM, quant.; (d) Select-Fluor®, AcOH, MeCN, 80°C, 22%; (e) 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, *n*-BuLi in Hex, THF, −78°C, quant.; (f) *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-methylpyrrolidine-1-carboxylate, CMBP, toluene, reflux, 57%–98%; (g) Pd(dppf)Cl_2 ·DCM, aq. K_2CO_3, 1,4-dioxane, reflux, 71%; (h) **9**, Xphos, $Pd_2(dba)_3$, Cs_2CO_3, *tert*-BuOH, reflux, 34%–56%; (i) TFA, DCM, 79%–88%; (j) aq. HCHO, $NaBH(OAc)_3$, MeOH, DCM, 69%–94%.

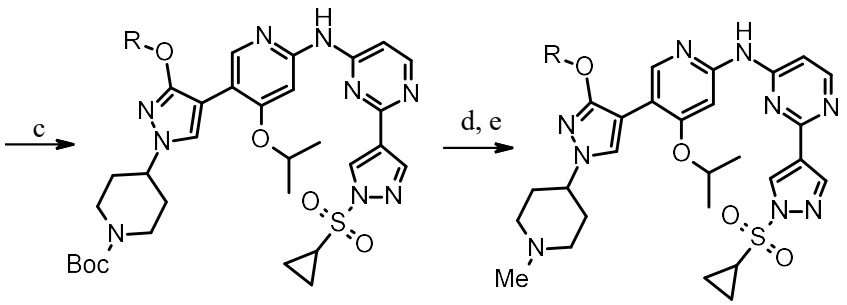
化合物 **60a** と **60b** は化合物 **55d** の合成法と同様の方法にて得られた (Scheme 2-4)。ヨードピラゾール誘導体 **50** と **56** に対してアルキル化反応によりピペリジン部位を導入し、**57a** と **57b** をそれぞれ得た。その後、鈴木カップリング反応、バックワルドアミノ化反応、Boc 基の除去、還元的アミノ化反応を経て、化合物 **60a** と **60b** を得た。



50: R = Me
56: R = CF_2H

57a: R = Me
57b: R = CF_2H

58a: R = Me
58b: R = CF_2H



59a: R = Me
59b: R = CF_2H

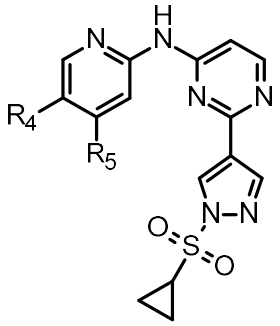
60a: R = Me
60b: R = CF_2H

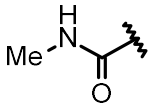
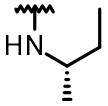
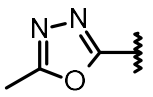
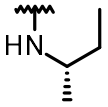
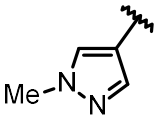
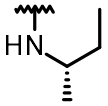
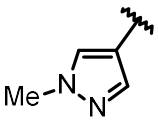
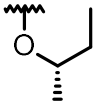
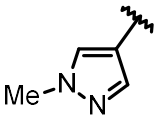
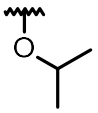
Scheme 2-4. Synthesis of compounds **60a** and **60b**. Reagents and conditions: (a) *tert*-butyl 4-methylsulfonyloxypiperidine-1-carboxylate, Cs_2CO_3, DMA, 100°C, 72%–84%; (b) **49**, $Pd(dppf)Cl_2$, aq. K_2CO_3, 1,4-dioxane, 100°C, 51%–70%; (c) **9**, Xphos, $Pd_2(dba)_3$, Cs_2CO_3, 1,4-dioxane, 90°C, 31%–47%; (d) TFA, DCM, 80%–85%; (e) aq. HCHO, $NaBH(OAc)_3$, MeOH, DCM, 58%–83%.

2-3 ビアリール骨格を有する誘導体の評価

ビアリール骨格を有する誘導体 **24**、**28**、**32a** および **32b** の無細胞系における EGFR del19/T790M/C797S および野生型 EGFR のリン酸化阻害活性を Table 2-1 に示す。1,3,4-オキサジアゾール誘導体 **24** とピラゾール誘導体 **28** はいずれも活性の減弱を示した。次いで、ピリジン環 4 位(R_5)の置換基効果について検討した。ピラゾール誘導体 **28** のピリジン環 4 位の窒素原子を酸素原子へと変換した化合物 **32a** は、リン酸化阻害活性の向上を示した。さらに、*sec*-ブチルオキシ基をイソプロピルオキシ基へと変換した **32b** は **32a** と同等の活性を示した。

Table 2-1. Structures-activity relationship of 5-position transformation. [a]



Compd.	R_4	R_5	D19/TM/CS [b] IC_{50} (nmol/L)	WT IC_{50} (nmol/L)
5d			3.6	45
24			13	215
28			14	275
32a			2.6	22
32b			2.4	24

[a] The IC_{50} values are for the enzymatic assay, the method of which is described in the experimental section. [b] del19/T790M/C797S.

2-4 塩基性官能基導入による活性向上

次に、更なる活性向上を目的とし、ピラゾール環窒素原子上の置換基変換を実施した。実際に誘導体の合成を開始する前に、化合物 **32b** と EGFR del19/T790M/C797S のドッキングシミュレーションを実施した (Figure 2-2)。その結果、化合物 **32b** のピラゾール環窒素原子上のメチル基はリガンド結合ポケットの外へ配向していることが示唆された。そのため、ピラゾール環上の窒素原子に導入する置換基としては親水性のものが好ましいと推察された。加えて、この位置に塩基性の官能基を導入することで活性が向上したという報告があることから[20]、塩基性置換基を導入することとした。その結果を Table 2-2 に示す。強塩基性の *N*-メチルピペリジン環を有する化合物 **35** では化合物 **32b** に比べて増殖抑制活性が 6

倍向上した。*N*-メチルピペリジン環と同じく強塩基性の *N*-メチルピロリジン環を有する (*S*)-および (*R*)-**44** や 1,5-ジメチルピロリジン環を有する **55a** も活性向上を示した一方で、他のヘテロ六員環、例えば、中性のテトラヒドロピラン環を有する **37** や弱塩基性のピリジン環を有する **40** では活性の向上は見られなかった。このことから、活性の向上には強い塩基性部位が必要であることが判った。

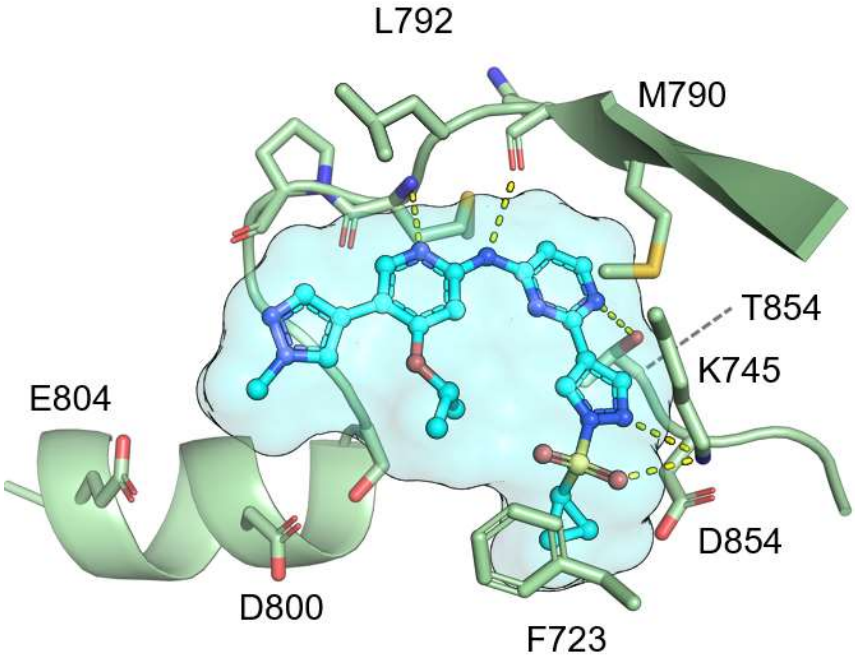
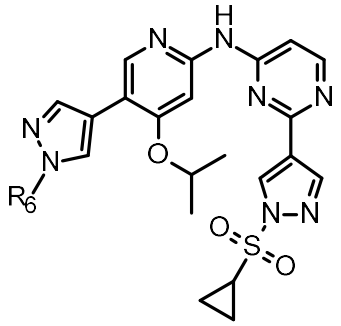
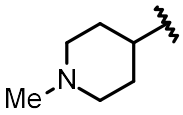
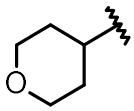
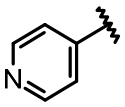
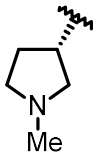
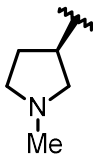
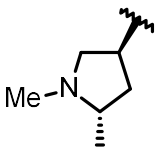


Figure 2-2. The modeled binding mode of compound **32b** with the EGFR C797S model. The secondary structure of EGFR is depicted as a cartoon model, with key residues shown as stick models. The compound **32b** is represented as a ball-and-stick model, with its molecular surface shown in transparent cyan. Hydrogen bonds to the compound are indicated by dashed yellow lines. L792, the gk+2 residue (Ghose's notation[21]), is shown with an orange molecular surface. For the sake of clarity, residues 696–722, 794–786, 808–853, and 858–1019 have been excluded from the depiction.

Table 2-2. Structures-activity relationship of 5-position transformation. [a, b]



Compd.	R_6	D19/TM/CS [c] IC_{50} (nmol/L)	WT [d] IC_{50} (nmol/L)	D19/TM/CS [c] GI_{50} (nmol/L)	WT [d] GI_{50} (nmol/L)
32b		2.4	24	60.8	879
35		2.1	8.5	9.6	203
37		2.7	21.2	45.9	353
40		20.4	237	601	>10000
(*S*)-**44**		1.7	9.7	19	311
(*R*)-**44**		1.6	9.8	21	318
55a		2.5	11	26	387

[a] The IC_{50} values are for the enzymatic assay, the method of which is described in the experimental section. [b] The GI_{50} values are for Ba/F3 proliferation assays, the method of which is described in the experimental section. [c] del19/T790M/C797S. [d] wildtype.

2-5 化合物 **55a** のキナーゼ選択性評価

化合物 **55a** のキナーゼ選択性をキナーゼパネルアッセイにて評価した。オフターゲット

キナーゼ阻害は毒性の原因となるため、キナーゼ阻害剤には一般に高いキナーゼ選択性が求められる[22]。化合物 **55a** の 161 キナーゼに対する阻害活性を評価し、その結果を Figure 2-3 に示した。化合物 **55a** は 161 キナーゼ中 48 キナーゼ (6 種類の変異 EGFR を含む) を阻害率 50%以上で阻害した。オシメルチニブは同じ条件で 10 キナーゼ (5 種類の変異 EGFR を含む) しか阻害しないため、キナーゼ選択性の改善が必要であると推察された。

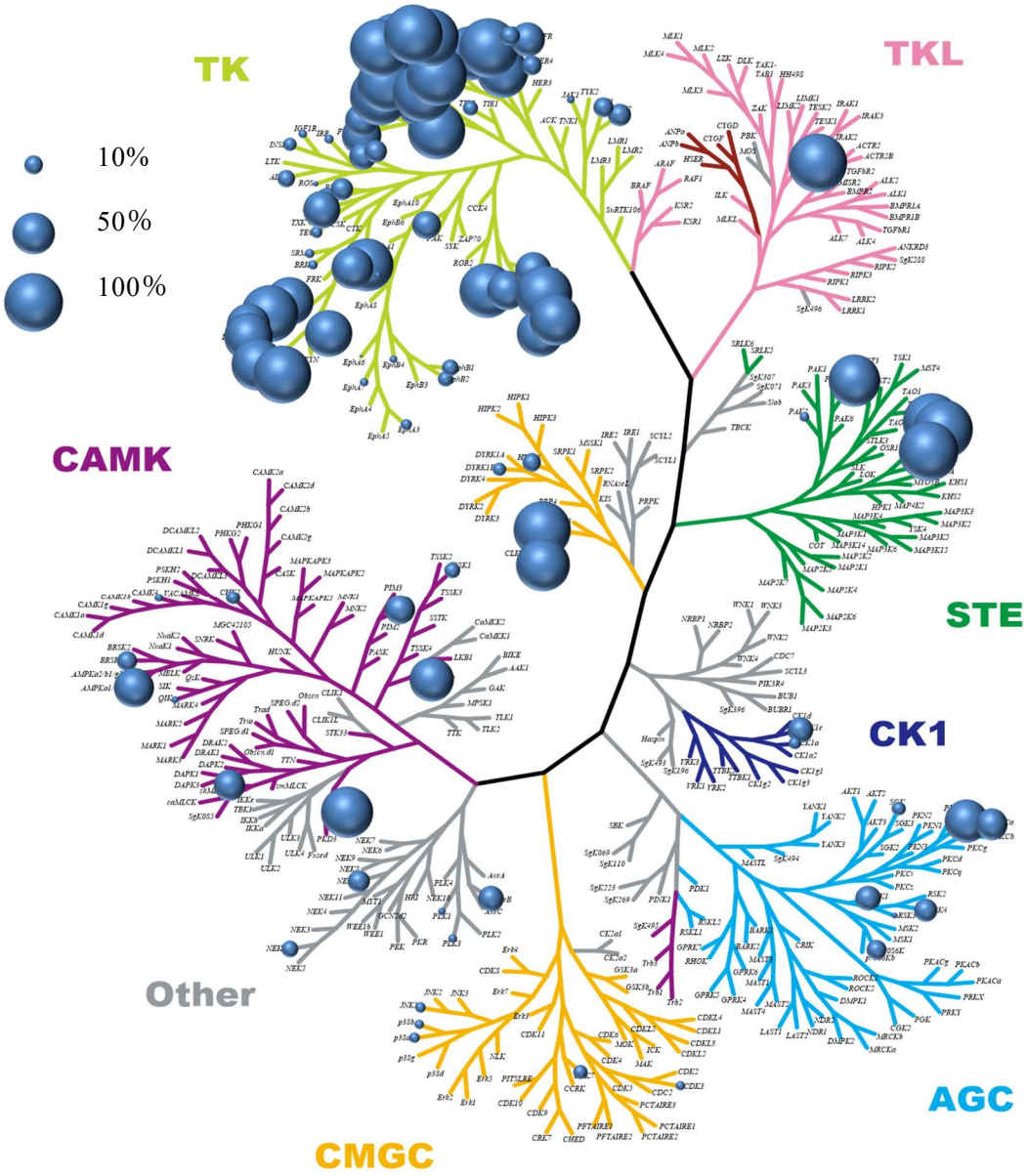


Figure 2-3. Kinome inhibition plot by compound **55a**. The inhibitory rate against selected 161 kinases was evaluated (compound conc.: 200 nmol/L, ATP conc.: 1 mmol/L) and is indicated as a corresponding bubble plotted in the phylogenetic tree of the human protein kinome. The size of the bubble indicates the inhibition rate (See experimental section for the specific values). AGC, kinases from the protein kinase A, G, and C families; CAMK, calcium/calmodulin-dependent protein kinases; CK1, casein kinase 1; CMGC: kinases from the cyclin-dependent kinase, MAPK, glycogen synthase kinase, and casein kinase II families; STE, homologs of yeast sterile 7, sterile 11, and sterile 20 kinases; TK, tyrosine kinases; TKL, tyrosine kinase like kinases.

2-6 キナーゼ選択性改善を目的としたピラゾール 3 位への置換基導入

キナーゼ選択性を改善するため、EGFR の Leu792 によって作り出される“selectivity pocket“に着目した。Leu792 はヒンジ部位に位置する残基であるが、EGFR 以外の多くのキナーゼではこの部分の残基は Leu よりも嵩高い Phe 又は Tyr であることが知られている。従って、EGFR を含む一部のキナーゼでのみ、この部位に置換基を導入することが可能である一方で、その他の多くのキナーゼでは立体障害により置換基を導入することができない。そのため、この部位の空間はキナーゼ選択性を向上させる目的でしばしば利用され、selectivity pocket と呼ばれている[23]。

selectivity pocket がどこに位置しているかを特定するため、化合物 **55a** と EGFR T790M/C797S/L858R のドッキングシミュレーションを行った(Figure 2-4)。その結果、化合物 **55a** のピラゾール環 3 位の先に selectivity pocket は位置しており、置換基を導入できる空間が存在することが示唆された。以上の結果から、キナーゼ選択性改善のため、ピラゾール 3 位への置換基の導入を実施した。

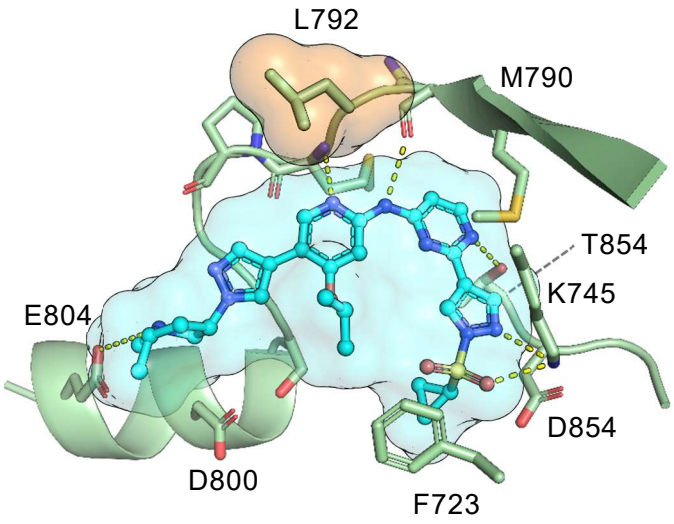
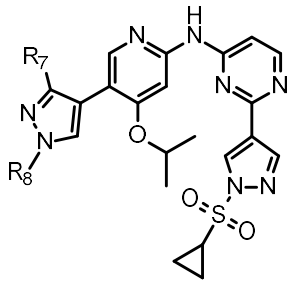
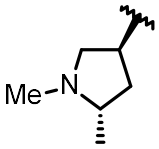
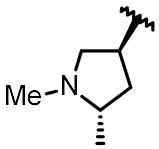
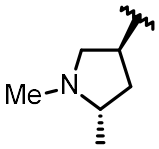
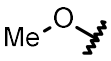
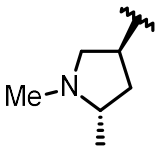
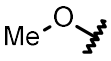
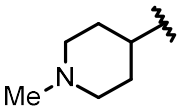
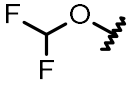
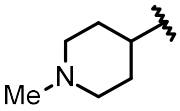


Figure 2-4. The modeled binding mode of compound **55a** with the EGFR C797S model. The representation style remains the same as in Figure 2-2.

その結果を Table 2-3 に示す。フルオロ基を 3 位に導入した化合物 **55b** はわずかな活性減弱を示した。メチル基を有する化合物 **55c** は大きな活性の減弱を示した一方で、メトキシ基を有する化合物 **55d** は活性を維持することが判った。塩基性部位については、前述の構造活性相関の通り、ピペリジン環 (**60a**) も許容された。 ピラゾール 3 位の置換基としては、メトキシ基の代わりにジフルオロメトキシ基でも強い活性が維持された (**60b**)。

Table 2-3. Structures-activity relationship of the pyrazole substituents. [a,b]



Compd.	R_7	R_8	D19 [c] IC_{50} (nmol/L)	WT [d] IC_{50} (nmol/L)	D19 [c] GI_{50} (nmol/L)	WT [d] GI_{50} (nmol/L)
55a			2.5	11	26	387
55b			3.0	13	43	856
55c			6.8	66	168	2294
55d			2.8	5.1	12	574
60a			1.4	3.7	6.5	565
60b DS06652923			2.0	6.0	9.4	760

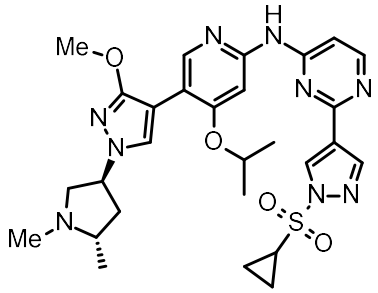
[a] The IC_{50} values are for the enzymatic assay, the method of which is described in the experimental section. [b] The GI_{50} values are for Ba/F3 proliferation assays, the method of which is described in the experimental section. [c] del19/T790M/C797S. [d] wildtype.

2-7 化合物 **55d**、**60a** および **60b** の *in vitro* 評価結果の比較

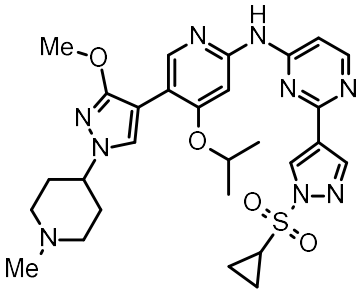
強い阻害活性を示した化合物 **55d**、**60a** および **60b** の *in vitro* 評価結果を Table 2-4 に示した。全ての化合物が del19/T790M/C797S 変異型 EGFR だけではなく、L858R/T790M/C797S 変異型 EGFR に対して強いリン酸化阻害活性を示した。また、これらの化合物の野生型

EGFR に対する選択性は同等であった。化合物 **55d** は他の二つの化合物に比べて、高い LogD を示した。この脂溶性の差を反映し、**60a** と **60b** は **55d** と比べて、高い肝ミクロソーム代謝安定性を示した。また、いずれの化合物も、酸性域では良好な溶解性を示したが、中性域における溶解性は乏しかった。MDCK 細胞における膜透過性については、化合物 **60b** が最も良好な値を示した。タンパク結合率については、いずれの化合物も非常に高い値を示した。以上の結果から、他の化合物よりも優れた代謝安定性と膜透過性を示した化合物 **60b** (DS06652923) を選抜し、更なる高次評価を実施した。

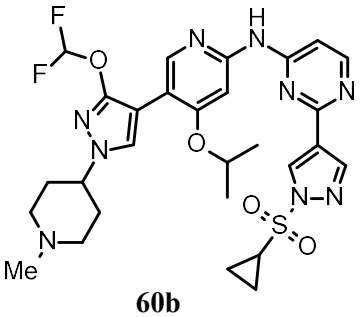
Table 2-4. *In vitro* pharmacological activity and ADME profile of **55d**, **60a**, and **60b**.[a,b]



55d



60a



60b

Compd.	**55d**	**60a**	**60b** (DS06652923)
D19[c]/LR[d]/WT[e] IC_{50} (nmol/L)	2.8/1.3/5.1	1.4/0.75/3.7	2.0/0.89/6.0
D19[c]/LR[d]/WT[e] GI_{50} (nmol/L)	12/23/574	6.5/18/565	9.4/13/760
LogD	5.4	4.4	4.6
Metabolic stability[f] [human/mouse] (%remaining)	1/20	24/48	18/55
Solubility [pH 1.2/pH6.8] (μg/mL)	>1100/0	>1100/15	1200/0
MDCK Papp (10^{-6} cm/s)	5.2	3.1	9.0
Plasma protein binding [human/mouse] (%)	>99.8/>99.8	>99.8/>99.8	>99.8/>99.8

[a]The IC_{50} values are for the enzymatic assay, the method of which is described in the experimental section. [b]The GI_{50} values are for the Ba/F3 proliferation assay, the method of which is described in the experimental section. [c]del19/T790M/C797S. [d]L858R/T790M/C797S. [e]Wildtype. [f]Remaining rate of tested compounds (1.0 μmol/L) after incubation for 0.5 h in human or mouse liver microsomes (0.5 mg/mL).

2-8 DS06652923 のキナーゼ選択性評価

DS06652923 のキナーゼ選択性プロファイルを Figure 2-5 に示す。DS06652923 は 161 キナーゼのうち、14 キナーゼ (6 種の変異 EGFR を含む) を阻害率 50%以上で阻害し、そのキナーゼ選択性は大きく改善していた。ドッキングシミュレーションにて DS06652923 の結合様式を解析したところ、ジフルオロメトキシ基は selectivity pocket を占有しており、このためにキナーゼ選択性が改善したと推察された。

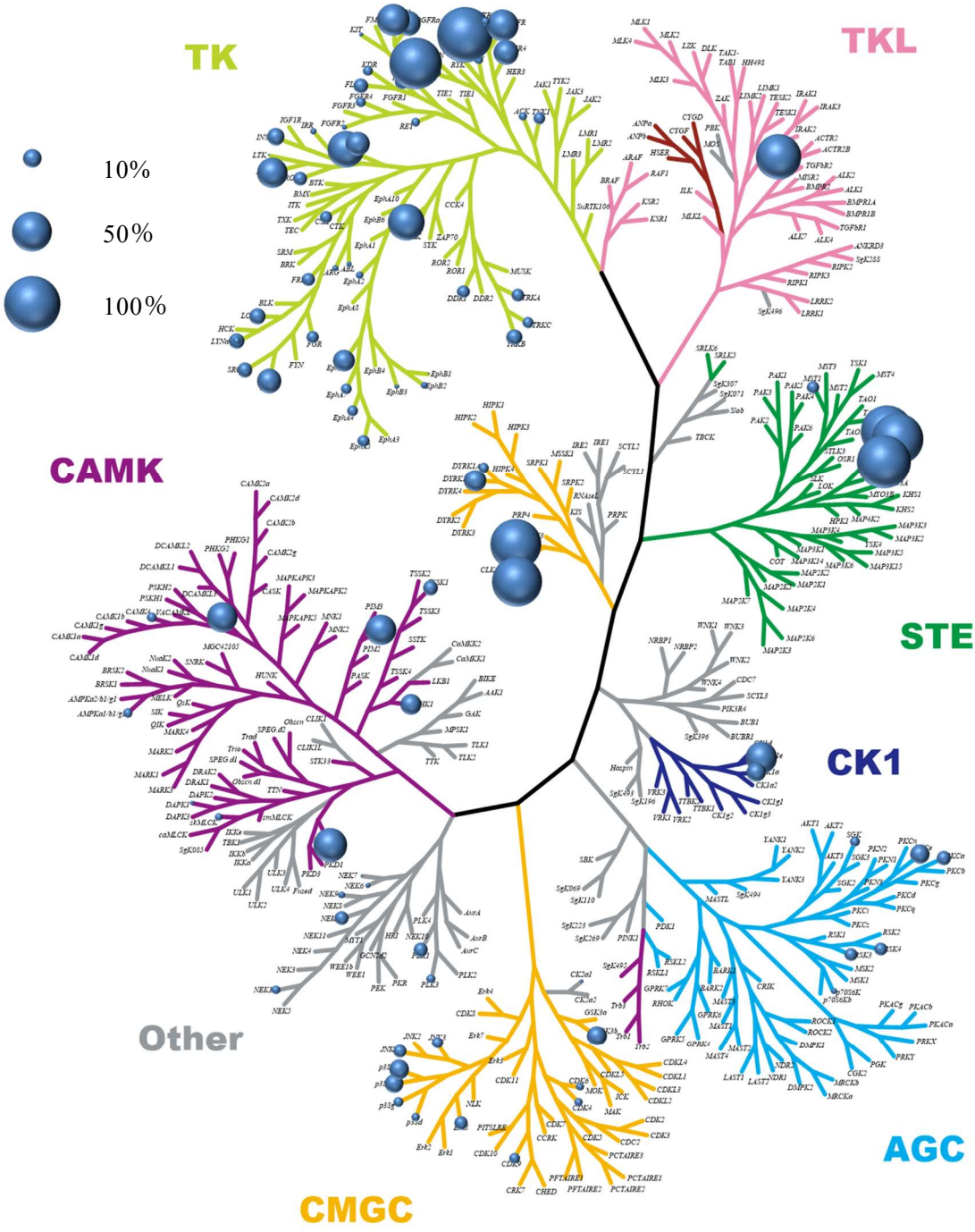


Figure 2-5. Kinome plot of inhibition by **60b** (DS06652923). The inhibitory rate against 161 selected kinases was evaluated (compound conc.: 200 nmol/L, ATP conc.: 1 mmol/L) and is indicated as a corresponding bubble plotted in the phylogenetic tree of the human protein kinase. The size of the bubble indicates the inhibition rate (see experimental section for the specific values).

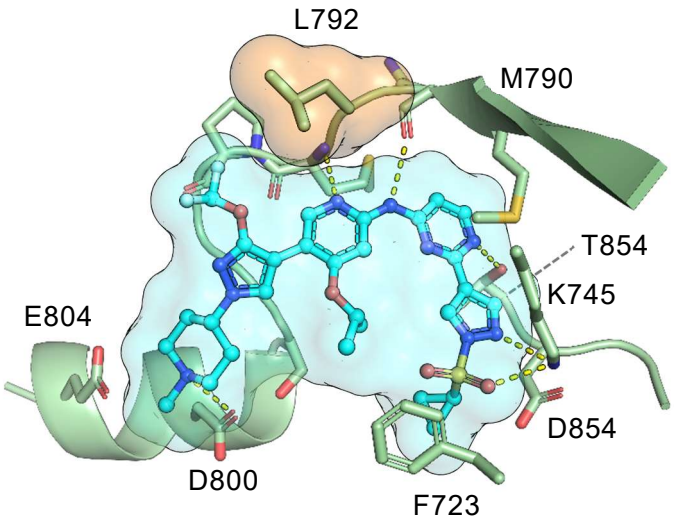


Figure 2-6. The modeled binding mode of compound **60b** (DS06652923) with the EGFR C797S model. The representation style is the same as in Figure 2-2.

2-9 DS06652923 の抗腫瘍効果

Ba/F3 アログラフトマウスモデルを用いて、DS06652923 の抗腫瘍効果を評価した。その結果を Figure 2-7 に示す。メス Balb/c 免疫不全マウスに Ba/F3 (EGFR del19/T790M/C797S) 細胞を移植し、DS06652923 を一日一回 (qd) 又は一日二回 (bid)、5 日間、経口投与した。その結果、全ての投与群で明確な抗腫瘍効果が確認された (Figure 2-7A)。その際、いずれの投与群においても体重減少や毒性の兆候は観察されなかった (Figure 2-7B)。また、化合物の最終投与から 6 時間後の腫瘍中リン酸化レベルをウェスタンブロッティングにて評価した。その結果、全ての投与群において、変異 EGFR のリン酸化は完全に阻害されていた (Figure 2-7C)。その際、腫瘍中および血漿中の両方に化合物が暴露していることを確認した。

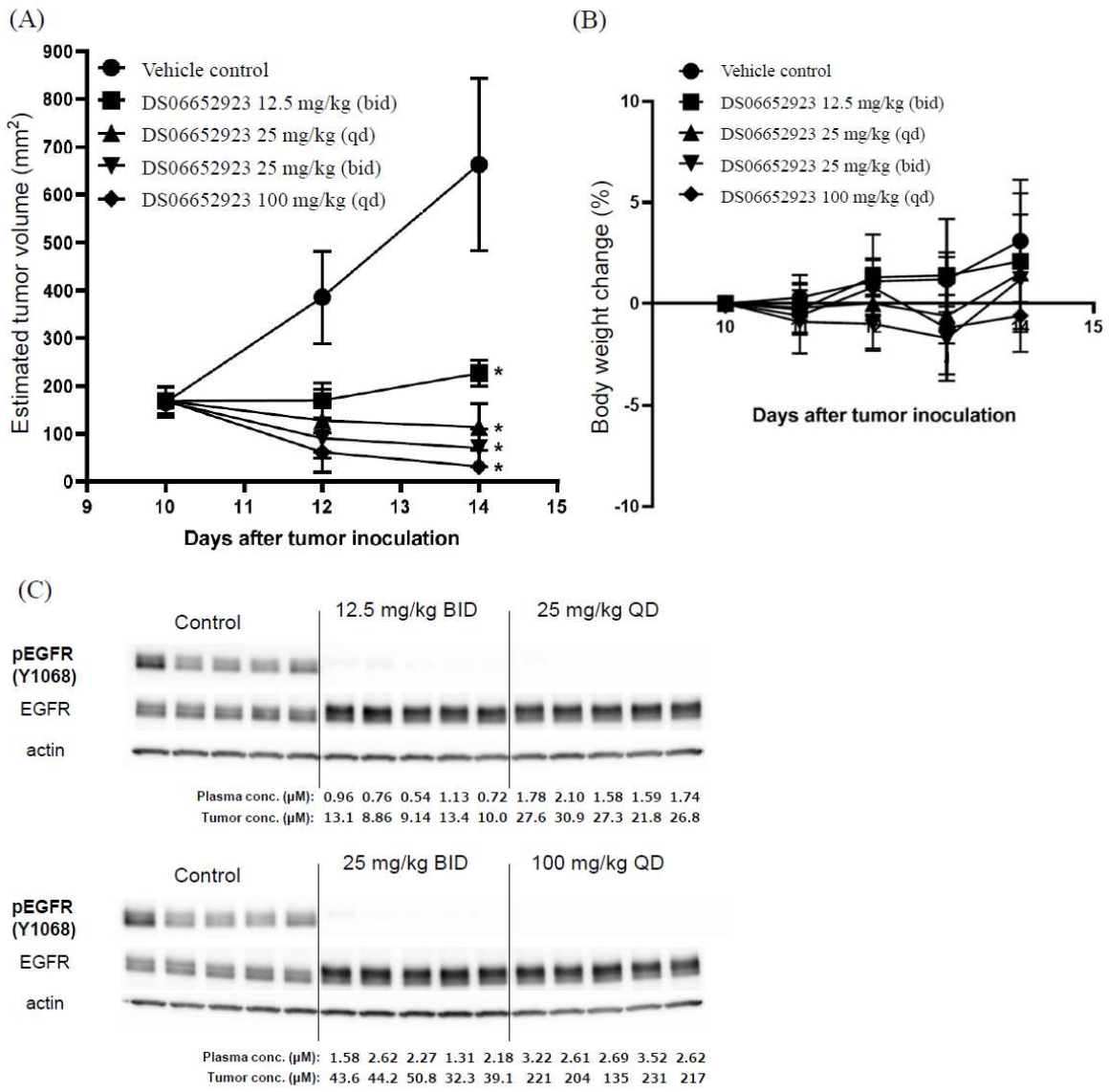


Figure 2-7. The results of antitumor study with DS06652923 in Balb/c nude mice bearing engineered Ba/F3 (EGFR del19/T790M/C797S) tumors. The compound at doses of 12.5 (bid), 25 (qd), 25 (bid), and 100 mg/kg (qd) and the vehicle (0.5% methylcellulose) were orally administered daily from day 10 to 14. (A) Antitumor activity of DS06652923. Each group included five animals and data are presented as mean ± standard error. The asterisk indicates a significant difference between the control group and the treatment group by the parametric Dunnett's test (*P < 0.05). (B) Effects on body weight changes of the mice during treatment with DS06652923. Each group included five animals and data are expressed as mean ± standard error. (C) Immunoblotting of phosphorylated EGFR (pEGFR), total EGFR (EGFR), and β-Actin (Actin) in Ba/F3-EGFR del19/T790M/C797S tumors. Tumors were collected 6 h after the final administration. Conc.: compound concentration.

2-10 小括

化合物 **5d** のアミド基をピラゾールへと変換することで新規ビアリール骨格を有する化合物 **32b** を取得した。ドッキングシミュレーションを活用し、溶媒和部位に塩基性部位を導入することでリン酸化阻害活性が向上した。さらに、ピラゾール 3 位へのジフルオロメトキシ基の導入により、EGFR 以外のキナーゼとの間で立体障害が生じ、キナーゼ選択性が大きく改善した。最適化された化合物 **60b** (DS06652923)の抗腫瘍効果を Ba/F3 アログラフトモデルにおいて評価したところ、一日一回、25 mg/kg の経口投与にて腫瘍の退縮を示した。

結論

本論文では、未だ有効な治療薬の無い三重変異 EGFR に対する新規阻害薬を発見し、複合体構造情報を指標にした構造最適化を経て、マウスへの経口投与により抗腫瘍効果を示す DS06652923 を創出した。

第 1 章では、既知の非共有結合型 EGFR 阻害薬から 4-アミノニコチンアミド骨格を獲得後、アミド部位を変換することで高活性な化合物 **5i** を獲得した。化合物 **5i** と EGFR L858R/T790M/C797S タンパク質との複合体構造情報から、新たに形成された水素結合と疎水性相互作用により活性が向上したことが示唆された。

第 2 章では、4-アミノニコチンアミド骨格のアミド部位をピラゾール環へと変換することで得られたビアリール骨格の構造最適化について報告した。溶媒和部位に塩基性構造を導入することにより活性の向上した化合物 **55a** を獲得した。また、ピラゾール 3 位にアルコキシ基を導入することにより、強い活性を維持したままキナーゼ選択性を改善することに成功した。いずれの変換もドッキングシミュレーションを活用した SBDD により達成された。得られた有望化合物の内、最適な *in vitro* ADME パラメーターを示した DS06652923 は、優れたキナーゼ選択性プロファイルと経口投与による抗腫瘍効果を示し、三重変異 EGFR 阻害薬として高い資質を示した。

EGFR を標的とした阻害薬は多く報告されているものの、三重変異 EGFR に対して有効な薬剤は未だ承認されていない。本研究において得られた知見が、新規 EGFR 阻害薬の創出に繋がることを強く願っている。

謝辞

学士課程および修士課程における指導教員として私の研究者としての基礎を築いて下さった東京大学大学院農学生命科学研究科　渡邉秀典名誉教授に感謝を申し上げます。

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博士論文の発表の機会を与えて下さり、終始ご指導、ご支援を賜りました、第一三共株式会社モダリティ第一研究所 青木一真参事、同社 研究イノベーション企画部　内藤博之主幹、同社　モダリティ第三研究所　北川麻弓所長、同　神子島佳子グループ長に厚く御礼申し上げます。

本研究遂行時の直属の上司であり、本研究の進め方に関して貴重なご助言を賜りました第一三共株式会社モダリティ第一研究所　田中直樹主幹に感謝を申し上げます。本研究遂行時の合成チームリーダーであり、プロジェクトを主導頂きました、第一三共株式会社薬事渉外部　百瀬孝幸担当課長に深く感謝いたします。

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薬理研究を主導して頂き、論文執筆にあたり多大なるご協力、ご助言を賜りました、第一三共株式会社研究イノベーション企画部　礒山毅主幹、同　岡田浩幸主査、同社　モダリティ第三研究所　長基康人専門研究員、同　富樫紀子上級研究員に心より感謝を申し上げます。また、化合物評価に関して貴重なご協力を賜りました、同社　バイオプロセス技術第一研究所　平本久美子副主任研究員、同　木原かをり研究員に深く感謝いたします。

X 線結晶構造解析および SBDD において貴重なご協力、ご助言を賜りました、第一三共株式会社モダリティ第一研究所　安松勲主任研究員、同　西河洋祐副主任研究員に感謝いたします。また、各種スペクトル測定や *in vitro* ADME データを取得して下さりました、同社　ヒットディスカバリー基盤研究所の皆様に深く感謝いたします。

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最後に、これまで長きに渡り私の研究生活を支えて下さりました、家族に心より感謝いたします。

実験の部

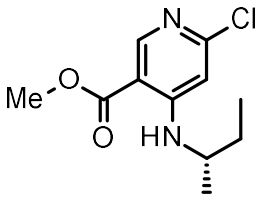
全ての動物実験は「第一三共株式会社動物実験に関する細則」に基づいて実施した。

1. Chemistry

General

Unless otherwise noted, commercial reagents and solvents were obtained from suppliers and used as purchased. Normal-phase column chromatography was performed on silica gel (SiO2) or amino silica gel (amino) using prepackaged cartridges. Analytical thin-layer chromatography (TLC) was performed on Merck pre-coated TLC glass sheets with silica gel 60 F_{254}. ^{1}H-NMR spectra were recorded on a JEOL JNM-EX400 spectrometer in the indicated solvent, and chemical shifts are given in ppm from tetramethylsilane as an internal standard. ESI/APCI mass spectra were recorded on Agilent Infinity 1260 series LC/MS. Purities of ≥95% were confirmed by LC/MS for all test compounds. Conditions were as follows: column: Develosil Combi-RP-5 2.0 mm × 50 mm, gradient elution: 0.1% HCO_2H–H_2O/0.1% HCO_2H–MeCN = 98/2–0/100 (v/v), flow rate: 1.2 mL/min, UV detection: 254 nm, column temperature: 40°C, and ionization: APCI/ESI. High-resolution mass spectra (HRMS) were obtained on an LC/MS system composed of the Waters Xevo Q-Tof MS system and Acuity UPLC system.

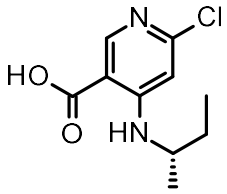
Methyl 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylate (S1)



To a solution of methyl 4,6-dichloronicotinate (15.2 g, 73.8 mmol) in 2-propanol (150 mL) were added (*S*)-(+)-2-aminobutane hydrochloride (10.5 g, 95.9 mmol) and DIPEA (38 mL, 220 mmol) at rt. After stirring at 70°C for 5 h, (*S*)-(+)-2-aminobutane hydrochloride (2.43 g, 22.1 mmol) and DIPEA (7.7 mL, 44 mmol) were added and stirred at 70°C for 6 h. The reaction mixture was cooled

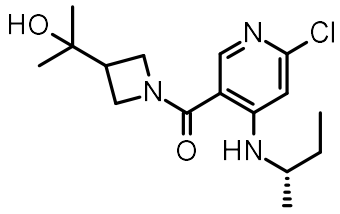
to rt and concentrated under reduced pressure. EtOAc was added to the residue and the organic layer was washed with sat. aq. $NaHCO_3$ and brine. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 5/95 (v/v)] to give the title compound as a colorless oil (12.9 g, 72% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, *J* = 7.6 Hz), 1.24 (3H, d, *J* = 6.1 Hz), 1.56-1.68 (2H, m), 3.44-3.53 (1H, m), 3.88 (3H, s), 6.54 (1H, s), 8.11 (1H, br s), 8.66 (1H, s).

4-[(2*S*)-Butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (6)



To a solution of methyl 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylate (**S1**) (12.8 g, 52.7 mmol) in 1,4-dioxane (130 mL) was added 1 mol/L aq. sodium hydroxide (130 mL, 130 mmol) at rt. After stirring for 2 h at rt, 1 mol/L aq. hydrochloric acid (130 mL, 130 mmol) was added and the resulting precipitate was filtrated to afford the title compound (11.4 g, 95% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 0.89 (3H, t, *J* = 7.4 Hz), 1.15 (3H, d, *J* = 6.1 Hz), 1.50-1.57 (2H, m), 3.63-3.74 (1H, m), 6.81 (1H, s), 8.23 (1H, d, *J* = 8.6 Hz), 8.50 (1H, s), 13.35 (1H, s).

{4-[(2*S*)-Butan-2-ylamino]-6-chloropyridin-3-yl}[3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (8a)

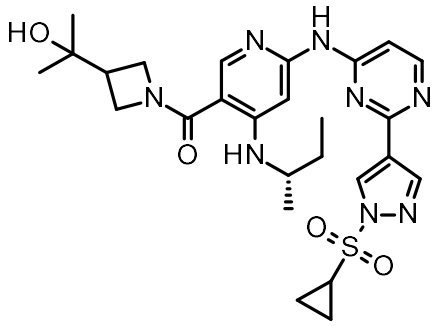


To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (100 mg, 0.437 mmol), 2-(azetidin-3-yl)propan-2-ol hydrochloride (99 mg, 0.66 mmol), and 3*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ol (18 mg, 0.13 mmol) in DMF (2.5 mL) were added DIPEA (0.150 mL, 0.875 mmol) and WSCI·HCl (126 mg, 0.656 mmol). After stirring at rt for 3 h, the mixture was diluted with

EtOAc, and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography [SiO_2, Hex/EtOAc = 2/1–4/1 (v/v)] afforded the title compound (112 mg, 79% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, t, *J* = 7.7 Hz), 1.16-1.25 (9H, m), 1.52-1.65 (2H, m), 2.64-2.71 (1H, m), 3.37-3.43 (1H, m), 4.03-4.38 (4H, m), 6.50 (1H, s), 7.95 (1H, s), 8.08 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{16}H_{25}ClN_3O_2$ $(M+H)^+$: 326.2. Found 326.2.

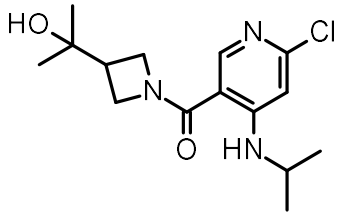
{4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)pyridin-3-yl}[3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (5a)



A mixture of {4-[(2*S*)-butan-2-ylamino]-6-chloropyridin-3-yl}[3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (**8a**) (112 mg, 0.344 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine[14] (91.0 mg, 0.34 mmol), tris(dibenzylideneacetone)dipalladium(0) (31 mg, 0.035 mmol), Xphos (66 mg, 0.14 mmol), and cesium carbonate (224 mg, 0.687 mmol) in 1,4-dioxane (3 mL) was stirred at reflux for 4 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc, filtered through celite, and concentrated in vacuo. Purification by column chromatography [SiO_2, DCM/MeOH = 92/8–88/12 (v/v)] afforded the title compound (72 mg, 38% yield) as a pale-yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 0.99 (3H, t, *J* = 7.4 Hz), 1.18-1.25 (8H, m), 1.29 (3H, d, *J* = 6.7 Hz), 1.49-1.55 (2H, m), 1.62-1.73 (2H, m), 2.66-2.73 (1H, m), 2.79-2.86 (1H, m), 3.50-3.59 (1H, m), 4.14-4.31 (4H, m), 6.99 (1H, br s), 7.24 (1H, d, *J* = 5.8 Hz), 7.43 (1H, s), 8.08-8.10 (1H, m), 8.12 (1H, s), 8.42 (1H, d, *J* = 5.8 Hz), 8.44 (1H, s), 8.64 (1H, s). HRMS (ESI): *m/z* calcd for $C_{26}H_{35}N_8O_4S$ $(M+H)^+$: 555.2502. Found 555.2498.

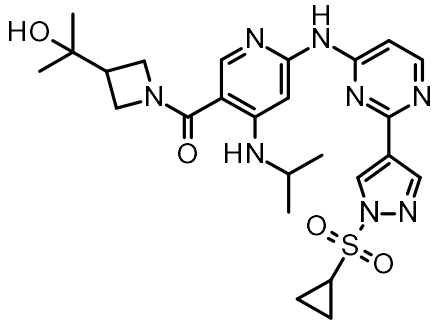
[6-Chloro-4-(propan-2-ylamino)pyridin-3-yl][3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (8b)



To a solution of 6-chloro-4-(propan-2-ylamino)pyridine-3-carboxylic acid[24] (**7**) (180 mg, 0.839 mmol), 2-(azetidin-3-yl)propan-2-ol hydrochloride (191 mg, 1.26 mmol), and 3*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ol (35 mg, 0.25 mmol) in DMF (3 mL) were added DIPEA (0.287 mL, 1.68 mmol) and WSCI·HCl (241 mg, 1.26 mmol). After stirring at rt for 2 h, the solution was diluted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and filtrated. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 98/2–95/5 (v/v)] to give the title compound (221 mg, 85% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.20 (6H, s), 1.25 (3H, s), 1.26 (3H, s), 1.53 (1H, s), 2.63-2.71 (1H, m), 3.56-3.66 (1H, m), 4.04-4.36 (4H, m), 6.51 (1H, s), 7.89-7.92 (1H, br m), 8.08 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{15}H_{23}ClN_3O_2$ $(M+H)^+$: 312.1. Found 312.2.

[6-({2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-ylamino)pyridin-3-yl][3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (5b)

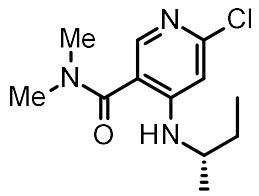


A mixture of [6-chloro-4-(propan-2-ylamino)pyridin-3-yl][3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (**8b**) (221 mg, 0.709 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (188 mg, 0.709 mmol), Xphos (135 mg, 0.284 mmol), tris(dibenzylideneacetone)dipalladium(0) (65 mg, 0.071 mmol), and cesium carbonate (462 mg,

1.42 mmol) in 1,4-dioxane (4 mL) was stirred at reflux for 5 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, EtOAc; DCM/MeOH = 2/98–4/96 (v/v); amino, DCM/MeOH = 99/1–97/3 (v/v)] to give the title compound (176 mg, 46% yield) as a pale-yellow solid.

^{1}H-NMR (DMSO-D_6) δ: 1.04 (6H, s), 1.23-1.30 (8H, m), 1.30-1.38 (2H, m), 2.52-2.63 (1H, m), 3.24-3.32 (1H, m), 3.68-3.78 (1H, m), 3.89-4.00 (2H, m), 4.16-4.35 (2H, m), 4.55 (1H, s), 7.39 (1H, br s), 7.45 (1H, br s), 7.99 (1H, d, *J* = 7.4 Hz), 8.13 (1H, s), 8.43-8.46 (2H, m), 8.63 (1H, s), 10.12 (1H, s). HRMS (ESI): *m/z* calcd for $C_{25}H_{33}N_8O_4S$ $(M+H)^+$: 541.2345. Found 541.2338.

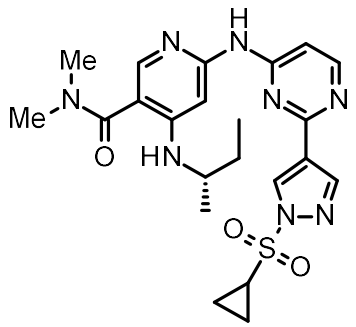
4-[(2*S*)-Butan-2-ylamino]-6-chloro-*N*,*N*-dimethylpyridine-3-carboxamide (8c)



To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (110 mg, 0.481 mmol) and 3*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ol (20 mg, 0.14 mmol) in DMF (2.5 mL) was added dimethylamine solution in THF (2.0 M, 0.481 mL, 0.962 mmol) and WSCI·HCl (138 mg, 0.722 mmol). After stirring at rt for 5 h, the solution was diluted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and filtrated. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 20/80–0/100 (v/v)] to give the title compound (73 mg, 59% yield) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, t, *J* = 7.4 Hz), 1.21 (3H, d, *J* = 6.1 Hz), 1.49-1.64 (2H, m), 3.10 (6H, s), 3.35-3.46 (1H, m), 6.24 (1H, br s), 6.52 (1H, s), 7.93 (1H, s).

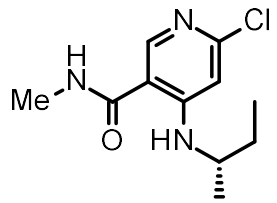
4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-*N*,*N*-dimethylpyridine-3-carboxamide (5c)



A mixture of 4-[(2*S*)-butan-2-ylamino]-6-chloro-*N*,*N*-dimethylpyridine-3-carboxamide (**8c**) (75 mg, 0.29 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (78 mg, 0.29 mmol), tris(dibenzylideneacetone)dipalladium(0) (27 mg, 0.029 mmol), Xphos (56 mg, 0.12 mmol), and cesium carbonate (191 mg, 0.587 mmol) in 1,4-dioxane (2.5 mL) was stirred at reflux for 6 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, EtOAc; amino, DCM/MeOH = 99/1–97/3 (v/v)] to give the title compound (59 mg, 42% yield) as a colorless amorphous solid.

^{1}H-NMR (DMSO-D_6) δ: 0.91 (3H, t, *J* = 7.1 Hz), 1.17-1.38 (7H, m), 1.53-1.64 (2H, m), 3.00 (6H, s), 3.22-3.31 (1H, m), 3.46-3.56 (1H, m), 6.23 (1H, d, *J* = 7.4 Hz), 7.28 (1H, s), 7.52 (1H, s), 7.91 (1H, br s), 8.42-8.47 (2H, m), 8.63 (1H, s), 10.06 (1H, s). HRMS (ESI): *m/z* calcd for $C_{22}H_{29}N_8O_3S$ $(M+H)^+$: 485.2083. Found 485.2076.

4-[(2*S*)-Butan-2-ylamino]-6-chloro-*N*-methylpyridine-3-carboxamide (8d)

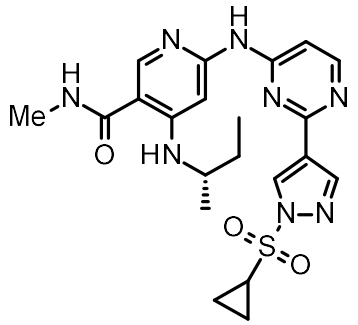


To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (345 mg, 1.51 mmol) and 3*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ol (62.0 mg, 0.453 mmol) in DMF (5 mL) were added methylamine solution in THF (2.0 M, 1.51 mL, 3.02 mmol) and WSCI·HCl (376 mg, 1.96 mmol) at rt. After stirring for 3 h, the reaction mixture was diluted with EtOAc and washed with water and brine. The organic layer was dried with Na_2SO_4 and filtered. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 60/40–40/60 (v/v)] to give

the title compound (343 mg, 94% yield) as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, *J* = 7.4 Hz), 1.22 (3H, d, *J* = 6.1 Hz), 1.53-1.68 (2H, m), 2.97 (3H, d, *J* = 4.3 Hz), 3.38-3.46 (1H, m), 6.14 (1H, br s), 6.50 (1H, s), 8.15 (1H, s), 8.28 (1H, br s).

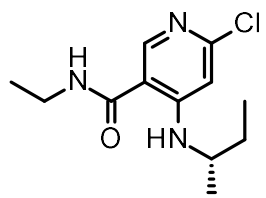
4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-*N*-methylpyridine-3-carboxamide (5d)



A mixture of 4-[(2*S*)-butan-2-ylamino]-6-chloro-*N*-methylpyridine-3-carboxamide (**8d**) (223 mg, 0.923 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (245 mg, 0.923 mmol), tris(dibenzylideneacetone)dipalladium(0) (127 mg, 0.138 mmol), Xphos (264 mg, 0.554 mmol), and cesium carbonate (601 mg, 1.85 mmol) in 1,4-dioxane (5 mL) was stirred at reflux for 5 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 97/3–92/8 (v/v); amino, EtOAc] to give the title compound (231 mg, 53% yield) as a colorless amorphous solid.

^{1}H-NMR (DMSO-D_6) δ: 0.92 (3H, t, *J* = 7.4 Hz), 1.21-1.30 (5H, m), 1.31-1.37 (2H, m), 1.53-1.66 (2H, m), 2.73 (3H, d, *J* = 4.3 Hz), 3.23-3.31 (1H, m), 3.52-3.60 (1H, m), 7.36-7.41 (2H, m), 8.35-8.39 (2H, m), 8.43-8.46 (2H, m), 8.61-8.64 (2H, m), 10.13 (1H, s). HRMS (ESI): *m/z* calcd for $C_{21}H_{27}N_8O_3S$ $(M+H)^+$: 471.1927. Found 471.1929.

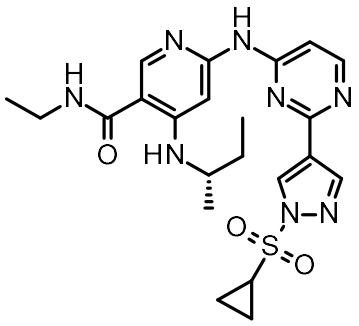
4-[(2*S*)-Butan-2-ylamino]-6-chloro-*N*-ethylpyridine-3-carboxamide (8e)



To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (700 mg, 3.06 mmol), ethylamine hydrochloride (499 mg, 6.12 mmol), and 3*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ol (129 mg, 0.918 mmol) in DMF (10 mL) were added DIPEA (1.05 mL, 6.12 mmol) and WSCI·HCl (763 mg, 3.98 mmol) at rt. After stirring at rt overnight, the solution was diluted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and filtrated. After evaporating the solvent, the residue was purified by column chromatography (SiO_2, EtOAc) to give the title compound (759 mg, 97% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, *J* = 7.4 Hz), 1.22 (3H, d, *J* = 6.1 Hz), 1.26 (3H, t, *J* = 7.4 Hz), 1.53-1.66 (2H, m), 3.38-3.50 (3H, m), 6.08 (1H, s), 6.50 (1H, s), 8.14 (1H, s), 8.25-8.30 (1H, m). MS(ESI/APCI) calcd for $C_{12}H_{19}ClN_3O$ $(M+H)^+$: 256.1. Found 256.2.

4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-*N*-ethylpyridine-3-carboxamide (5e)

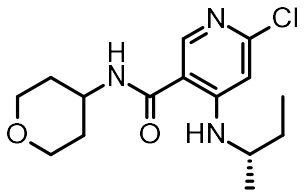


A mixture of 4-[(2*S*)-butan-2-ylamino]-6-chloro-*N*-ethylpyridine-3-carboxamide (**8e**) (117 mg, 0.457 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (121 mg, 0.457 mmol), tris(dibenzylideneacetone)dipalladium(0) (42 mg, 0.046 mmol), Xphos (87 mg, 0.18 mmol), and cesium carbonate (298 mg, 0.915 mmol) in 1,4-dioxane (3.5 mL) was stirred at reflux for 6 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, EtOAc, DCM/MeOH = 97/3–92/8 (v/v); amino, DCM/MeOH = 99/1–97/3 (v/v)] to give the title compound (130 mg, 59% yield) as a yellow amorphous solid.

^{1}H-NMR (DMSO-D_6) δ: 0.92 (3H, t, *J* = 7.4 Hz), 1.12 (3H, t, *J* = 7.1 Hz), 1.17-1.31 (5H, m), 1.31-1.38 (2H, m), 1.53-1.68 (2H, m), 3.21-3.31 (3H, m), 3.52-3.62 (1H, m), 7.36-7.44 (2H, m), 8.36-8.47 (4H, m), 8.60-8.65 (2H, m), 10.13 (1H, s). HRMS (ESI): *m/z* calcd for $C_{22}H_{29}N_8O_3S$ $(M+H)^+$:

485.2083. Found 485.2112.

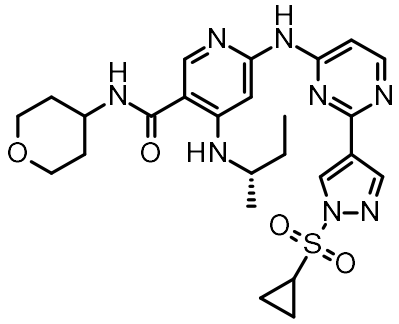
4-[(2*S*)-Butan-2-ylamino]-6-chloro-*N*-(tetrahydro-2*H*-pyran-4-yl)pyridine-3-carboxamide (8f)



To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (114 mg, 0.499 mmol) in DMF (3 mL) were added DIPEA (0.128 mL, 0.750 mmol), 4-aminotetrahydropyran (0.078 mL, 0.75 mmol), and DMT-MM (208 mg, 0.750 mmol). After stirring at rt for 3 h, sat. aq. $NaHCO_3$ was added and extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 65/35 (v/v)] to give the title compound (154 mg, 99% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, *J* = 7.4 Hz), 1.22 (3H, d, *J* = 6.7 Hz), 1.54-1.67 (4H, m), 1.96-2.02 (2H, m), 3.38-3.47 (1H, m), 3.48-3.56 (2H, m), 3.98-4.04 (2H, m), 4.07-4.17 (1H, m), 5.92 (1H, d, *J* = 7.4 Hz), 6.51 (1H, s), 8.14 (1H, s), 8.23 (1H, d, *J* = 7.4 Hz).

4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-*N*-(tetrahydro-2*H*-pyran-4-yl)pyridine-3-carboxamide (5f)

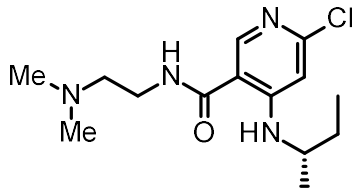


A mixture of 4-[(2*S*)-butan-2-ylamino]-6-chloro-*N*-(tetrahydro-2*H*-pyran-4-yl)pyridine-3-carboxamide (**8f**) (154 mg, 0.494 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (131 mg, 0.494 mmol), cesium carbonate (322 mg, 0.988 mmol), Xphos (47 mg, 0.099 mmol),

and tris(dibenzylideneacetone)dipalladium(0) (45 mg, 0.049 mmol) in 1,4-dioxane (5 mL) was stirred at reflux for 5.5 h under a N_2 atmosphere. After cooling down to rt, sat. aq. $NaHCO_3$ was added and extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the residue was purified by column chromatography [amino, Hex/EtOAc = 50/50 (v/v); SiO_2, DCM/MeOH = 50/1–20/1 (v/v)] to give the title compound (157 mg, 59% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, *J* = 7.4 Hz), 1.18-1.26 (2H, m), 1.31 (3H, d, *J* = 6.1 Hz), 1.50-1.75 (6H, m), 1.98-2.04 (2H, m), 2.79-2.87 (1H, m), 3.49-3.62 (3H, m), 3.99-4.05 (2H, m), 4.11-4.20 (1H, m), 5.87 (1H, d, *J* = 7.4 Hz), 7.08 (1H, s), 7.17 (1H, d, *J* = 5.5 Hz), 7.37 (1H, s), 8.18 (1H, s), 8.32 (1H, d, *J* = 7.4 Hz), 8.43 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.65 (1H, s). HRMS (ESI): *m/z* calcd for $C_{25}H_{33}N_8O_4S$ $(M+H)^+$: 541.2345. Found 541.2372.

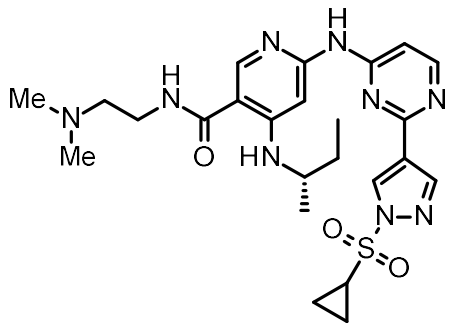
4-[(2*S*)-Butan-2-ylamino]-6-chloro-*N*-[2-(dimethylamino)ethyl]pyridine-3-carboxamide (8g)



To a solution of 4-[(2*S*)-Butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (114 mg, 0.500 mmol) and *N*,*N*-dimethylethylenediamine (0.080 mL, 0.75 mmol) in DMF (5 mL) were added DIPEA (0.128 mL, 0.750 mmol) and DMT-MM (208 mg, 0.750 mmol). After stirring at rt overnight, sat. aq. $NaHCO_3$ was added and the mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 80/20 (v/v)] to give the title compound (132 mg, 88% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, *J* = 7.7 Hz), 1.22 (3H, d, *J* = 6.1 Hz), 1.53-1.66 (2H, m), 2.26 (6H, s), 2.50 (2H, t, *J* = 5.8 Hz), 3.39-3.46 (3H, m), 6.50 (1H, s), 6.83 (1H, br s), 8.19 (1H, s), 8.30 (1H, br s).

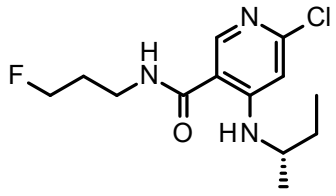
4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-*N*-[2-(dimethylamino)ethyl]pyridine-3-carboxamide (5g)



A mixture of 4-[(2*S*)-butan-2-ylamino]-6-chloro-*N*-[2-(dimethylamino)ethyl]pyridine-3-carboxamide (**8g**) (132 mg, 0.442 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (117 mg, 0.442 mmol), cesium carbonate (288 mg, 0.883 mmol), Xphos (42 mg, 0.088 mmol), and tris(dibenzylideneacetone)dipalladium(0) (41 mg, 0.044 mmol) in *tert*-BuOH (5 mL) was stirred at reflux for 3 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through celite. After evaporating the solvent, the residue was purified by column chromatography [amino, DCM/MeOH = 100/1–50/1 (v/v)] to give the title compound (83 mg, 36% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, *J* = 7.4 Hz), 1.19-1.25 (2H, m), 1.30 (3H, d, *J* = 6.7 Hz), 1.50-1.55 (2H, m), 1.64-1.73 (2H, m), 2.27 (6H, s), 2.52 (2H, t, *J* = 5.8 Hz), 2.80-2.86 (1H, m), 3.43-3.47 (2H, m), 3.52-3.60 (1H, m), 6.78 (1H, br s), 6.98 (1H, br s), 7.25 (1H, s), 7.42 (1H, br s), 8.23 (1H, s), 8.40-8.45 (3H, m), 8.65 (1H, s). HRMS (ESI): *m/z* calcd for $C_{24}H_{34}N_9O_3S$ $(M+H)^+$: 528.2505. Found 528.2504.

4-[(2*S*)-Butan-2-ylamino]-6-chloro-*N*-(3-fluoropropyl)pyridine-3-carboxamide (8h)

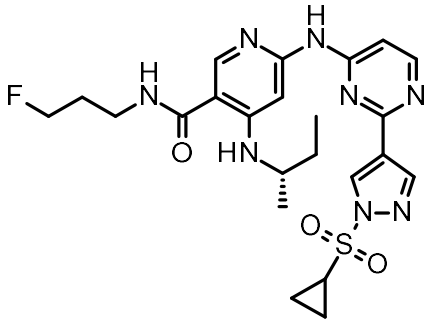


To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (114 mg, 0.500 mmol) and 3-fluoropropylamine hydrochloride (85 mg, 0.75 mmol) in DMF (5 mL) were added DIPEA (0.128 mL, 0.750 mmol) and DMT-MM (208 mg, 0.750 mmol). After stirring at rt for 17 h, sat. aq. $NaHCO_3$ was added and the mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced

pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 80/20–50/50 (v/v)] to give the title compound (144 mg, quant.) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, *J* = 7.7 Hz), 1.22 (3H, d, *J* = 6.7 Hz), 1.56-1.62 (2H, m), 1.95-2.10 (2H, m), 3.37-3.47 (1H, m), 3.58-3.61 (2H, m), 4.54 (1H, t, *J* = 5.5 Hz), 4.66 (1H, t, *J* = 5.5 Hz), 6.50 (2H, br s), 8.17 (1H, s), 8.27 (1H, d, *J* = 7.4 Hz). MS (ESI/APCI): *m/z* calcd for $C_{13}H_{20}ClFN_3O$ $(M+H)^+$: 288.1. Found 288.1.

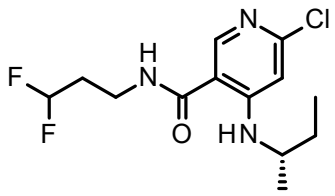
4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-*N*-(3-fluoropropyl)pyridine-3-carboxamide (5h)



A mixture of 4-[(2*S*)-butan-2-ylamino]-6-chloro-*N*-(3-fluoropropyl)pyridine-3-carboxamide (**8h**) (144 mg, 0.500 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (133 mg, 0.500 mmol), cesium carbonate (326 mg, 1.00 mmol), Xphos (48 mg, 0.10 mmol), and tris(dibenzylideneacetone)dipalladium(0) (46 mg, 0.050 mmol) in *tert*-BuOH (5 mL) was stirred at reflux for 3.5 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 50/1–30/1 (v/v)] to give the title compound (133 mg, 66%) as a pale-yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, *J* = 7.4 Hz), 1.21-1.23 (2H, m), 1.31 (3H, d, *J* = 6.7 Hz), 1.51-1.54 (2H, m), 1.62-1.73 (2H, m), 1.98-2.11 (2H, m), 2.80-2.86 (1H, m), 3.56-3.60 (3H, m), 4.63 (2H, dt, *J* = 47.2, 5.5 Hz), 6.26 (1H, s), 7.06 (1H, s), 7.19 (1H, d, *J* = 6.1 Hz), 7.36 (1H, s), 8.19 (1H, s), 8.35 (1H, d, *J* = 7.4 Hz), 8.43 (1H, d, *J* = 6.1 Hz), 8.45 (1H, s), 8.65 (1H, s). HRMS (ESI): *m/z* calcd for $C_{23}H_{30}FN_8O_3S$ $(M+H)^+$: 517.2146. Found 517.2141.

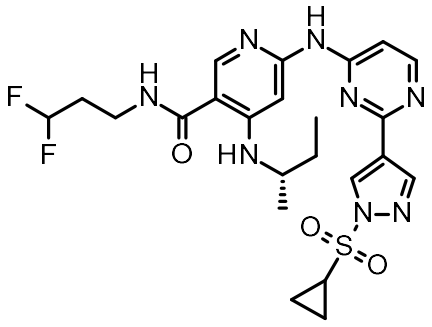
4-[(2*S*)-Butan-2-ylamino]-6-chloro-*N*-(3,3-difluoropropyl)pyridine-3-carboxamide (8i)



To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (686 mg, 3.00 mmol) and 3,3-difluoropropan-1-amine hydrochloride (395 mg, 3.00 mmol) in DMF (10 mL) were added DIPEA (0.514 mL, 3.00 mmol) and DMT-MM (996 mg, 3.60 mmol). After stirring at rt for 6 h, sat. aq. NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 80/20–75/25 (v/v)] to give the title compound (676 mg, 74% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, *J* = 7.4 Hz), 1.23 (3H, d, *J* = 6.7 Hz), 1.60-1.64 (2H, m), 2.13-2.26 (2H, m), 3.39-3.46 (1H, m), 3.63 (2H, dd, *J* = 12.9, 6.1 Hz), 5.98 (1H, tt, *J* = 56.1, 4.0 Hz), 6.26 (1H, s), 6.51 (1H, s), 8.14 (1H, s), 8.21 (1H, d, *J* = 6.1 Hz).

4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-*N*-(3,3-difluoropropyl)pyridine-3-carboxamide (5i)

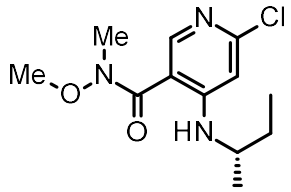


A mixture of 4-[(2*S*)-butan-2-ylamino]-6-chloro-*N*-(3,3-difluoropropyl)pyridine-3-carboxamide (**8i**) (676 mg, 2.21 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (531 mg, 2.00 mmol), cesium carbonate (1.30 g, 4.00 mmol), Xphos (191 mg, 0.400 mmol), and tris(dibenzylideneacetone)dipalladium(0) (183 mg, 0.200 mmol) in *tert*-BuOH (20 mL) was stirred at reflux for 4 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 50/1–30/1 (v/v); amino, DCM/MeOH = 50/1–30/1 (v/v)] to

give the title compound (810 mg, 76% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, *J* = 7.4 Hz), 1.21-1.23 (2H, m), 1.31 (3H, d, *J* = 6.1 Hz), 1.52-1.54 (2H, m), 1.65-1.72 (2H, m), 2.14-2.27 (2H, m), 2.80-2.86 (1H, m), 3.57-3.64 (3H, m), 6.00 (1H, tt, *J* = 56.1, 4.0 Hz), 6.22 (1H, t, *J* = 5.5 Hz), 7.08 (1H, s), 7.18 (1H, d, *J* = 5.5 Hz), 7.39 (1H, s), 8.18 (1H, s), 8.31 (1H, d, *J* = 7.4 Hz), 8.43 (1H, d, *J* = 5.5 Hz), 8.44 (1H, s), 8.65 (1H, s). HRMS (ESI): *m/z* calcd for $C_{23}H_{29}F_2N_8O_3S$ $(M+H)^+$: 535.2051. Found 535.2052.

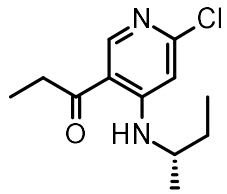
4-[(2*S*)-Butan-2-ylamino]-6-chloro-*N*-methoxy-*N*-methylpyridine-3-carboxamide (10)



To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (229 mg, 1.00 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (146 mg, 1.50 mmol) in DMF (5 mL) were added DIPEA (0.514 mL, 3.00 mmol) and DMT-MM (415 mg, 1.50 mmol) at rt. After stirring at rt overnight, sat. aq. $NaHCO_3$ was added and the mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 50/50 (v/v)] to give the title compound (272 mg, quant.) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, t, *J* = 7.7 Hz), 1.21 (3H, d, *J* = 6.7 Hz), 1.51-1.63 (2H, m), 3.35 (3H, s), 3.38-3.47 (1H, m), 3.60 (3H, s), 6.53 (1H, s), 6.91 (1H, d, *J* = 7.4 Hz), 8.34 (1H, s).

1-{4-[(2*S*)-Butan-2-ylamino]-6-chloropyridin-3-yl}propan-1-one (11)

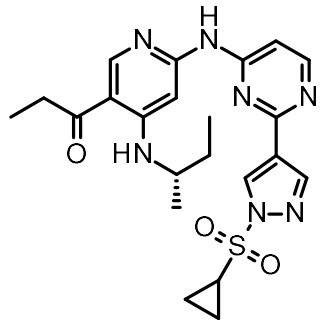


To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloro-*N*-methoxy-*N*-methylpyridine-3-carboxamide (**10**) (136 mg, 0.500 mmol) in THF (5 mL) was added ethyl magnesium bromide solution (3.0 M in diethyl ether, 0.50 mL, 1.5 mmol) at 0°C. After stirring for 4.5 h, sat. aq. NH_4Cl was added and the

mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 80/20 (v/v)] to give the title compound (97 mg, 81% yield) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, *J* = 7.4 Hz), 1.21 (3H, t, *J* = 7.2 Hz), 1.24 (3H, d, *J* = 6.7 Hz), 1.55-1.69 (2H, m), 2.98 (2H, q, *J* = 7.2 Hz), 3.44-3.54 (1H, m), 6.57 (1H, s), 8.64 (1H, s), 9.22 (1H, d, *J* = 4.9 Hz).

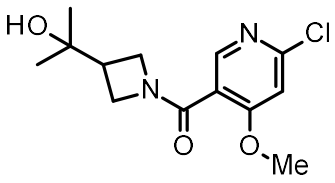
1-{4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)pyridin-3-yl}propan-1-one (12)



A mixture of 1-{4-[(2*S*)-butan-2-ylamino]-6-chloropyridin-3-yl}propan-1-one (**11**) (97 mg, 0.40 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (107 mg, 0.403 mmol), cesium carbonate (263 mg, 0.806 mmol), Xphos (38 mg, 0.081 mmol), and tris(dibenzylideneacetone)dipalladium(0) (37 mg, 0.040 mmol) in *tert*-BuOH (5 mL) was stirred at reflux for 3 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through celite. After evaporating the solvent, the residue was purified by column chromatography [amino, Hex/EtOAc = 75/25–50/50 (v/v)] to give the title compound (87 mg, 46% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, *J* = 7.4 Hz), 1.20-1.25 (5H, m), 1.33 (3H, d, *J* = 6.7 Hz), 1.51-1.55 (2H, m), 1.64-1.77 (2H, m), 2.79-2.87 (1H, m), 2.98 (2H, q, *J* = 7.2 Hz), 3.60-3.69 (1H, m), 7.15 (1H, d, *J* = 5.5 Hz), 7.18 (1H, s), 7.43 (1H, br s), 8.45 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.65 (1H, s), 8.67 (1H, s), 9.33 (1H, d, *J* = 7.4 Hz). HRMS (ESI): *m/z* calcd for $C_{22}H_{28}N_7O_3S$ $(M+H)^+$: 469.1974. Found 470.1975.

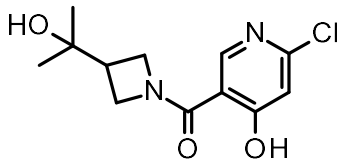
(6-Chloro-4-methoxypyridin-3-yl)[3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (14)



To a suspension of 2-(azetidin-3-yl)propan-2-ol hydrochloride (152 mg, 1.00 mmol) and 6-chloro-4-methoxynicotinic acid (**13**, purchased from Key Organics Ltd.) (188 mg, 1.00 mmol) in DMF (3 mL) were added DIPEA (0.205 mL, 1.20 mmol) and DMT-MM (354 mg, 1.20 mmol). After stirring at rt for 5 h, sat. aq. $NaHCO_3$ was added and extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 50/1–30/1 (v/v)] to give the title compound (215 mg, 76% yield) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 1.16 (3H, s), 1.22 (3H, s), 1.42 (1H, s), 2.62-2.69 (1H, m), 3.91-3.95 (5H, m), 4.04-4.10 (1H, m), 4.11-4.18 (1H, m), 6.88 (1H, s), 8.27 (1H, s).

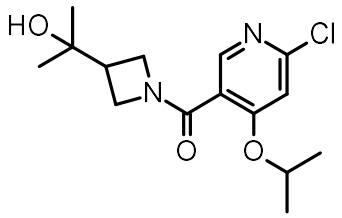
(6-Chloro-4-hydroxypyridin-3-yl)[3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (15)



To a solution of (6-chloro-4-methoxypyridin-3-yl)[3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (**14**) (215 mg, 0.755 mmol) in dichloromethane (10 mL) was added boron tribromide (17% in DCM, 2.27 mL, 2.27 mmol) at rt. After stirring at rt for 1.5 h, MeOH was added and the mixture was concentrated. Purification by column chromatography [SiO_2, Hex/EtOAc = 75/25 (v/v)] afforded the title compound (35 mg, 17% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.23 (6H, s), 1.37 (1H, s), 2.73-2.82 (1H, m), 4.07-4.31 (2H, m), 4.40-4.60 (2H, m), 6.93 (1H, s), 8.38 (1H, s), 13.41 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{12}H_{16}ClN_2O_3$ $(M+H)^+$: 271.1. Found 271.2.

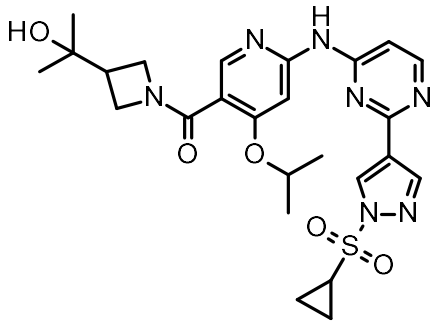
[6-Chloro-4-(propan-2-yloxy)pyridin-3-yl][3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (16)



To a solution of (6-chloro-4-hydroxypyridin-3-yl)[3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (**15**) (35.0 mg, 0.129 mmol) in DMF (3 mL) were added 2-iodopropane (0.026 mL, 0.26 mmol) and potassium carbonate (36 mg, 0.26 mmol) at rt. The mixture was stirred for 3 days and diluted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and filtrated. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 50/1–20/1 (v/v)] to give the title compound (40.0 mg, 99% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.15 (3H, s), 1.22 (3H, s), 1.41 (3H, d, *J* = 5.7 Hz), 1.42 (3H, d, *J* = 5.7 Hz), 2.60-2.69 (1H, m), 3.87-3.98 (2H, m), 4.03-4.17 (2H, m), 4.64-4.73 (1H, m), 6.82 (1H, s), 8.26 (1H, s).

[6-({2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl][3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (17)

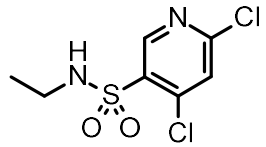


To a solution of [6-chloro-4-(propan-2-yloxy)pyridin-3-yl][3-(2-hydroxypropan-2-yl)azetidin-1-yl]methanone (**16**) (40.0 mg, 0.128 mmol) and 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (34 mg, 0.128 mmol) in 1,4-dioxane (5 mL) were added tris(dibenzylideneacetone)dipalladium(0) (12 mg, 0.013 mmol), Xphos (12 mg, 0.026 mmol), and cesium carbonate (83 mg, 0.26 mmol) at rt. The mixture was stirred at reflux for 2 h and cooled to rt. Brine was added to the mixture and it was extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure.

Purification by column chromatography [amino, Hex/EtOAc = 75/25–50/50 (v/v), DCM/MeOH = 50/1 (v/v); SiO_2, DCM/MeOH = 30/1–10/1 (v/v)] afforded the title compound (16 mg, 23% yield) as a colorless solid.

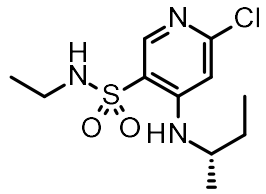
^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, s), 1.21-1.27 (4H, m), 1.38 (1H, s), 1.47-1.56 (9H, m), 2.61-2.69 (1H, m), 2.80-2.87 (1H, m), 3.95-4.08 (3H, m), 4.13-4.20 (1H, m), 4.80-4.88 (1H, m), 7.01 (1H, d, *J* = 5.7 Hz), 7.57 (1H, s), 7.72 (1H, s), 8.27 (1H, s), 8.44 (1H, s), 8.44 (1H, d, *J* = 5.7 Hz), 8.63 (1H, s). HRMS (ESI): *m/z* calcd for $C_{25}H_{32}N_7O_5S$ $(M+H)^+$: 542.2186. Found 542.2186.

4,6-Dichloro-*N*-ethylpyridine-3-sulfonamide (19)



To a solution of 4,6-dichloropyridine-3-sulfonyl chloride (**18**, purchased from Enamine Ltd.) (246 mg, 1.00 mmol) in DCM (10 mL) were added ethylamine hydrochloride (82 mg, 1.0 mmol) and DIPEA (0.342 mL, 2.00 mmol) at rt. After stirring at rt for 6 h, sat. aq. NH_4Cl was added and the mixture was extracted with DCM. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the title compound (255 mg, quant.) was obtained and used for the next reaction without further purification.

4-[(2*S*)-Butan-2-ylamino]-6-chloro-*N*-ethylpyridine-3-sulfonamide (20)

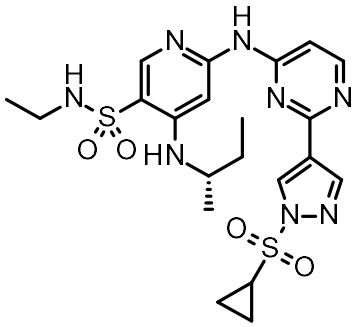


To a solution of 4,6-dichloro-*N*-ethylpyridine-3-sulfonamide (**19**) (255 mg, 1.00 mmol) in ethanol (5 mL) was added (*S*)-(+)-2-aminobutane (0.200 mL, 1.98 mmol) at rt. After stirring at 50°C for 9 h, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography [SiO_2, Hex/EtOAc = 80/20–65/35 (v/v)] to give the title compound (197 mg, 68% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, *J* = 7.4 Hz), 1.13 (3H, t, *J* = 7.4 Hz), 1.25 (3H, d, *J* = 6.1 Hz),

1.56-1.66 (2H, m), 2.98-3.07 (2H, m), 3.44-3.54 (1H, m), 4.42-4.48 (1H, m), 6.40-6.47 (1H, m), 6.59 (1H, s), 8.46 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{11}H_{19}ClN_3O_2S$ $(M+H)^+$: 292.1. Found 292.1.

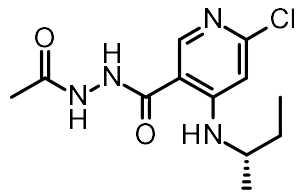
4-[(2*S*)-Butan-2-ylamino]-6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-*N*-ethylpyridine-3-sulfonamide (21)



A mixture of 4-[(2*S*)-butan-2-ylamino]-6-chloro-*N*-ethylpyridine-3-sulfonamide (**20**) (197 mg, 0.675 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (179 mg, 0.675 mmol), cesium carbonate (440 mg, 1.35 mmol), Xphos (64 mg, 0.14 mmol), and tris(dibenzylideneacetone)dipalladium(0) (62 mg, 0.0.68 mmol) in *tert*-BuOH (6 mL) was stirred at reflux for 3.5 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 50/1 (v/v); Hex/EtOAc = 50/50–75/25 (v/v)] to give the title compound (122 mg, 35% yield) as a colorless solid.

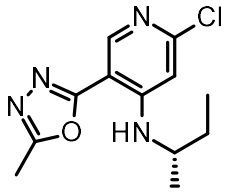
^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, *J* = 7.7 Hz), 1.13 (3H, t, *J* = 7.1 Hz), 1.19-1.26 (2H, m), 1.33 (3H, d, *J* = 6.1 Hz), 1.50-1.56 (2H, m), 1.61-1.80 (2H, m), 2.79-2.87 (1H, m), 2.98-3.07 (2H, m), 3.61-3.70 (1H, m), 4.43 (1H, t, *J* = 6.1 Hz), 6.37 (1H, d, *J* = 7.4 Hz), 7.11 (1H, d, *J* = 6.1 Hz), 7.31 (1H, s), 7.54 (1H, s), 8.44-8.48 (3H, m), 8.64 (1H, s). HRMS (ESI): *m/z* calcd for $C_{21}H_{29}N_8O_4S_2$ $(M+H)^+$: 521.1753. Found 521.1749.

***N′*-Acetyl-4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carbohydrazide (22)**



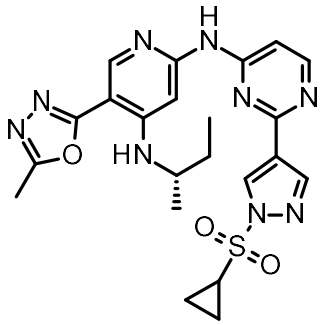
To a solution of 4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carboxylic acid (**6**) (300 mg, 1.31 mmol), 3*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ol (54 mg, 0.39 mmol), and acetohydrazide (117 mg, 1.57 mmol) in DMF (4 mL) was added WSCI·HCl (327 mg, 1.71 mmol). After stirring at rt overnight, the reaction mixture was diluted with EtOAc and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the title compound (373 mg) as a colorless oil and used for the next reaction without further purification.

_N_-[(2_S_)-Butan-2-yl]-2-chloro-5-(5-methyl-1,3,4-oxadiazol-2-yl)pyridin-4-amine (23)



To a solution of N′-acetyl-4-[(2*S*)-butan-2-ylamino]-6-chloropyridine-3-carbohydrazide (**22**) (373 mg) and triethylamine (0.365 mL, 2.62 mmol) in DCM (5 mL) was added *p*-toluenesulfonyl chloride (300 mg, 1.57 mmol) at 0°C and stirred at rt for 20 h. The reaction mixture was diluted with DCM, washed with brine, and dried over anhydrous Na_2SO_4. After concentration under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 75/25 (v/v)–50/50 (v/v)] to give the title compound (330 mg, 1.24 mmol, 94% yield in 2 steps) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, *J* = 7.4 Hz), 1.30 (3H, d, *J* = 6.7 Hz), 1.60-1.77 (2H, m), 2.64 (3H, s), 3.54-3.62 (1H, m), 6.64 (1H, s), 8.00-8.06 (1H, m), 8.55 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{12}H_{16}ClN_4O$ $(M+H)^+$ 267.1. Found 267.1.

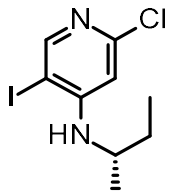
N^4-[(2_S_)-Butan-2-yl]-N^2-{2-[1-(cyclopropylsulfonyl)-1_H_-pyrazol-4-yl]pyrimidin-4-yl}-5-(5-methyl-1,3,4-oxadiazol-2-yl)pyridine-2,4-diamine (24)



A mixture of *N*-[(2*S*)-butan-2-yl]-2-chloro-5-(5-methyl-1,3,4-oxadiazol-2-yl)pyridin-4-amine (**23**) (120 mg, 0.450 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (119 mg, 0.450 mmol), tris(dibenzylideneacetone)dipalladium(0) (62 mg, 0.068 mmol), Xphos (129 mg, 0.270 mmol), and Cs_2CO_3 (293 mg, 0.900 mmol) in 1,4-dioxane (2.5 mL) was stirred at reflux for 5 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [amino, DCM/MeOH = 99/1–97/3 (v/v)] to give the title compound (76 mg, 0.15 mmol, 34% yield) as a colorless solid.

^{1}H-NMR (DMSO-D_6) δ: 0.96 (3H, t, *J* = 7.4 Hz), 1.24-1.39 (7H, m), 1.62-1.78 (2H, m), 2.58 (3H, s), 3.25-3.37 (1H, m), 3.71-3.81 (1H, m), 7.39 (1H, br s), 7.62 (1H, br s), 7.78 (1H, d, *J* = 7.4 Hz), 8.46-8.51 (3H, m), 8.66 (1H, s), 10.33 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.17, 10.20, 10.91, 19.86, 29.11, 31.28, 49.69, 92.45, 99.76, 106.56, 125.59, 131.24, 144.42, 147.93, 152.13, 154.40, 156.63, 158.93, 159.19, 161.57, 163.43. HRMS (ESI): *m/z* calcd for $C_{22}H_{26}N_9O_3S$ $(M+H)^+$ 496.1881. Found 496.1882.

_N_-[(2_S_)-Butan-2-yl]-2-chloro-5-iodopyridin-4-amine (26)

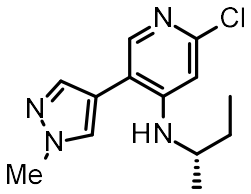


To a solution of 2-chloro-5-iodopyridin-4-amine (**25**)[25] (254 mg, 1.00 mmol) in DMF (4 mL) were added NaH (60 mass%, 120 mg, 3.00 mmol) and a solution of (2*R*)-butan-2-yl 4-methylbenzenesulfonate[26] (457 mg, 2.00 mmol) in DMF (1 mL). After stirring at 80°C for 1.5 h, the reaction mixture was cooled down to rt. Water was added to the reaction mixture and extracted

with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 90/10 (v/v)] to give the title compound (311 mg, 1.00 mmol, qunat.) as a pale-yellow oil.

^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, *J* = 7.4 Hz), 1.25 (3H, d, *J* = 6.1 Hz), 1.54-1.67 (2H, m), 3.42-3.53 (1H, m), 4.59-4.66 (1H, m), 6.39 (1H, s), 8.27 (1H, s).

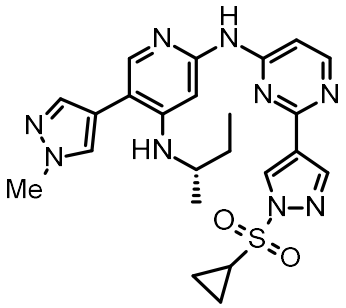
_N_-[(2_S_)-Butan-2-yl]-2-chloro-5-(1-methyl-1_H_-pyrazol-4-yl)pyridin-4-amine (27)



To a mixture of *N*-[(2*S*)-butan-2-yl]-2-chloro-5-iodopyridin-4-amine (**26**) (311 mg, 1.00 mmol), 1-methylpyrazole-4-boronic acid pinacol ester (312 mg, 1.50 mmol), K_2CO_3 (415 mg, 3.00 mmol) in DMF (4 mL) and water (1 mL) was added Pd(dppf)Cl_2·DCM (82 mg, 0.10 mmol). After stirring at 100°C for 3.5 h, the reaction mixture was cooled down to rt. Sat. aq. $NaHCO_3$ was added and the mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After being concentrated under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 80/20 (v/v)] to give the title compound (260 mg, 0.982 mmol, 98% yield) as a yellow oil.

^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, t, *J* = 7.4 Hz), 1.17 (3H, d, *J* = 6.7 Hz), 1.47-1.59 (2H, m), 3.39-3.49 (1H, m), 3.99 (3H, s), 4.42-4.49 (1H, m), 6.48 (1H, s), 7.45 (1H, s), 7.57 (1H, s), 7.82 (1H, s).

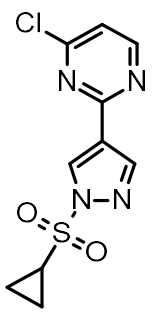
N^4-[(2_S_)-Butan-2-yl]-N^2-{2-[1-(cyclopropylsulfonyl)-1_H_-pyrazol-4-yl]pyrimidin-4-yl}-5-(1-methyl-1_H_-pyrazol-4-yl)pyridine-2,4-diamine (28)



To a mixture of *N*-[(2*S*)-butan-2-yl]-2-chloro-5-(1-methyl-1*H*-pyrazol-4-yl)pyridin-4-amine (**27**) (260 mg, 0.982 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (261 mg, 0.982 mmol), and Cs_2CO_3 (640 mg, 1.96 mmol) in *tert*-BuOH (10 mL) were added Xphos (94 mg, 0.20 mmol) and tris(dibenzylideneacetone)dipalladium(0) (90 mg, 0.098 mmol). After stirring at reflux for 4 h, the reaction mixture was cooled down to rt. Sat. aq. $NaHCO_3$ was added and the mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After concentration under reduced pressure, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 40/1–30/1 (v/v); amino, Hex/EtOAc =1/3 (v/v)] to give the title compound (86 mg, 0.17 mmol, 18% yield) as a pale-yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, t, *J* = 7.4 Hz), 1.18-1.29 (5H, m), 1.51-1.66 (4H, m), 2.79-2.85 (1H, m), 3.53-3.61 (1H, m), 4.00 (3H, s), 4.45 (1H, d, *J* = 8.0 Hz), 6.95-6.99 (1H, m), 7.37-7.41 (1H, m), 7.46 (1H, s), 7.60 (1H, s), 7.83 (1H, s), 8.40 (1H, d, *J* = 6.1 Hz), 8.45 (1H, s), 8.65 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.10, 10.17, 19.90, 29.10, 31.27, 39.17, 49.29, 93.33, 106.10, 110.03, 115.71, 125.79, 129.03, 131.20, 138.66, 144.46, 146.95, 152.08, 152.84, 156.27, 158.76, 159.49. HRMS (ESI): *m/z* calcd for $C_{23}H_{28}N_9O_2S$ $(M+H)^+$ 494.2088. Found 494.2087.

4-Chloro-2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidine (29)

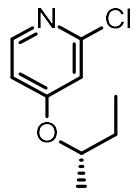


To a mixture of 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (1.15 g, 4.33

mmol), $CuCl_2$ (907 mg, 6.74 mmol), and anhydrous $MgSO_4$ (19.7 mg, 0.164 mmol) in acetonitrile (20 mL) was added *tert*-butyl nitrite (0.924 mL, 7.80 mmol) and stirred at 60°C for 7 h. After cooling down to 0°C, aq. HCl (1.0 mol/L, 20 mL) was added to the reaction mixture. After stirring for 5 min, DCM and aq. NaOH (1.0 mol/L, 20 mL) were added. The mixture was filtered through Celite and the filtrate was extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. DCM was added to the residue and an insoluble solid was filtered out. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 78/22–57/43 (v/v)] to give the title compound (422 mg, 1.48 mmol, 34% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.18-1.27 (2H, m), 1.50-1.58 (2H, m), 2.81-2.88 (1H, m), 7.22 (1H, d, J = 5.5 Hz), 8.47 (1H, s), 8.60 (1H, d, J = 5.5 Hz), 8.73 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{10}H_{10}ClN_4O_2S$ $(M+H)^+$ 285.0. Found 285.1.

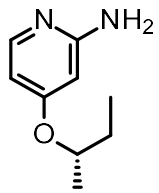
4-[(2*S*)-Butan-2-yloxy]-2-chloropyridine (S2)



To a solution of (*S*)-(+)-2-butanol (2.32 g, 31.3 mmol) in DMF (27 mL) was added NaH (55 mass%, 1.79 g, 41.0 mmol) at 0°C. After stirring at 0°C for 15 min, 2,4-dichloropyridine (4.00 g, 27.0 mmol) was added to the reaction mixture. The mixture was stirred at 0°C for 1 h and warmed up to rt. After stirring at rt for 45 min, sat. aq. NH_4Cl was added and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by column chromatography [SiO_2, Hex/EtOAc = 100/0–80/20 (v/v)] afforded the title compound (4.45 g, 24.0 mmol, 89% yield) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, J = 7.6 Hz), 1.33 (3H, d, J = 6.1 Hz), 1.60-1.81 (2H, m), 4.34-4.42 (1H, m), 6.71 (1H, dd, J = 6.1, 2.4 Hz), 6.80 (1H, d, J = 2.4 Hz), 8.16 (1H, d, J = 6.1 Hz). MS (ESI/APCI): *m/z* calcd for $C_9H_{13}ClNO$ $(M+H)^+$ 186.1. Found 186.2.

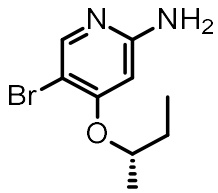
4-[(2*S*)-Butan-2-yloxy]pyridin-2-amine (S3)



To a mixture of 4-[(2*S*)-butan-2-yloxy]-2-chloropyridine (**S2**) (4.41 g, 23.8 mmol), tris(dibenzylideneacetone)dipalladium(0) (1.09 g, 1.19 mmol), and Xphos (1.14 g, 2.38 mmol) in THF (47 mL) was added lithium bis(trimethylsilyl)amide (1.17 mol/L in THF, 26 mL, 30.4 mmol) at rt. After stirring at 60°C for 2.5 h, sat. aq. $NaHCO_3$ and water were added, and the mixture was filtrated. The filtrate was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by column chromatography [SiO_2, EtOAc/MeOH = 99/1–95/5 (v/v)] afforded the title compound (3.55 g, 21.4 mmol, 90% yield) as a pale-yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, t, *J* = 7.6 Hz), 1.29 (3H, d, *J* = 6.1 Hz), 1.56-1.79 (2H, m), 4.27-4.38 (3H, m), 5.96 (1H, d, *J* = 2.4 Hz), 6.23 (1H, dd, *J* = 5.5, 2.4 Hz), 7.88 (1H, d, *J* = 5.5 Hz). MS (ESI/APCI): *m/z* calcd for $C_9H_{15}N_2O$ $(M+H)^+$ 167.1. Found 167.2.

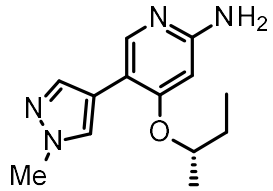
5-Bromo-4-[(2*S*)-butan-2-yloxy]pyridin-2-amine (30a)



To a solution of 4-[(2*S*)-butan-2-yloxy]pyridin-2-amine (**S3**) (2.31 g, 13.9 mmol) in acetonitrile (35 mL) was added *N*-bromosuccinimide (2.47 g, 13.9 mmol) at 0°C. After stirring at 0°C for 40 min, aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$ were added and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by column chromatography [SiO_2, Hex/EtOAc = 41/59–20/80 (v/v)] afforded the title compound (2.94 g, 12.0 mmol, 86% yield) as an orange solid.

^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, *J* = 7.3 Hz), 1.35 (3H, d, *J* = 6.1 Hz), 1.63-1.85 (2H, m), 4.29-4.38 (1H, m), 4.43 (2H, br s), 5.97 (1H, s), 8.02 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_9H_{14}BrN_2O$ $[M(^{79}Br)+H]^+$ 245.0, $[M(^{81}Br)+H]^+$ 247.0. Found 245.1, 247.1.

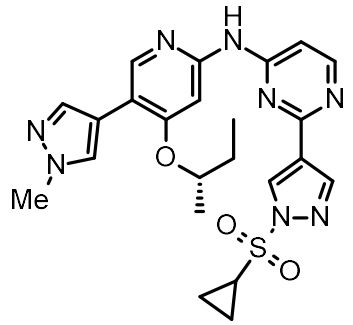
4-[(2*S*)-Butan-2-yloxy]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridin-2-amine (31a)



A mixture of 5-bromo-4-[(2*S*)-butan-2-yloxy]pyridin-2-amine (**30a**) (107 mg, 0.438 mmol), tris(dibenzylidene-acetone)dipalladium(0) (39.8 mg, 0.0435 mmol), Xphos (38.3 mg, 0.0803 mmol), 1-methylpyrazole-4-boronic acid pinacol ester (122 mg, 0.588 mmol), and aq. K_2CO_3 (2.0 mol/L, 0.548 mL, 1.10 mmol) in 1,4-dioxane (4 mL) was stirred at 85°C for 4 h under a N_2 atmosphere. After cooling down to rt, the reaction mixture was diluted with EtOAc, washed with brine, and dried over anhydrous Na_2SO_4. After concentration under reduced pressure, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 95/5–93/7 (v/v)] to give the title compound (90.2 mg, 0.366 mmol, 84% yield) as a pale-yellow oil.

^{1}H-NMR ($CDCl_3$) δ: 0.99 (3H, t, *J* = 7.3 Hz), 1.36 (3H, d, *J* = 6.1 Hz), 1.66-1.89 (2H, m), 3.93 (3H, s), 4.30-4.45 (3H, m), 6.02 (1H, s), 7.65 (1H, s), 7.80 (1H, s), 8.12 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{13}H_{19}N_4O$ $(M+H)^+$ 247.2. Found 247.3.

***N*-{4-[(2*S*)-Butan-2-yloxy]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridin-2-yl}-2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (32a)**

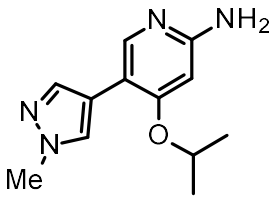


A mixture of 4-chloro-2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidine (**29**) (108 mg, 0.380 mmol), 4-[(2*S*)-butan-2-yloxy]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridin-2-amine (**31a**) (90.2 mg, 0.366 mmol), Xphos (33.5 mg, 0.0703 mmol), tris(dibenzylideneacetone)dipalladium(0) (33.6 mg, 0.0367 mmol), and Cs_2CO_3 (295 mg, 0.906 mmol) in 1,4-dioxane (2 mL) was stirred at 85°C for 4 h under a N_2 atmosphere. Xphos (27.7 mg, 0.0581 mmol), tris(dibenzylideneacetone)dipalladium(0)

(25.2 mg, 0.0275 mmol), and Cs_2CO_3 (105.3 mg, 0.3232 mmol) were added and the reaction mixture was stirred at 85°C for 6 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, EtOAc/MeOH = 92/8–90/10 (v/v)] to give the title compound (89.1 mg, 0.180 mmol, 49% yield) as a colorless solid.

^{1}H-NMR (DMSO-D_6) δ: 0.94 (3H, t, *J* = 7.3 Hz), 1.17-1.36 (4H, m), 1.39 (3H, d, *J* = 5.5 Hz), 1.68-1.90 (2H, m), 3.21-3.30 (1H, m), 3.86 (3H, s), 4.59-4.71 (1H, m), 7.34-7.50 (1H, m), 7.69-7.82 (1H, m), 7.89 (1H, s), 8.04 (1H, s), 8.38-8.46 (3H, m), 8.62 (1H, s), 10.18 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.15, 9.66, 19.02, 28.86, 31.26, 39.05, 75.74, 97.09, 106.22, 114.25, 115.58, 125.69, 128.67, 131.17, 137.43, 144.39, 145.74, 152.10, 156.41, 158.75, 159.21, 161.68. HRMS (ESI): *m/z* calcd for $C_{23}H_{27}N_8O_3S$ $(M+H)^+$ 495.1929. Found 495.1923.

5-(1-Methyl-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridin-2-amine (31b)

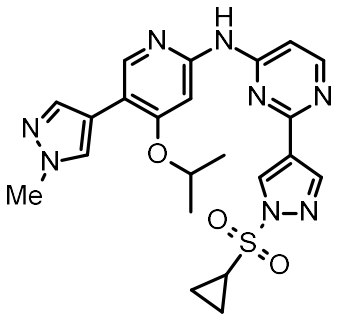


A mixture of 5-bromo-4-(propan-2-yloxy)pyridin-2-amine (**30b**)[27] (85.1 mg, 0.368 mmol), tris(dibenzylidene-acetone)dipalladium(0) (32.8 mg, 0.0358 mmol), Xphos (35.3 mg, 0.0737 mmol), 1-methylpyrazole-4-boronic acid pinacol ester (116 mg, 0.556 mmol), and aq. K_2CO_3 (2.0 mol/L, 0.368 mL, 0.737 mmol) in 1,4-dioxane (2 mL) was stirred at 80°C for 4 h under a N_2 atmosphere. Xphos (15.8 mg, 0.0331 mmol), tris(dibenzylideneacetone)dipalladium(0) (13.6 mg, 0.0149 mmol), 1-methylpyrazole-4-boronic acid pinacol (47.6 mg, 0.229 mmol), and aq. K_2CO_3 (2.0 mol/L, 0.184 mL, 0.368 mmol) were added to the mixture. After stirring at 80°C for 40 min, the reaction mixture was cooled down to rt. Sat. aq. $NaHCO_3$ was added and the mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After concentration under reduced pressure, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 92/8–87/13 (v/v); amino, DCM/MeOH = 99/1–97/3 (v/v)] to give the title compound (55.1 mg, 0.237 mmol, 64% yield) as a pale-yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 1.42 (6H, d, *J* = 6.1 Hz), 3.94 (3H, s), 4.34 (2H, br s), 4.60-4.69 (1H, m), 6.03

(1H, s), 7.66 (1H, s), 7.80 (1H, s), 8.13 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{12}H_{17}N_4O$ $(M+H)^+$ 233.1. Found 233.2.

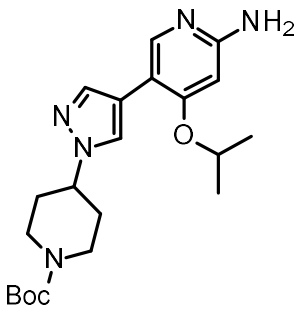
2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-(1-methyl-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (32b)



A mixture of 4-chloro-2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidine (**29**) (83.2 mg, 0.292 mmol), 5-(1-methyl-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridin-2-amine (**31b**) (55.1 mg, 0.237 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (28.5 mg, 0.0493 mmol), tris(dibenzylideneacetone)dipalladium(0) (24.9 mg, 0.0272 mmol), and Cs_2CO_3 (166 mg, 0.511 mmol) in 1,4-dioxane (2 mL) was stirred at 85°C for 5.5 h under a N_2 atmosphere. 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (24.2 mg, 0.0418 mmol), tris(dibenzylideneacetone)dipalladium(0) (26.1 mg, 0.0285 mmol), and Cs_2CO_3 (73.5 mg, 0.226 mmol) were added and the reaction mixture was stirred at 95°C for 2 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with EtOAc and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [amino, EtOAc/MeOH = 100/0–99/1 (v/v); SiO_2, DCM/MeOH = 97/3–95/5 (v/v)] to give the title compound (45.4 mg, 0.0945 mmol, 40% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.18-1.27 (2H, m), 1.49-1.57 (8H, m), 2.79-2.89 (1H, m), 3.97 (3H, s), 4.81-4.95 (1H, m), 7.04 (1H, d, *J* = 5.5 Hz), 7.48 (1H, s), 7.68 (1H, s), 7.80 (1H, s), 7.89 (1H, s), 8.37 (1H, s), 8.42 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.16, 21.82, 31.26, 39.05, 70.89, 97.13, 106.25, 106.25-161.39, 114.16, 115.53, 125.70, 128.73, 131.12, 137.36, 144.38, 145.67, 152.12, 156.32, 158.72, 159.21, 161.39. HRMS (ESI): *m/z* calcd for $C_{22}H_{25}N_8O_3S$ $(M+H)^+$ 481.1772. Found 481.1768.

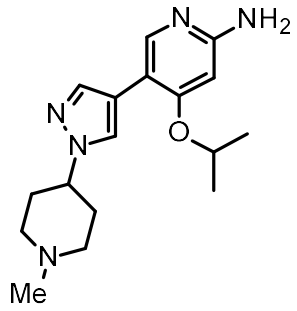
***tert*-Butyl 4-{4-[6-amino-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (33)**



A mixture of 5-bromo-4-(propan-2-yloxy)pyridin-2-amine (**30b**) (409 mg, 1.77 mmol), tris(dibenzylideneacetone)dipalladium(0) (152 mg, 0.166 mmol), Xphos (166 mg, 0.349 mmol), *tert*-butyl 4-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (826 mg, 2.19 mmol), and aq. K_2CO_3 (2.0 mol/L, 2.0 mL, 4.0 mmol) in 1,4-dioxane (8 mL) was stirred at reflux for 4 h under a N_2 atmosphere. Xphos (59.0 mg, 0.124 mmol), tris(dibenzylideneacetone)dipalladium(0) (48.7 mg, 0.0532 mmol), and *tert*-butyl 4-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (27.1 mg, 0.0718 mmol) were added to the mixture. After stirring at reflux for 2 h, the reaction mixture was cooled down to rt. The reaction mixture was diluted with EtOAc, washed with brine, and dried over anhydrous Na_2SO_4. After concentration under reduced pressure, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 97/3–95/5 (v/v)] to give the title compound (446 mg, 1.11 mmol, 63% yield) as a pale-yellow amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.42 (6H, d, J = 6.1 Hz), 1.48 (9H, s), 1.78-2.02 (2H, m), 2.12-2.20 (2H, m), 2.82-2.99 (2H, m), 4.15-4.44 (5H, m), 4.60-4.68 (1H, m), 6.03 (1H, s), 7.73 (1H, s), 7.83 (1H, s), 8.12 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{21}H_{32}N_5O_3$ $(M+H)^+$ 402.3. Found 402.3.

5-[1-(1-Methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-amine (34)



Step 1: To a solution of *tert*-butyl 4-{4-[6-amino-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (**33**) (153 mg, 0.382 mmol) and pyridine (0.0922 mL, 1.15 mmol) in DCM (1 mL) was added acetyl chloride (0.0407 mL, 0.573 mmol) at rt. After stirring for 1 h, 1.0 mol/L aq. HCl was added and the mixture was extracted with DCM. The organic layer was washed with 1.0 mol/L aq. HCl, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by column chromatography [SiO_2, Hex/EtOAc = 34/66–0/100 (v/v); EtOAc/MeOH = 100/0–90/10(v/v)] afforded *tert*-butyl 4-{4-[6-acetamido-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (163 mg, 0.368 mmol, 96% yield) as a red amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (6H, d, *J* = 6.1 Hz), 1.48 (9H, s), 1.89-2.02 (2H, m), 2.12-2.22 (2H, m), 2.21 (3H, s), 2.85-2.99 (2H, m), 4.19-4.36 (3H, m), 4.80-4.88 (1H, m), 7.85 (1H, s), 7.91 (1H, s), 7.92 (1H, s), 8.10 (1H, br s), 8.29 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{23}H_{34}N_5O_4$ $(M+H)^+$ 444.3. Found 444.2.

Step 2: To a solution of *tert*-butyl 4-{4-[6-acetamido-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (163 mg, 0.368 mmol) in 1,4-dioxane (2 mL) was added HCl (4 mol/L in 1,4-dioxane, 2 mL, 8 mmol) at rt. After stirring for 2 h, the reaction mixture was concentrated. The residue was washed with EtOAc to afford *N*-{5-[1-(piperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}acetamide hydrochloride (163 mg) as a colorless solid. The compound was used for the next reaction without further purification.

^{1}H-NMR (DMSO-D_6) δ: 1.44 (6H, d, *J* = 6.1 Hz), 2.13-2.23 (7H, m), 3.01-3.13 (2H, m), 3.36-3.43 (2H, m), 4.53-4.61 (1H, m), 4.79-4.86 (1H, m), 7.60 (1H, s), 8.01 (1H, s), 8.22 (1H, s), 8.45 (1H, s), 8.94 (1H, s), 9.10 (1H, br s), 11.62 (1H, br s). MS (ESI/APCI): *m/z* calcd for $C_{18}H_{26}N_5O_2$ $(M+H)^+$ 344.2. Found 344.2.

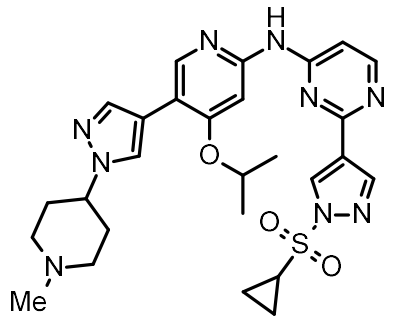
Step 3: To a solution of *N*-{5-[1-(piperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}acetamide hydrochloride (163 mg) and DIPEA (0.315 mL, 1.84 mmol) in MeOH (2 mL) was added aq. formaldehyde (37 mass%, 0.0415 mL, 0.552 mmol) at rt. After stirring for 15 min, $NaBH(OAc)_3$ (119 mg, 0.563 mmol) was added to the reaction mixture. After stirring at rt for 1 h, water was added and the mixture was extracted with DCM. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography [amino, EtOAc/MeOH = 100/0–95/5 (v/v)] afforded *N*-{5-[1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}acetamide (85.1 mg, 0.238 mmol, 65% yield in 2 steps) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.45 (6H, d, *J* = 6.1 Hz), 2.01-2.24 (9H, m), 2.35 (3H, s), 2.97-3.03 (2H, m), 4.11-4.22 (1H, m), 4.78-4.87 (1H, m), 7.89 (2H, s), 7.91 (1H, s), 8.10 (1H, br s), 8.30 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{19}H_{28}N_5O_2$ $(M+H)^+$ 358.2. Found 358.2.

Step 4: To a solution of *N*-{5-[1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}acetamide (600 mg, 1.68 mmol) in MeOH (10 mL) was added HCl (4 mol/L in 1,4-dioxane, 10 mL, 40 mmol) at rt. After stirring at 60°C for 2.5 h, the reaction mixture was cooled to rt and evaporated. Sat. aq. $NaHCO_3$ was added to the residue and extracted with DCM and 10% DCM/MeOH. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give 5-[1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-amine (**34**) (533 mg, quant.) as a colorless solid and used for the next reaction without further purification.

^{1}H-NMR ($CDCl_3$) δ: 1.41 (6H, d, *J* = 6.1 Hz), 1.99-2.25 (6H, m), 2.35 (3H, s), 2.95-3.05 (2H, m), 4.09-4.21 (1H, m), 4.37 (2H, br s), 4.57-4.71 (1H, m), 6.03 (1H, s), 7.77 (1H, s), 7.81 (1H, s), 8.13 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{17}H_{26}N_5O$ $(M+H)^+$ 316.2. Found 316.2.

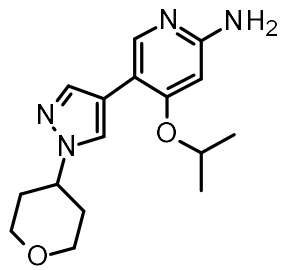
2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{5-[1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}pyrimidin-4-amine (35)



A mixture of 5-[1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-amine (**34**) (533 mg), 4-chloro-2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidine (**29**) (487 mg, 1.71 mmol), tris(dibenzylideneacetone)dipalladium(0) (157 mg, 0.172 mmol), Xphos (163 mg, 0.343 mmol), and Cs_2CO_3 (1.35 g, 4.15 mmol) in *tert*-BuOH (17 mL) was stirred at 95°C for 1.5 h under a N_2 atmosphere. After cooling down to rt, the reaction mixture was diluted with DCM and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [amino, EtOAc/MeOH = 100/0–98/2 (v/v)] to give the title compound (659 mg, 1.17 mmol, 70% yield in 2 steps) as a pale-yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 1.13-1.33 (2H, m), 1.49-1.58 (8H, m), 2.00-2.29 (6H, m), 2.36 (3H, s), 2.78-2.88 (1H, m), 2.95-3.06 (2H, m), 4.09-4.27 (1H, m), 4.81-4.95 (1H, m), 7.04 (1H, d, *J* = 6.1 Hz), 7.51 (1H, s), 7.68 (1H, s), 7.90 (1H, s), 7.91 (1H, s), 8.38 (1H, s), 8.41 (1H, d, *J* = 6.1 Hz), 8.45 (1H, s), 8.64 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.15, 21.86, 31.26, 32.61, 46.02, 54.67, 59.05, 70.89, 97.10, 106.22, 114.30, 115.09, 125.08, 125.73, 131.12, 136.75, 144.39, 145.68, 152.05, 156.36, 158.75, 159.19, 161.34. HRMS (ESI): *m/z* calcd for $C_{27}H_{34}N_9O_3S$ $(M+H)^+$ 564.2507. Found 564.2509.

4-(Propan-2-yloxy)-5-[1-(tetrahydro-2H-pyran-4-yl)-1*H*-pyrazol-4-yl]pyridin-2-amine (36)

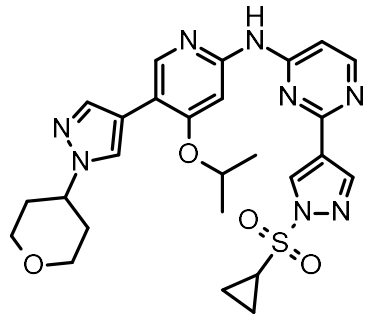


A mixture of 5-bromo-4-(propan-2-yloxy)pyridin-2-amine (**30b**) (202 mg, 0.874 mmol), 1-(tetrahydro-2H-pyran-4-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (489 mg,

1.76 mmol), tris(dibenzylideneacetone)dipalladium(0) (81 mg, 0.088 mmol), Xphos (84 mg, 0.18 mmol), and aq. K_2CO_3 (2.0 mol/L, 0.9 mL, 1.8 mmol) in 1,4-dioxane (4.3 mL) was stirred at reflux for 3.5 h. After cooling down to rt, the reaction mixture was diluted with EtOAc, washed with brine, and concentrated under reduced pressure. The residue was purified by column chromatography (amino, EtOAc) to give the title compound (184 mg, 0.609 mmol, 70% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.42 (6H, d, *J* = 6.1 Hz), 2.05-2.18 (4H, m), 3.57 (2H, td, *J* = 11.4, 3.1 Hz), 4.10-4.16 (2H, m), 4.32-4.42 (3H, m), 4.60-4.69 (1H, m), 6.03 (1H, s), 7.76 (1H, s), 7.84 (1H, s), 8.14 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{16}H_{23}N_4O_2$ $(M+H)^+$ 303.2. Found 303.2.

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{4-(propan-2-yloxy)-5-[1-(tetrahydro-2H-pyran-4-yl)-1*H*-pyrazol-4-yl]pyridin-2-yl}pyrimidin-4-amine (37)

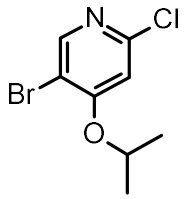


A mixture of 4-(propan-2-yloxy)-5-[1-(tetrahydro-2H-pyran-4-yl)-1*H*-pyrazol-4-yl]pyridin-2-amine (**36**) (184 mg, 0.609 mmol), 4-chloro-2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidine (**29**) (179 mg, 0.629 mmol), Xphos (59 mg, 0.12 mmol), tris(dibenzylideneacetone)dipalladium(0) (58 mg, 0.063 mmol), and Cs_2CO_3 (494 mg, 1.52 mmol) in *tert*-BuOH (3.0 mL) was stirred at reflux for 90 min. After cooling down to rt, the mixture was diluted with DCM and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, EtOAc/MeOH = 100/0–96/4 (v/v)] to give the title compound (200 mg, 0.363 mmol, 60% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.19-1.27 (2H, m), 1.51-1.57 (8H, m), 2.08-2.20 (4H, m), 2.80-2.87 (1H, m), 3.59 (2H, td, *J* = 11.4, 3.3 Hz), 4.12-4.18 (2H, m), 4.36-4.45 (1H, m), 4.83-4.94 (1H, m), 7.02 (1H, d, *J* = 5.5 Hz), 7.67 (1H, s), 7.72 (1H, s), 7.90 (1H, s), 7.93 (1H, s), 8.39 (1H, s), 8.41 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.18, 21.86, 31.27, 33.29, 58.16, 66.82, 70.93, 97.11, 106.25, 114.16, 115.16, 125.25, 125.70, 131.14, 137.09, 144.39, 145.73, 152.08,

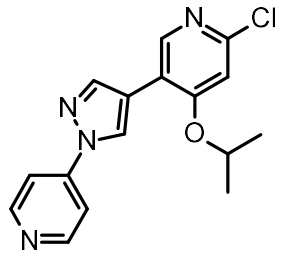
156.37, 158.75, 159.17, 161.38. HRMS (ESI): *m/z* calcd for $C_{26}H_{31}N_8O_4S$ $(M+H)^+$ 551.2191. Found 551.2191.

5-Bromo-2-chloro-4-(propan-2-yloxy)pyridine (38)



To a suspension of NaH (55 mass%, 4.33 g, 99.2 mmol) in DMF (66 mL) was added 2-propanol (5.56 mL, 72.7 mmol) dropwise at 0°C. After stirring at the same temperature for 1 h, 5-bromo-2,4-dichloropyridine (15.0 g, 66.1 mmol) was added and stirred for 30 min. The reaction mixture was warmed to rt and stirred for 30 min. Sat. aq. $NaHCO_3$ was added to the reaction mixture at 0°C and extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography [SiO_2, Hex/EtOAc = 67/33–25/75 (v/v); Hex/DCM = 100/0–25/75 (v/v)] to give the title compound (14.2 g, 56.6 mmol, 86% yield) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (6H, d, *J* = 6.1 Hz), 4.61-4.71 (1H, m), 6.79 (1H, s), 8.33 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_8H_{10}BrClNO$ $[M(^{79}Br)+H]^+$ 250.0, $[M(^{81}Br)+H]^+$ 252.0. Found 250.0, 252.0.

2-Chloro-4-(propan-2-yloxy)-5-[1-(pyridin-4-yl)-1*H*-pyrazol-4-yl]pyridine (39)

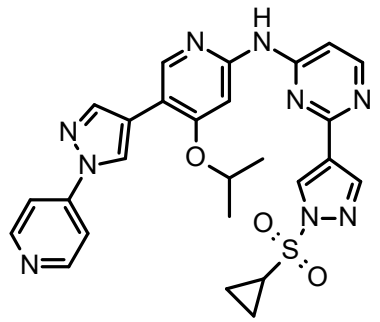


To a solution of 5-bromo-2-chloro-4-(propan-2-yloxy)pyridine (**38**) (300 mg, 1.20 mmol) and 4-[4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-1-yl]pyridine (390 mg, 1.44 mmol) in 1,4-dioxane (10 mL) were added Pd(dppf)Cl_2 ·DCM (97.8 mg, 0.120 mmol), water (2.0 mL), and K_2CO_3 (497 mg, 3.59 mmol). After stirring at 90°C for 3 h, the reaction mixture was cooled down to rt and water was added. The mixture was extracted with EtOAc, washed with brine, dried over anhydrous

Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography [SiO_2, Hex/EtOAc = 50/50–0/100 (v/v)] to give the title compound (250 mg, 0.794 mmol, 66% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.51 (6H, d, *J* = 6.1 Hz), 4.74-4.84 (1H, m), 6.90 (1H, s), 7.65-7.68 (2H, m), 8.17 (1H, s), 8.45 (1H, s), 8.48 (1H, s), 8.69-8.71 (2H, m).

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{4-(propan-2-yloxy)-5-[1-(pyridin-4-yl)-1*H*-pyrazol-4-yl]pyridin-2-yl}pyrimidin-4-amine (40)

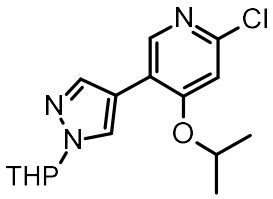


A mixture of 2-chloro-4-(propan-2-yloxy)-5-[1-(pyridin-4-yl)-1*H*-pyrazol-4-yl]pyridine (**39**) (248 mg, 0.788 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (230 mg, 0.867 mmol), Cs_2CO_3 (770 mg, 2.36 mmol), tris(dibenzylideneacetone)dipalladium(0) (144 mg, 0.158 mmol), and Xphos (150 mg, 0.315 mmol) in *tert*-BuOH (10 mL) was stirred at reflux for 4 h. After cooling down to rt, water was added to the reaction mixture. The mixture was extracted with EtOAc and the organic layer was filtered through Celite, washed with brine, and dried over anhydrous $MgSO_4$. After evaporating the solvent, the residue was purified by column chromatography [amino, Hex/EtOAc = 40/60–0/100 (v/v)]. The crude product was washed with EtOAc to give the title compound (81.3 mg, 0.150 mmol, 19% yield) as a colorless solid.

^{1}H-NMR (DMSO-D_6) δ: 1.25-1.38 (4H, m), 1.51 (6H, d, *J* = 6.1 Hz), 3.28-3.30 (1H, m), 4.93-4.99 (1H, m), 7.40 (1H, br s), 7.89-7.90 (2H, m), 7.96 (1H, br s), 8.37 (1H, s), 8.46-8.48 (2H, m), 8.61 (1H, s), 8.67-8.69 (3H, m), 9.02 (1H, s), 10.35 (1H, s). ^{1}H-NMR (DMSO-D_6) δ: 1.25-1.38 (4H, m), 1.51 (6H, d, *J* = 6.1 Hz), 3.28-3.30 (1H, m), 4.93-4.99 (1H, m), 7.40 (1H, br s), 7.89-7.90 (2H, m), 7.96 (1H, br s), 8.37 (1H, s), 8.46-8.48 (2H, m), 8.61 (1H, s), 8.67-8.69 (3H, m), 9.02 (1H, s), 10.35 (1H, s). ^{13}C-NMR (DMSO-D_6) δ: 6.81, 21.44, 30.73, 70.60, 96.75, 106.78, 111.65, 111.98, 118.71, 125.13, 125.43, 131.07, 141.39, 143.89, 145.18, 146.61, 151.14, 153.34, 156.27, 157.95, 159.31,

160.80. HRMS (ESI): *m/z* calcd for $C_{28}H_{36}N_9O_5S$ $(M+H)^+$ 544.1881. Found 544.1852.

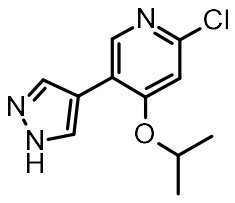
2-Chloro-4-(propan-2-yloxy)-5-[1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl]pyridine (41a)



A mixture of 5-bromo-2-chloro-4-(propan-2-yloxy)pyridine (**38**) (1.16 g, 4.62 mmol), 1-(tetrahydro-2*H*-pyran-2-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1.90 g, 6.83 mmol), aq. K_2CO_3 (2.0 mol/L, 6 mL, 12 mmol), tris(dibenzylideneacetone)dipalladium(0) (411 mg, 0.449 mmol), and Xphos (441 mg, 0.926 mmol) in 1,4-dioxane (24 mL) was stirred at reflux for 2.5 h. After cooling down to rt, the reaction mixture was diluted with EtOAc, washed with brine, and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 74/26–53/47 (v/v)] to give the title compound (517 mg, 1.61 mmol, 35% yield) as a yellow oil.

^{1}H-NMR ($CDCl_3$) δ: 1.45 (6H, d, *J* = 6.1 Hz), 1.56-1.79 (3H, m), 1.98-2.23 (3H, m), 3.74 (1H, td, *J* = 11.1, 3.0 Hz), 4.06-4.12 (1H, m), 4.67-4.76 (1H, m), 5.42 (1H, dd, *J* = 9.1, 3.0 Hz), 6.84 (1H, s), 7.96 (1H, s), 8.01 (1H, s), 8.40 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{16}H_{21}ClN_3O_2$ $(M+H)^+$ 322.1. Found 322.1.

2-Chloro-4-(propan-2-yloxy)-5-(1*H*-pyrazol-4-yl)pyridine (41b)

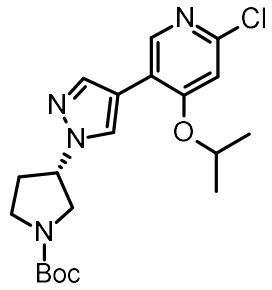


To a solution of 2-chloro-4-(propan-2-yloxy)-5-[1-(tetrahydro-2H-pyran-2-yl)-1*H*-pyrazol-4-yl]pyridine (**41a**) (1.71 g, 4.49 mmol) and triethylsilane (1.07 mL, 6.73 mmol) in DCM was added TFA (5.0 mL, 65 mmol) at rt. After stirring for 3 h, sat. aq. $NaHCO_3$ was added. The mixture was extracted with DCM, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure.

Purification by column chromatography [SiO_2, Hex/EtOAc = 34/66–25/75 (v/v)] afforded the title compound (817 mg, 3.44 mmol, 77% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.46 (6H, d, J = 6.1 Hz), 4.69-4.79 (1H, m), 6.86 (1H, s), 8.03 (2H, s), 8.45 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{11}H_{13}ClN_3O$ $(M+H)^+$ 238.1. Found 238.1.

tert-Butyl (3*S*)-3-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}pyrrolidine-1-carboxylate ((*S*)-42)

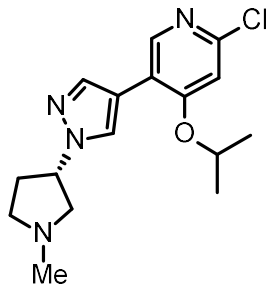


To a solution of (*R*)-1-(*tert*-butoxycarbonyl)-3-pyrrolidinol (710 mg, 3.79 mmol) and triethylamine (0.734 mL, 5.30 mmol) in THF (8.0 mL) was added methanesulfonic anhydride (792 mg, 4.55 mmol) at 0°C. After stirring at rt for 1.5 h, methanesulfonic anhydride (220 mg, 1.26 mmol) was added and the mixture was stirred for 30 min. Sat. aq. $NaHCO_3$ was added to the reaction mixture and the mixture was extracted with EtOAc. The organic layer was washed with 1 mol/L aq. HCl, water, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the corresponding mesylate.

A suspension of the mesylate, 2-chloro-4-(propan-2-yloxy)-5-(1H-pyrazol-4-yl)pyridine (**41b**) (600 mg, 2.52 mmol), and Cs_2CO_3 (2.00 g, 6.31 mmol) in DMA (8.0 mL) was stirred at 100°C for 3 h. After cooling down to rt, sat. aq. $NaHCO_3$ was added to the reaction mixture. The mixture was extracted with EtOAc and the organic layer was washed with 1 mol/L aq. HCl, water, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by column chromatography [SiO_2, Hex/EtOAc = 40/60–25/75 (v/v)] afforded the title compound (1.02 g, 2.51 mmol, 99% yield) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 1.44-1.49 (15H, m), 2.37-2.45 (2H, m), 3.42-3.92 (4H, m), 4.69-4.76 (1H, m), 4.90-4.96 (1H, m), 6.84 (1H, s), 7.86 (1H, s), 7.92 (1H, s), 8.40 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{20}H_{28}ClN_4O_3$ $(M+H)^+$ 407.2. Found 407.2.

2-Chloro-5-{1-[(3*S*)-1-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridine ((*S*)-43)



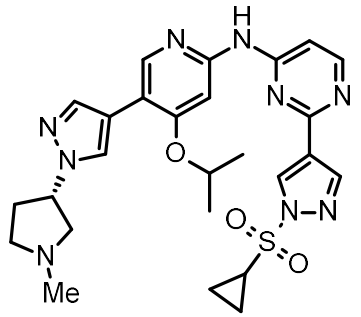
Step 1: To a solution of *tert*-butyl (3*S*)-3-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}pyrrolidine-1-carboxylate ((*S*)-**42**) (400 mg, 0.983 mmol) in 1,4-dioxane (2 mL) was added HCl (4 mol/L in 1,4-dioxane, 2 mL, 8 mmol) at rt. After stirring at rt for 3.5 h, the reaction mixture was concentrated to give 2-chloro-4-(propan-2-yloxy)-5-{1-[(3*S*)-pyrrolidin-3-yl]-1*H*-pyrazol-4-yl}pyridine hydrochloride (381 mg, quant.) as a colorless amorphous solid and used for the next reaction without further purification.

MS (ESI/APCI): *m/z* calcd for $C_{15}H_{20}ClN_4O$ $(M+H)^+$ 307.1. Found 307.1.

Step 2: To a solution of 2-chloro-4-(propan-2-yloxy)-5-{1-[(3*S*)-pyrrolidin-3-yl]-1*H*-pyrazol-4-yl}pyridine hydrochloride (381 mg) and DIPEA (0.720 mL, 4.21 mmol) in MeOH (4 mL) was added aq. formaldehyde (37 mass%, 0.0948 mL, 1.26 mmol) at rt. After stirring at rt for 10 min, $NaBH(OAc)_3$ (263 mg, 1.24 mmol) was added to the reaction mixture. After stirring for 5 h at rt, the reaction mixture was quenched by sat. aq. $NaHCO_3$ and extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography [amino, Hex/EtOAc = 34/66–0/100 (v/v)] afforded 2-chloro-5-{1-[(3S)-1-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridine ((*S*)-**43**) (203 mg, 0.632 mmol, 64%) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 1.46 (6H, d, *J* = 6.1 Hz), 2.17-2.25 (1H, m), 2.43 (3H, s), 2.45-2.61 (2H, m), 2.84-2.97 (3H, m), 4.69-4.76 (1H, m), 4.89-4.96 (1H, m), 6.83 (1H, s), 7.90 (1H, s), 8.01 (1H, s), 8.40 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{16}H_{22}ClN_4O$ $(M+H)^+$ 321.1. Found 321.1.

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*)-1-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine ((*S*)-44)

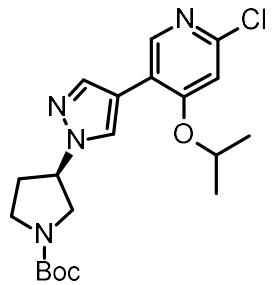


A mixture of 2-chloro-5-{1-[(3*S*)-1-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridine ((*S*)-**43**) (203 mg, 0.632 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (170 mg, 0.642 mmol), tris(dibenzylideneacetone)dipalladium(0) (54.6 mg, 0.0596 mmol), Xphos (62.4 mg, 0.131 mmol), and Cs_2CO_3 (411 mg, 1.26 mmol) in *tert*-BuOH (6 mL) was stirred at reflux for 5 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with DCM and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [amino, EtOAc/MeOH = 100/0–98/2 (v/v)] to give the title compound (90.1 mg, 0.164 mmol, 26% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.15-1.36 (2H, m), 1.48-1.59 (8H, m), 2.15-2.29 (1H, m), 2.44 (3H, s), 2.45-2.66 (2H, m), 2.75-3.00 (4H, m), 4.81-5.00 (2H, m), 7.03 (1H, d, *J* = 5.5 Hz), 7.53 (1H, s), 7.69 (1H, s), 7.91 (1H, s), 8.02 (1H, s), 8.38 (1H, s), 8.41 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s).

^{13}C-NMR ($CDCl_3$) δ: 7.15, 21.82, 31.26, 32.77, 41.84, 55.34, 61.20, 62.33, 70.89, 97.12, 106.26, 114.28, 115.35, 125.73, 126.56, 131.14, 137.20, 144.39, 145.68, 152.02, 156.33, 158.74, 159.20, 161.37. HRMS (ESI): *m/z* calcd for $C_{26}H_{32}N_9O_3S$ $(M+H)^+$ 550.2351. Found 550.2329.

tert-Butyl (3*R*)-3-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}pyrrolidine-1-carboxylate ((*R*)-42)



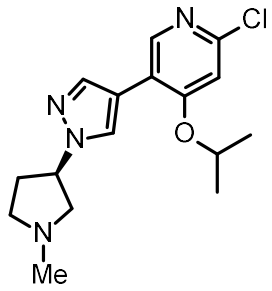
To a solution of (*S*)-1-Boc-3-pyrrolidinol (710 mg, 3.79 mmol) and triethylamine (0.735 mL, 5.30 mmol) in THF (8.0 mL) was added methanesulfonic anhydride (792 mg, 4.55 mmol) at 0°C. After

stirring at rt for 90 min, methanesulfonic anhydride (220 mg, 1.26 mmol) was added and the mixture was stirred for 30 min. Sat. aq. $NaHCO_3$ was then added to the reaction mixture and the mixture was extracted with EtOAc. The organic layer was washed with 1 mol/L aq. HCl, water, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the corresponding mesylate.

A suspension of the mesylate, 2-chloro-4-(propan-2-yloxy)-5-(1H-pyrazol-4-yl)pyridine (**41b**) (591 mg, 2.49 mmol), and Cs_2CO_3 (1.96 g, 6.02 mmol) in DMA (8.0 mL) was stirred at 100°C for 3 h. After cooling down to rt, sat. aq. $NaHCO_3$ was added to the reaction mixture. The mixture was extracted with EtOAc and the organic layer was washed with 1 mol/L aq. HCl, water, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by column chromatography [SiO_2, Hex/EtOAc = 40/60–25/75 (v/v)] afforded the title compound (1.01 g, 2.48 mmol, qunat.) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 1.44-1.49 (15H, m), 2.37-2.45 (2H, m), 3.42-3.92 (4H, m), 4.69-4.76 (1H, m), 4.90-4.96 (1H, m), 6.84 (1H, s), 7.86 (1H, s), 7.92 (1H, s), 8.40 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{20}H_{28}ClN_4O_3$ $(M+H)^+$ 407.2. Found 407.2.

2-Chloro-5-{1-[(3*R*)-1-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridine ((*R*)-43)



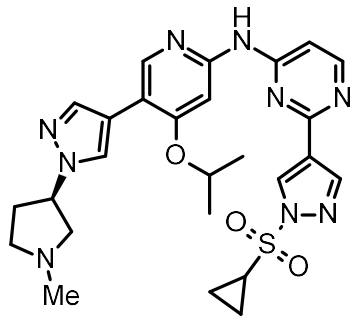
Step 1: To a solution of *tert*-butyl (3*R*)-3-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}pyrrolidine-1-carboxylate ((*R*)-**42**) (370 mg, 0.909 mmol) in 1,4-dioxane (2 mL) was added HCl (4 mol/L in 1,4-dioxane, 2 mL, 8 mmol) at rt. After stirring at rt for 50 min, the reaction mixture was concentrated to give 2-chloro-4-(propan-2-yloxy)-5-{1-[(3*R*)-pyrrolidin-3-yl]-1*H*-pyrazol-4-yl}pyridine hydrochloride (394 mg, quant.) as a colorless amorphous solid and used for the next reaction without further purification.

MS (ESI/APCI): *m/z* calcd for $C_{15}H_{20}ClN_4O$ $(M+H)^+$ 307.1. Found 307.1.

Step 2: To a solution of 2-chloro-4-(propan-2-yloxy)-5-{1-[(3*R*)-pyrrolidin-3-yl]-1*H*-pyrazol-4-yl}pyridine hydrochloride (394 mg) and DIPEA (0.687 mL, 3.94 mmol) in MeOH (3 mL) was added aq. Formaldehyde (37 mass%, 0.090 mL, 1.2 mmol) at rt. After stirring at rt for 10 min, $NaBH(Oac)_3$ (259 mg, 1.22 mmol) was added to the reaction mixture. After stirring for 50 min at rt, the reaction mixture was quenched by sat. aq. $NaHCO_3$ and extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography [amino, Hex/EtOAc = 25/75–10/90 (v/v)] afforded 2-chloro-5-{1-[(3*R*)-1-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridine ((*R*)-**25**) (275 mg, 0.857 mmol, 94%, containing impurities) as a colorless oil.

MS (ESI/APCI): *m/z* calcd for $C_{16}H_{22}ClN_4O$ $(M+H)^+$ 321.1. Found 321.2.

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*R*)-1-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine ((*R*)-44)



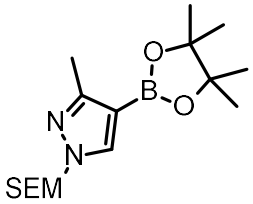
A mixture of 2-chloro-5-{1-[(3*R*)-1-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridine ((*R*)-**43**) (275 mg, 0.857 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (242 mg, 0.913 mmol), tris(dibenzylideneacetone)dipalladium(0) (75.4 mg, 0.0823 mmol), Xphos (81.7 mg, 0.171 mmol), and Cs_2CO_3 (699 mg, 2.15 mmol) in *tert*-BuOH (8.5 mL) was stirred at reflux for 4 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with DCM and purified by column chromatography [amino, EtOAc/MeOH = 100/0–96/4 (v/v)] to give the title compound (122 mg, 0.222 mmol, 26%) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.15-1.36 (2H, m), 1.48-1.59 (8H, m), 2.15-2.29 (1H, m), 2.44 (3H, s), 2.45-2.66 (2H, m), 2.75-3.00 (4H, m), 4.81-5.00 (2H, m), 7.03 (1H, d, *J* = 5.5 Hz), 7.63 (1H, s), 7.69 (1H, s), 7.91 (1H, s), 8.02 (1H, s), 8.38 (1H, s), 8.41 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s).

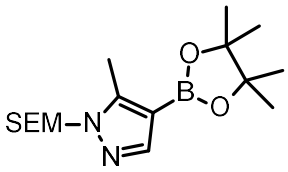
^{13}C-NMR ($CDCl_3$) δ: 7.15, 21.82, 31.26, 32.80, 41.87, 55.37, 61.26, 62.44, 70.88, 97.15, 106.23,

114.33, 115.35, 125.75, 126.50, 131.13, 137.16, 144.39, 145.68, 152.07, 156.35, 158.75, 159.21, 161.37. HRMS (ESI): *m/z* calcd for $C_{26}H_{32}N_9O_3S$ $(M+H)^+$ 550.2351. Found 550.2330.

3-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrazole (46a)



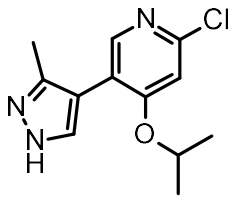
5-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrazole (46b)



To a solution of 3-methylpyrazole-4-boronic acid pinacol ester (2.00 g, 9.61 mmol) and DIPEA (3.30 mL, 19.2 mmol) in DCM (40 mL) was added SEMCl (2.02 mL, 11.5 mmol) at 0°C. The reaction mixture was stirred at rt until the starting material was completely consumed. Sat. aq. NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layer was washed with water and brine. After drying over anhydrous Na_2SO_4, the mixture was concentrated under reduced pressure. Purification by column chromatography [SiO_2, Hex/EtOAc = 100/0–67/33 (v/v)] afforded a 1:1 mixture of the title compounds (3.24 g, 9.58 mmol, 99% yield) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: -0.03 (4.5H, s), -0.02 (4.5H, s), 0.86-0.93 (2H, m), 1.31 (6H, s), 1.31 (6H, s), 2.40 (1.5H, s), 2.50 (1.5H, s), 3.52-3.57 (2H, m), 5.33 (1H, s), 5.41 (1H, s), 7.68 (0.5H, s), 7.75 (0.5H, s).

2-Chloro-5-(3-methyl-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridine (47)



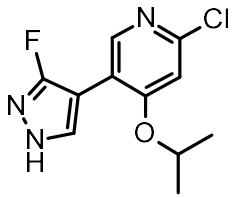
Step 1: To a mixture of 5-bromo-2-chloro-4-(propan-2-yloxy)pyridine (**38**) (2.28 g, 9.10 mmol), sodium carbonate (1.16 g, 10.9 mmol), water (15 mL), 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrazole (**46a**), and 5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrazole (**46b**) (1:1 mixture, 3.24 g, 9.58 mmol) in 1,4-dioxane (40 mL) was added Pd(dppf)Cl_2·DCM (743 mg, 0.910 mmol). The flask was evacuated and purged with nitrogen, heated to 105°C, and stirred for 2.5 h. The reaction mixture was cooled to room temperature and diluted with EtOAc. The organic phase was washed with water and brine, dried with $MgSO_4$, and concentrated. Purification by column chromatography [SiO_2, Hex/EtOAc = 95/5–60/40 (v/v)] afforded a 1:1 mixture of 2-chloro-5-(3-methyl-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridine and 2-chloro-5-(5-methyl-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridine (2.40 g, 6.28 mmol, 69% yield) as a pale-yellow oil.

^{1}H-NMR ($CDCl_3$) δ: -0.02 (4.5H, s), -0.01 (4.5H, s), 0.90-0.96 (2H, m), 1.36 (3H, d, *J* = 6.1 Hz), 1.38 (3H, d, *J* = 6.1 Hz), 2.31 (1.5H, s), 2.35 (1.5H, s), 3.59-3.65 (2H, m), 4.64-4.72 (1H, m), 5.40 (1H, s), 5.48 (1H, s), 6.86 (1H, s), 7.58 (0.5H, s), 7.66 (0.5H, s), 8.11 (0.5H, s), 8.19 (0.5H, s).

Step 2: To a solution of 2-chloro-5-(3-methyl-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridine and 2-chloro-5-(5-methyl-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridine (1:1 mixture, 910 mg, 2.38 mmol) in DCM (10 mL) was added TFA (5 mL). After stirring for 4 h at rt, the reaction mixture was concentrated and diluted with DCM. The organic layer was washed with 1 mol/L aq. K_2CO_3, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by column chromatography [SiO_2, DCM/MeOH = 97/3–92/8 (v/v)] afforded the title compound (609 mg, 2.42 mmol, qunat.) as a colorless amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.38 (6H, d, *J* = 6.1 Hz), 2.34 (3H, s), 4.62-4.72 (1H, m), 6.85 (1H, s), 7.68 (1H, s), 8.15 (1H, s).

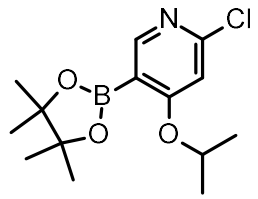
2-Chloro-5-(3-fluoro-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridine (48)



To a solution of 2-chloro-4-(propan-2-yloxy)-5-(1H-pyrazol-4-yl)pyridine (**41b**) (300 mg, 1.26 mmol) in acetonitrile (4 mL) were added Select-Fluor® (894 mg, 2.52 mmol) and acetic acid (0.16 mL, 2.8 mmol) at rt. After stirring at 80°C for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was diluted with EtOAc and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography [SiO_2, Hex/EtOAc = 60/40–40/60 (v/v)] to give the title compound (71.0 mg, 0.278 mmol, 22% yield) as a yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 1.44 (6H, d, *J* = 6.1 Hz), 4.69-4.79 (1H, m), 6.86 (1H, s), 7.90 (1H, d, *J* = 1.8 Hz), 8.52 (1H, s), 9.57 (1H, br s). MS (ESI/APCI): *m/z* calcd for $C_{11}H_{12}ClFN_3O$ $(M+H)^+$ 256.1. Found 256.1.

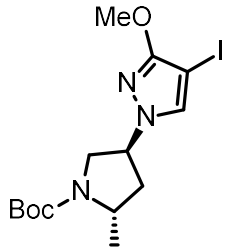
2-Chloro-4-(propan-2-yloxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (49)



To a solution of 5-bromo-2-chloro-4-(propan-2-yloxy)pyridine (**38**) (3.0 g, 12 mmol) and 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.0 mL, 15 mmol) in tetrahydrofuran (40 mL) was added *n*-butyllithium (2.6 mol/L in Hex, 7.0 mL, 19 mmol) dropwise at –78°C. After stirring at −78°C for 1 h, sat. aq. NH_4Cl was added to the reaction mixture and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the title compound (3.6 g, quant., containing impurities) as a red oil and used for the next reaction without further purification.

^{1}H-NMR ($CDCl_3$) δ: 1.34 (12H, s), 1.38 (6H, d, *J* = 6.1 Hz), 4.53-4.62 (1H, m), 6.74 (1H, s), 8.38 (1H, s).

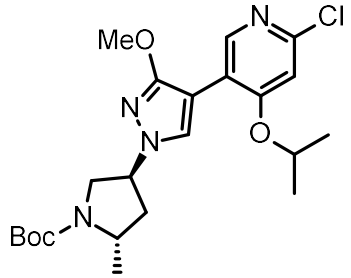
***tert*-Butyl (2*S*,4*S*)-4-(4-iodo-3-methoxy-1*H*-pyrazol-1-yl)-2-methylpyrrolidine-1-carboxylate (51)**



To a suspension of 4-iodo-3-methoxy-1*H*-pyrazole (**50**)[28] (500 mg, 2.23 mmol) and *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-methylpyrrolidine-1-carboxylate (674 mg, 3.35 mmol) in toluene (10 mL) was added CMBP (1.62 g, 6.70 mmol) at rt. After stirring at reflux for 5 h, the reaction mixture was concentrated. Purification by column chromatography [SiO_2, Hex/EtOAc = 90/10–50/50 (v/v)] afforded the title compound (832 mg, 2.04 mmol, 92% yield) as a colorless amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.25-1.27 (3H, br m), 1.46 (9H, s), 1.94-1.97 (1H, m), 2.52-2.54 (1H, br m), 3.69-3.72 (2H, m), 3.92 (3H, s), 4.08-4.15 (1H, br m), 4.67-4.69 (1H, m), 7.23 (1H, s).

***tert*-Butyl (2*S*,4*S*)-4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-methoxy-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (52)**

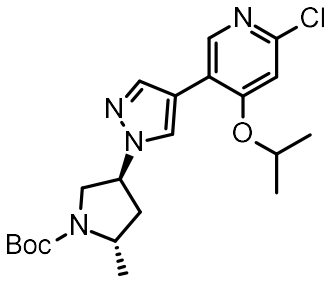


A mixture of *tert*-butyl (2*S*,4*S*)-4-(4-iodo-3-methoxy-1*H*-pyrazol-1-yl)-2-methylpyrrolidine-1-carboxylate (**51**) (830 mg, 2.04 mmol), 2-chloro-4-(propan-2-yloxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**49**) (1.16 g, crude), K_2CO_3 (845 mg, 6.11 mmol), and Pd(dppf)Cl_2·DCM (166 mg, 0.204 mmol) in 1,4-dioxane (20 mL) and water (4.0 mL) was stirred at reflux for 4 h. After cooling down to rt, water was added to the reaction mixture. The mixture was extracted with EtOAc, washed with brine, and dried over anhydrous $MgSO_4$. After concentration under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 90/10 (v/v)–50/50 (v/v)] to give the title compound (656 mg, 1.45 mmol, 71% yield) as a pale-

yellow amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.30-1.30 (3H, br m), 1.41-1.45 (15H, m), 1.99-2.02 (1H, m), 2.58-2.61 (1H, br m), 3.76-3.84 (2H, br m), 3.98 (3H, s), 4.05-4.19 (1H, br m), 4.67-4.73 (2H, m), 6.79 (1H, s), 7.76 (1H, s), 8.79 (1H, s).

***tert*-Butyl (2*S*,4*S*)-4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (53a)**

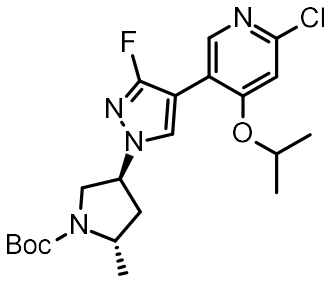


To a solution of 2-chloro-4-(propan-2-yloxy)-5-(1H-pyrazol-4-yl)pyridine (**41b**) (1.60 g, 6.73 mmol) and *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-methylpyrrolidine-1-carboxylate (1.63 g, 8.08 mmol) in toluene (30 mL) was added CMBP (3.5 mL, 13.5 mmol) at rt. After stirring at reflux for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography [SiO_2, Hex/EtOAc = 85/15–60/40 (v/v)] to give the title compound (2.78 g, 6.60 mmol, 98% yield) as a pale-yellow oil.

^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, d, J = 5.5 Hz), 1.40-1.52 (15H, m), 2.06-2.15 (1H, m), 2.58-2.72 (1H, m), 3.72-3.94 (2H, m), 4.03-4.23 (1H, m), 4.68-4.76 (1H, m), 4.90-4.99 (1H, m), 6.84 (1H, s), 7.83 (1H, s), 7.91 (1H, s), 8.38 (1H, s).

MS (ESI/APCI): *m/z* calcd for $C_{21}H_{30}ClN_4O_3$ $(M+H)^+$ 421.2. Found 421.2.

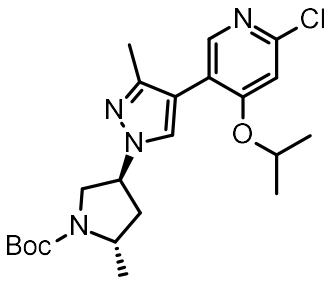
***tert*-Butyl (2*S*,4*S*)-4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-fluoro-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (53b)**



To a solution of 2-chloro-5-(3-fluoro-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridine (**48**) (70 mg, 0.27 mmol) in toluene (3 mL) were added *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-methylpyrrolidine-1-carboxylate (66 mg, 0.33 mmol) and CMBP (0.140 mL, 0.548 mmol). After stirring at reflux for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography [SiO_2, Hex/EtOAc = 80/20–60/40 (v/v)] to give the title compound (69 mg, 0.16 mmol, 57% yield) as a pale-yellow oil.

^{1}H-NMR ($CDCl_3$) δ: 1.26-1.33 (3H, m), 1.41-1.51 (15H, m), 1.99-2.09 (1H, m), 2.54-2.71 (1H, m), 3.68-3.96 (2H, m), 3.98-4.27 (1H, m), 4.69-4.79 (2H, m), 6.84 (1H, s), 7.72 (1H, d, *J* = 1.8 Hz), 8.47 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{21}H_{29}ClFN_4O_3$ $(M+H)^+$ 439.2. Found 439.2.

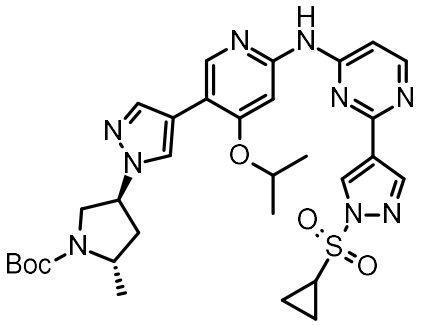
tert-Butyl (2*S*,4*S*)-4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-methyl-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (53c)



A mixture of 2-chloro-5-(3-methyl-1*H*-pyrazol-4-yl)-4-(propan-2-yloxy)pyridine (**47**) (4.32 g, 17.2 mmol), *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-methylpyrrolidine-1-carboxylate (3.80 g, 18.9 mmol), and CMBP (9.0 mL, 34 mmol) in toluene (60 mL) was stirred at reflux for 2.5 h. *tert*-Butyl (2*S*,4*R*)-4-hydroxy-2-methylpyrrolidine-1-carboxylate (690 mg, 3.43 mmol) and CMBP (1.8 mL, 6.9 mmol) were added and the reaction mixture was stirred at reflux for 1 h. The reaction mixture was concentrated, and the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 65/35–55/45 (v/v)] to give the title compound (4.43 g, 10.2 mmol, 59% yield) as a colorless amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.27-1.33 (3H, m), 1.39 (6H, d, *J* = 6.1 Hz), 1.46 (9H, s), 2.00-2.13 (1H, m), 2.28 (3H, s), 2.54-2.69 (1H, m), 3.68-3.95 (2H, m), 3.98-4.27 (1H, m), 4.61-4.72 (1H, m), 4.84-4.93 (1H, m), 6.84 (1H, s), 7.50 (1H, s), 8.14 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{22}H_{32}ClN_4O_3$ $(M+H)^+$ 435.2. Found 435.2.

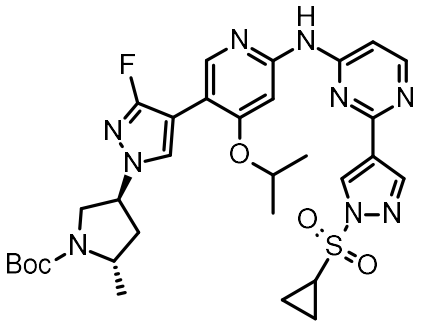
***tert*-Butyl (2*S*,4*S*)-4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (54a)**



A mixture of *tert*-butyl (2*S*,4*S*)-4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (**53a**) (944 mg, 2.24 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (653 mg, 2.46 mmol), Xphos (215 mg, 0.451 mmol), tris(dibenzylideneacetone)dipalladium(0) (225 mg, 0.246 mmol), and Cs_2CO_3 (1.81 g, 5.56 mmol) in *tert*-BuOH (11 mL) was stirred at reflux for 3 h under a N_2 atmosphere. After cooling down to rt, the reaction mixture was diluted with DCM and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, EtOAc/MeOH = 100/0–96/4(v/v)] to give the title compound (809 mg, 1.25 mmol, 56% yield) as a colorless amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.19-1.29 (2H, m), 1.32 (3H, d, *J* = 5.5 Hz), 1.39-1.58 (17H, m), 2.03-2.16 (1H, m), 2.60-2.74 (1H, m), 2.80-2.87 (1H, m), 3.70-3.94 (2H, m), 4.03-4.29 (1H, m), 4.84-5.01 (2H, m), 7.04 (1H, d, *J* = 5.5 Hz), 7.55 (1H, s), 7.70 (1H, br s), 7.85 (1H, s), 7.93 (1H, s), 8.36 (1H, s), 8.42 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{31}H_{40}N_9O_5S$ $(M+H)^+$ 650.3. Found 650.3.

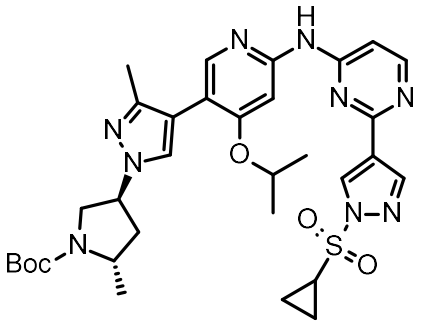
***tert*-Butyl (2S,4*S*)-4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-fluoro-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (54b)**



A mixture of *tert*-butyl (2*S*,4*S*)-4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-fluoro-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (**53b**) (69 mg, 0.16 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (42 mg, 0.16 mmol), tris(dibenzylideneacetone)dipalladium(0) (14 mg, 0.016 mmol), Xphos (19 mg, 0.039 mmol), and Cs_2CO_3 (102 mg, 0.314 mmol) in *tert*-BuOH (2 mL) was stirred at reflux for 3 h under a N_2 atmosphere. After cooling down to rt, the mixture was diluted with DCM and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 25/75–0/100 (v/v)] to give the title compound (46 mg, 0.069 mmol, 44% yield) as a colorless amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.20-1.35 (5H, m), 1.41-1.57 (17H, m), 2.01-2.10 (1H, m), 2.57-2.71 (1H, m), 2.80-2.88 (1H, m), 3.70-3.93 (2H, m), 4.02-4.24 (1H, m), 4.72-4.82 (1H, m), 4.83-4.92 (1H, m), 7.04 (1H, d, *J* = 5.5 Hz), 7.54 (1H, s), 7.69-7.75 (2H, m), 8.41-8.46 (3H, m), 8.64 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{31}H_{39}FN_9O_5S$ $(M+H)^+$ 668.3. Found 668.3.

tert-Butyl (2*S*,4*S*)-4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-methyl-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (54c)

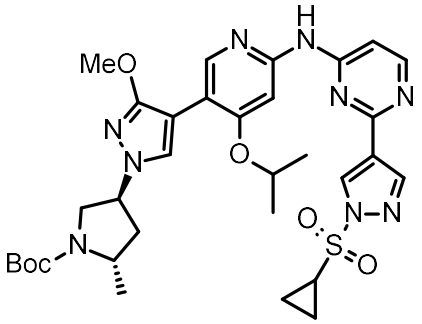


A mixture of *tert*-butyl (2*S*,4*S*)-4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-methyl-1*H*-

pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (**53c**) (4.43 g, 10.2 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (2.63 g, 9.91 mmol), Xphos (970 mg, 2.03 mmol), tris(dibenzylideneacetone)dipalladium(0) (980 mg, 1.07 mmol), and Cs_2CO_3 (8.35 g, 25.6 mmol) in *tert*-BuOH (51 mL) was stirred at reflux for 3 h. After cooling down to rt, the mixture was diluted with DCM and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, DCM/MeOH = 98/2–95/5 (v/v)] to give the title compound (2.75 g, 4.14 mmol, 41% yield) as a yellow amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.18-1.34 (5H, m), 1.40-1.56 (17H, m), 2.04-2.15 (1H, m), 2.32 (3H, s), 2.57-2.68 (1H, m), 2.80-2.88 (1H, m), 3.69-3.93 (2H, m), 4.00-4.23 (1H, m), 4.77-4.86 (1H, m), 4.86-4.95 (1H, m), 7.08 (1H, d, J = 5.5 Hz), 7.52 (1H, s), 7.67 (1H, br s), 7.71 (1H, s), 8.11 (1H, s), 8.42 (1H, d, J = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{32}H_{42}N_9O_5S$ $(M+H)^+$ 664.3. Found 664.3.

***tert*-Butyl (2*S*,4*S*)-4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-methoxy-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (54d)**

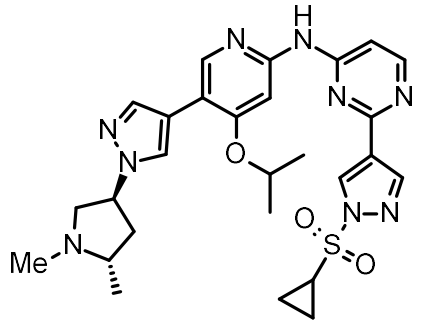


A mixture of *tert*-butyl (2*S*,4*S*)-4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-methoxy-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (**52**) (654 mg, 1.45 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (385 mg, 1.45 mmol), tris(dibenzylideneacetone)dipalladium(0) (13.3 mg, 0.145 mmol), Xphos (27.7 mg, 0.580 mmol), and Cs_2CO_3 (945 mg, 2.90 mmol) in *tert*-BuOH (10 mL) was stirred at reflux for 6 h. After cooling down to rt, water was added to the reaction mixture. The mixture was extracted with EtOAc, washed with brine, and dried over anhydrous $MgSO_4$. After concentration under reduced pressure, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 80/20–0/100 (v/v)] to give

the title compound (339 mg, 0.499 mmol, 34% yield) as a colorless amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.20-1.25 (2H, m), 1.31-1.32 (3H, br m), 1.49 (9H, br s), 1.52-1.55 (8H, m), 2.03-2.04 (1H, br m), 2.60 (1H, br s), 2.82-2.84 (1H, m), 3.77-3.84 (2H, br m), 4.00 (3H, s), 4.07-4.18 (2H, br m), 4.74-4.76 (1H, m), 4.83-4.89 (1H, m), 7.05-7.06 (1H, br m), 7.68 (1H, br s), 7.79 (1H, s), 7.92 (1H, br s), 8.39 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s), 8.77 (1H, s).

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-1,5-dimethylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (55a)



Step 1: To a solution of *tert*-butyl (2*S*,4*S*)-4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (**54a**) (1.27 g, 1.95 mmol) in DCM (10 mL) was added TFA (10 mL) at rt. After stirring at rt for 1 h, the reaction mixture was concentrated under reduced pressure and quenched by sat. aq. $NaHCO_3$. The mixture was extracted with DCM/MeOH, washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by column chromatography [amino, DCM/MeOH = 99/1–97/3 (v/v)] afforded 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-5-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (943 mg, 1.72 mmol, 88% yield) as a pink solid.

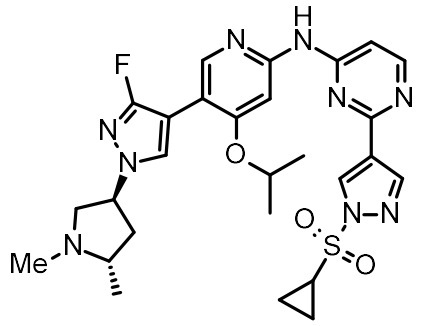
^{1}H-NMR ($CDCl_3$) δ: 1.20-1.32 (5H, m), 1.51-1.57 (8H, m), 1.81-1.90 (1H, m), 2.38-2.45 (1H, m), 2.80-2.87 (1H, m), 3.27 (1H, dd, *J* = 11.7, 4.9 Hz), 3.49-3.67 (2H, m), 4.84-4.94 (2H, m), 7.03 (1H, d, *J* = 5.5 Hz), 7.65 (1H, s), 7.70 (1H, br s), 7.88 (1H, s), 7.92 (1H, s), 8.37 (1H, s), 8.41 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{26}H_{32}N_9O_3S$ $(M+H)^+$ 550.2. Found 550.3.

Step 2: To a solution of 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-5-

methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (197 mg, 0.358 mmol) in MeOH (4 mL) were added aq. formaldehyde (37mass%, 0.040 mL, 0.532 mmol) and $NaBH(OAc)_3$ (110 mg, 0.519 mmol) in sequence at rt. After stirring at rt for 2 h, the reaction mixture was quenched by sat. aq. $NaHCO_3$ and extracted with DCM. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by column chromatography [SiO_2, EtOAc/MeOH = 100/0–98/2 (v/v); DCM/MeOH = 80/20] afforded 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-1,5-dimethylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (**54a**) (139 mg, 0.247 mmol, 69% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.18-1.27 (5H, m), 1.51-1.57 (8H, m), 2.04-2.13 (1H, m), 2.38-2.46 (4H, m), 2.66-2.76 (2H, m), 2.80-2.87 (1H, m), 3.60 (1H, dd, *J* = 9.5, 7.6 Hz), 4.85-4.93 (2H, m), 7.02 (1H, d, *J* = 5.5 Hz), 7.64 (1H, s), 7.71 (1H, br s), 7.87 (1H, s), 7.94 (1H, s), 8.37 (1H, s), 8.41 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.16, 18.36, 21.84, 31.26, 39.43, 40.46, 58.52, 60.59, 63.19, 70.92, 97.12, 106.27, 114.10, 115.13, 125.70, 127.09, 131.14, 137.61, 144.38, 145.67, 152.08, 156.33, 158.74, 159.19, 161.38. HRMS (ESI): *m/z* calcd for $C_{27}H_{34}N_9O_3S$ $(M+H)^+$ 564.2507. Found 564.2489.

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-1,5-dimethylpyrrolidin-3-yl]-3-fluoro-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (55b)

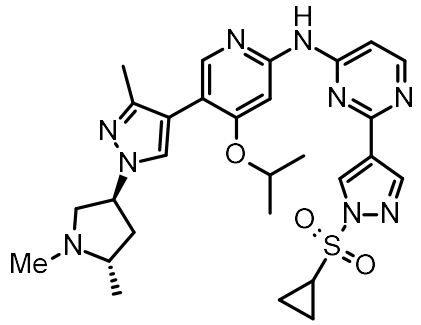


Step 1: To a solution of *tert*-butyl (2*S*,4*S*)-4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-fluoro-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (**54b**) (44 mg, 0.066 mmol) in DCM (1 mL) was added TFA (0.5 mL). After stirring at rt for 30 min, the reaction mixture was diluted with DCM. The mixture was washed with aq. 1 mol/L K_2CO_3, dried over anhydrous Na_2SO_4, and concentrated. The residue was

purified by column chromatography (amino, DCM/MeOH) to give 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{3-fluoro-1-[(3*S*,5*S*)-5-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (33 mg, 0.058 mmol, 88% yield) as a colorless solid.
^{1}H-NMR (DMSO-D_6) δ: 1.11 (3H, d, *J* = 6.7 Hz), 1.22-1.39 (4H, m), 1.44 (6H, d, *J* = 6.1 Hz), 1.63-1.73 (1H, m), 2.11-2.19 (1H, m), 2.90 (1H, dd, *J* = 11.6, 5.5 Hz), 3.25-3.41 (3H, m), 4.77-4.84 (1H, m), 4.85-4.93 (1H, m), 7.36-7.44 (1H, m), 7.89 (1H, br s), 8.00 (1H, d, *J* = 2.4 Hz), 8.27 (1H, s), 8.43-8.47 (2H, m), 8.65 (1H, s), 10.30 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{26}H_{31}FN_9O_3S$ $(M+H)^+$ 568.2. Found 568.2.

Step 2: To a solution of 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{3-fluoro-1-[(3*S*,5*S*)-5-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (25 mg, 0.044 mmol) in DCM (0.5 mL and MeOH (0.5 mL) were added aq. formaldehyde (37 mass%, 0.018 mL, 0.22 mmol) and $NaBH(OAc)_3$ (19 mg, 0.088 mmol). After stirring at rt for 90 min, the reaction was quenched by aq. 1.0 mol/L K_2CO_3. The mixture was diluted with DCM, washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography [SiO_2, DCM/MeOH = 99/1–97/3 (v/v)] to give 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-1,5-dimethylpyrrolidin-3-yl]-3-fluoro-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (**55b**) (24 mg, 0.041 mmol, 94% yield) as a colorless solid.
^{1}H-NMR (DMSO-D_6) δ: 1.08 (3H, d, *J* = 5.5 Hz), 1.21-1.39 (4H, m), 1.44 (6H, d, *J* = 6.1 Hz), 1.84-1.93 (1H, m), 2.18-2.29 (4H, m), 2.40-2.57 (2H, m), 3.25-3.33 (1H, m), 3.36-3.45 (1H, m), 4.79-4.94 (2H, m), 7.38-7.43 (1H, m), 7.89 (1H, br s), 8.02 (1H, d, *J* = 2.4 Hz), 8.28 (1H, s), 8.44-8.47 (2H, m), 8.65 (1H, s), 10.30 (1H, s). 13C-NMR (CDCl3) δ: 7.16, 18.35, 21.81, 31.26, 39.38, 40.05, 59.08, 60.39, 62.70, 71.05, 97.08, 97.94 (d, *J* = 17.3 Hz), 106.40, 111.86 (d, *J* = 5.8 Hz), 125.75, 129.95, 131.09, 144.38, 146.63 (d, *J* = 8.7 Hz), 152.42, 156.36, 158.70, 159.26, 160.39 (d, *J* = 246.6 Hz), 161.35. HRMS (ESI): *m/z* calcd for $C_{27}H_{33}FN_9O_3S$ $(M+H)^+$ 582.2413. Found 582.2395.

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-1,5-dimethylpyrrolidin-3-yl]-3-methyl-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (55c)



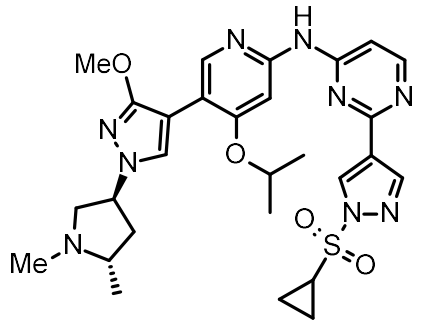
Step 1: To a solution of *tert*-butyl (2*S*,4*S*)-4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-methyl-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (**54c**) (2.75 g, 4.14 mmol) in DCM (20 mL) was added TFA (14 mL). After stirring at rt for 45 min, sat. aq. $NaHCO_3$ was added to the reaction mixture and extracted with DCM/MeOH. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated. Purification by column chromatography [amino, DCM/MeOH = 99/1–96/4 (v/v)] afforded 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{3-methyl-1-[(3*S*,5*S*)-5-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (1.85 g, 3.28 mmol, 79% yield) as a yellow amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.20-1.26 (5H, m), 1.47 (6H, d, *J* = 6.1 Hz), 1.51-1.56 (2H, m), 1.80-1.89 (1H, m), 2.33 (3H, s), 2.35-2.42 (1H, m), 2.80-2.87 (1H, m), 3.25 (1H, dd, *J* = 11.6, 4.9 Hz), 3.50-3.64 (2H, m), 4.79-4.87 (2H, m), 7.08 (1H, d, *J* = 5.5 Hz), 7.57 (1H, s), 7.64-7.67 (2H, m), 8.12 (1H, s), 8.42 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{27}H_{34}N_9O_3S$ $(M+H)^+$ 564.3. Found 564.3.

Step 2: To a solution of 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{3-methyl-1-[(3*S*,5*S*)-5-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (1.85 g, 3.28 mmol) in MeOH (15 mL) and DCM (15 mL) were added aq. formaldehyde (37 mass%, 0.370 mL, 4.92 mmol) and $NaBH(OAc)_3$ (1.04 g, 4.91 mmol). After stirring at rt for 40 min, sat. aq. $NaHCO_3$ was added to the reaction mixture and extracted with DCM/MeOH. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification by column chromatography [amino, DCM/MeOH = 100/0–97/3 (v/v)] afforded 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-1,5-dimethylpyrrolidin-3-yl]-3-methyl-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (**55c**) (1.68 g, 2.91 mmol, 89% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, d, *J* = 6.1 Hz), 1.19-1.27 (2H, m), 1.47 (6H, d, *J* = 6.1 Hz), 1.51-1.56 (2H, m), 2.01-2.11 (1H, m), 2.33 (3H, s), 2.36-2.44 (4H, m), 2.61-2.71 (2H, m), 2.80-2.87 (1H, m), 3.54-3.59 (1H, m), 4.78-4.87 (2H, m), 7.08 (1H, d, *J* = 5.5 Hz), 7.53-7.57 (2H, m), 7.64 (1H, s), 8.12 (1H, s), 8.42 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.15, 13.61, 18.53, 21.70, 31.26, 39.48, 40.31, 58.28, 60.48, 63.27, 70.62, 97.05, 106.26, 112.95, 114.96, 125.71, 128.71, 131.13, 144.38, 146.57, 148.00, 152.60, 156.40, 158.78, 159.26, 162.35. HRMS (ESI): *m/z* calcd for $C_{28}H_{36}N_9O_3S$ $(M+H)^+$ 578.2664. Found 578.2647.

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-1,5-dimethylpyrrolidin-3-yl]-3-methoxy-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (55d)



Step 1: To a solution of *tert*-butyl (2*S*,4*S*)-4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-methoxy-1*H*-pyrazol-1-yl}-2-methylpyrrolidine-1-carboxylate (**54d**) (339 mg, 0.499 mmol) in DCM (5.0 mL) was added TFA (1.91 mL) at rt. After stirring at rt for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography [amino, EtOAc/MeOH = 100/0–80/20 (v/v)] to give 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{3-methoxy-1-[(3*S*,5*S*)-5-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (247 mg, 0.426 mmol, 85% yield) as a colorless solid.

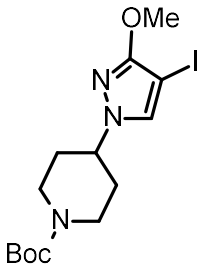
^{1}H-NMR ($CDCl_3$) δ: 1.20-1.28 (5H, m), 1.52-1.55 (8H, m), 1.76-1.80 (1H, m), 2.33-2.36 (1H, m), 2.82-2.84 (1H, m), 3.22-3.25 (1H, m), 3.44-3.47 (1H, m), 3.57-3.61 (1H, m), 4.00 (3H, s), 4.68-4.72 (1H, m), 4.83-4.89 (1H, m), 7.05-7.06 (1H, br m), 7.65 (1H, br s), 7.73 (1H, br s), 7.82 (1H, s), 8.39 (1H, d, *J* = 6.1 Hz), 8.45 (1H, s), 8.64 (1H, s), 8.78 (1H, s).

Step 2: To a solution of 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{3-methoxy-1-[(3*S*,5*S*)-

5-methylpyrrolidin-3-yl]-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (138 mg, 0.238 mmol) in MeOH (0.5 mL) and DCM (5.0 mL) was added aq. formaldehyde (37 mass%, 0.0537 mL, 0.714 mmol). After stirring at rt for 30 min, $NaBH(OAc)_3$ (101 mg, 0.476 mmol) was added to the reaction mixture. After stirring at rt for 16 h, sat. aq. $NaHCO_3$ and water were added to the reaction mixture and the mixture was extracted with DCM. The organic layer was dried over anhydrous $MgSO_4$. After evaporating the solvent, the residue was purified by column chromatography [amino, EtOAc/DCM/MeOH = 95/0/5–85/10/5 (v/v/v)]. The crude product was washed with Et_2O to give 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-[5-{1-[(3*S*,5*S*)-1,5-dimethylpyrrolidin-3-yl]-3-methoxy-1*H*-pyrazol-4-yl}-4-(propan-2-yloxy)pyridin-2-yl]pyrimidin-4-amine (**55d**) (122 mg, 0.205 mmol, 86% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.17-1.28 (5H, m), 1.52-1.54 (8H, m), 1.96-2.05 (1H, m), 2.34-2.36 (4H, m), 2.62-2.66 (2H, m), 2.81-2.85 (1H, m), 3.52-3.55 (1H, m), 4.01 (3H, s), 4.67-4.70 (1H, m), 4.83-4.90 (1H, m), 7.05 (1H, d, *J* =5.5 Hz), 7.66 (1H, br s), 7.82 (1H, s), 7.86 (1H, br s), 8.39 (1H, d, *J* =5.5 Hz), 8.45 (1H, s), 8.64 (1H, s), 8.79 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.15, 18.51, 21.90, 31.27, 39.58, 40.23, 56.20, 58.53, 60.44, 62.79, 70.76, 97.13, 98.67, 106.48, 113.88, 125.90, 129.57, 131.05, 144.41, 146.66, 151.56, 156.00, 158.66, 159.45, 160.36, 160.97. HRMS (ESI): *m/z* calcd for $C_{28}H_{36}N_9O_4S$ $(M+H)^+$ 594.2613. Found 594.2600.

***tert*-Butyl 4-(4-iodo-3-methoxy-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (57a)**

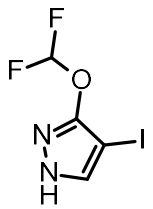


A mixture of 4-iodo-3-methoxy-1*H*-pyrazole (**50**) (300 mg, 1.34 mmol), *tert*-butyl 4-methylsulfonyloxypiperidine-1-carboxylate (487 mg, 1.74 mmol), and Cs_2CO_3 (1.10 g, 3.35 mmol) in DMA (7.0 mL) was stirred at 100°C for 2 h. After cooling down to 0°C, water was added to the reaction mixture. The mixture was extracted with Et_2O and the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 75/25–60/40 (v/v)] to give the title compound (392

mg, 0.963 mmol, 72% yield) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.75-1.87 (2H, m), 2.03-2.11 (2H, m), 2.79-2.93 (2H, m), 3.92 (3H, s), 4.01-4.34 (3H, m), 7.24 (1H, s).

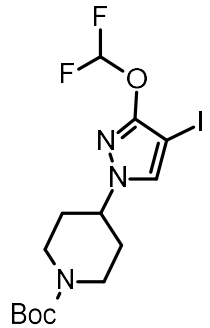
3-(Difluoromethoxy)-4-iodo-1*H*-pyrazole (56)



To a solution of 3-(difluoromethoxy)-1*H*-pyrazole[29] (920 mg, 6.86 mmol) in DMF (22 mL) was added *N*-iodosuccinimide (1.72 g, 7.64 mmol) at 0°C. After stirring at 0°C for 2 h, water was added to the reaction mixture. The mixture was extracted with EtOAc, and the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. Purification by column chromatography [SiO_2, Hex/EtOAc = 75/25–60/40 (v/v)] afforded the mixture of the title compounds (1.55 g, 5.96 mmol, 87% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 6.92 (1H, t, J = 72.9 Hz), 7.51 (1H, s), 9.66 (1H, br s). MS (ESI/APCI): *m/z* calcd for $C_4H_4F_2IN_2O$ $(M+H)^+$ 260.9. Found 261.

***tert*-Butyl 4-[3-(difluoromethoxy)-4-iodo-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (57b)**

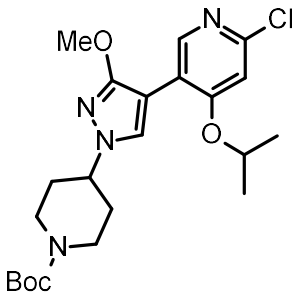


A mixture of 3-(difluoromethoxy)-4-iodo-1*H*-pyrazole (**56**) (300 mg, 1.15 mmol), *tert*-butyl 4-methylsulfonyl-oxypiperidine-1-carboxylate (430 mg, 1.54 mmol), and Cs_2CO_3 (923 mg, 2.83 mmol) in DMA (5.0 mL) was stirred at 100°C for 2 h. After cooling down to rt, water was added to the reaction mixture. The mixture was extracted with EtOAc and the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was

purified by column chromatography [SiO_2, Hex/EtOAc = 75/25–65/35 (v/v)] to give the title compound (430 mg, 0.970 mmol, 84% yield) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.78-1.89 (2H, m), 2.04-2.10 (2H, m), 2.77-2.92 (2H, m), 4.06-4.31 (3H, m), 6.89 (1H, t, *J* = 72.9 Hz), 7.33 (1H, s).

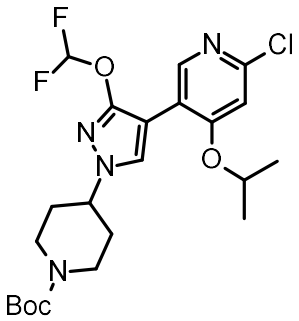
tert-Butyl 4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-methoxy-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (58a)



A mixture of *tert*-butyl 4-(4-iodo-3-methoxy-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (**57a**) (392 mg, 0.963 mol), 2-chloro-4-(propan-2-yloxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**49**) (580 mg, crude), Pd(dppf)Cl_2 (142 mg, 0.194 mol), and aq. K_2CO_3 (2.0 mol/L, 1.2 mL, 2.4 mmol) in 1,4-dioxane (5.0 mL) was stirred at 100°C for 1 h. After cooling down to rt, the reaction mixture was diluted with EtOAc, washed with water and brine, and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 65/35–55/45 (v/v)] to give the title compound (223 mg, 0.494 mmol, 51% yield) as a brown solid.

^{1}H-NMR ($CDCl_3$) δ: 1.44 (6H, d, *J* = 6.1 Hz), 1.48 (9H, s), 1.79-1.92 (2H, m), 2.10-2.17 (2H, m), 2.84-2.96 (2H, m), 3.98 (3H, s), 4.05-4.36 (3H, m), 4.67-4.75 (1H, m), 6.79 (1H, s), 7.78 (1H, s), 8.81 (1H, s).

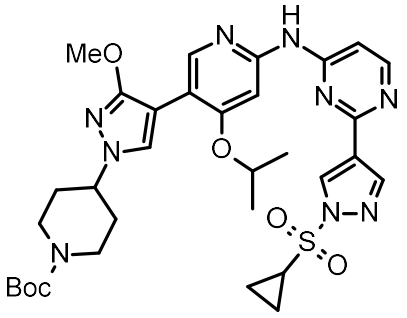
tert-Butyl 4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-(difluoromethoxy)-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (58b)



A mixture of [3-(difluoromethoxy)-4-iodo-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (**59b**) (420 mg, 0.948 mol), 2-chloro-4-(propan-2-yloxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**49**) (423 mg, crude), $Pd(dppf)Cl_2$ (138 mg, 0.189 mol), and aq. K_2CO_3 (2.0 mol/L, 1.2 mL, 2.4 mmol) in 1,4-dioxane (5.0 mL) was stirred at 100°C for 1 h. After cooling down to rt, the reaction mixture was diluted with EtOAc, washed with water and brine, and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 67/33–55/45 (v/v)] to give the title compound (325 mg, 0.667 mmol, 70% yield) as a brown amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.43 (6H, d, *J* = 6.1 Hz), 1.48 (9H, s), 1.78-1.95 (2H, m), 2.09-2.18 (2H, m), 2.82-2.97 (2H, m), 4.07-4.35 (3H, m), 4.65-4.75 (1H, m), 6.83 (1H, s), 7.04 (1H, t, *J* = 73.2 Hz), 7.73 (1H, s), 8.57 (1H, s).

tert-Butyl 4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-methoxy-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (59a)

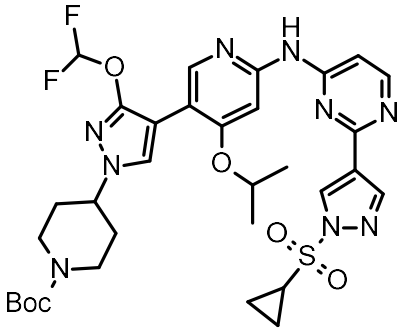


tert-Butyl 4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-methoxy-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (**58a**) (220 mg, 0.488 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (144 mg, 0.543 mmol), tris(dibenzylideneacetone)dipalladium(0) (92 mg, 0.10 mmol), Xphos (91 mg, 0.19 mmol), and Cs_2CO_3 (398 mg, 1.22 mmol) in 1,4-dioxane (3.0

mL) were stirred at reflux for 2.5 h. After cooling down to rt, the mixture was diluted with DCM and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, $CHCl_3$/MeOH = 98/2–95/5 (v/v); amino, $CHCl_3$/MeOH = 100/0–99/1 (v/v)] to give the title compound (157 mg, 0.231 mmol, 47% yield) as a yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 1.20-1.26 (2H, m), 1.47-1.56 (17H, m), 1.80-1.94 (2H, m), 2.12-2.20 (2H, m), 2.79-2.99 (3H, m), 4.01 (3H, s), 4.06-4.35 (3H, m), 4.79-4.91 (1H, m), 7.05 (1H, d, *J* = 5.5 Hz), 7.66 (1H, s), 7.75 (1H, s), 7.80 (1H, s), 8.39 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s), 8.78 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{32}H_{42}N_9O_6S$ $(M+H)^+$ 680.3. Found 680.3.

tert-Butyl 4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-(difluoromethoxy)-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (59b)

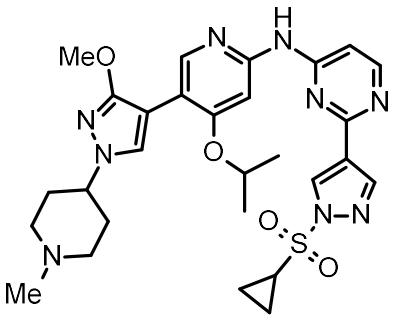


A mixture of *tert*-butyl 4-{4-[6-chloro-4-(propan-2-yloxy)pyridin-3-yl]-3-(difluoromethoxy)-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (**58b**) (17.0 g, 35.0 mmol), 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-amine (**9**) (9.28 g, 35.0 mmol), tris(dibenzylideneacetone)dipalladium(0) (3.20 g, 3.50 mmol), Xphos (4.17 g, 8.75 mmol), and Cs_2CO_3 (22.8 g, 70.0 mmol) in 1,4-dioxane (340 mL) was stirred at 90°C for 2 h. After cooling down to rt, the mixture was diluted with DCM and filtered through Celite. After evaporating the solvent, the residue was purified by column chromatography [SiO_2, Hex/EtOAc = 30/70–0/100 (v/v)]. The crude product was washed with Hex/EtOAc to give the title compound (7.70 g, 10.8 mmol, 31% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.20-1.27 (2H, m), 1.49 (9H, s), 1.51-1.56 (8H, m), 1.83-1.96 (2H, m), 2.11-2.19 (2H, m), 2.80-2.99 (3H, m), 4.10-4.34 (3H, m), 4.83-4.91 (1H, m), 7.04 (1H, d, *J* = 5.5 Hz), 7.08 (1H, t, *J* = 74.2 Hz), 7.73-7.78 (2H, m), 8.05 (1H, s), 8.42 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.57 (1H, s), 8.64 (1H, s). MS (ESI/APCI): *m/z* calcd for $C_{32}H_{40}F_2N_9O_6S$ $(M+H)^+$ 716.3. Found

716.3.

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{5-[3-methoxy-1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}pyrimidin-4-amine (60a)



Step 1: To a solution of *tert*-butyl 4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-methoxy-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (**59a**) (150 mg, 0.221 mmol) in DCM (1.0 mL) was added TFA (1.0 mL) at rt. After stirring at rt for 30 min, the reaction mixture was quenched by sat. aq. $NaHCO_3$. The mixture was extracted with MeOH/$CHCl_3$ and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was purified by column chromatography [amino, $CHCl_3$/MeOH = 98/2–97/3 (v/v)] to give 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{5-[3-methoxy-1-(piperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}pyrimidin-4-amine (102 mg, 0.176 mmol, 80% yield) as a colorless solid.

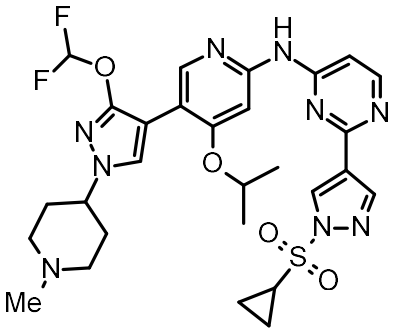
^{1}H-NMR ($CDCl_3$) δ: 1.19-1.26 (2H, m), 1.50-1.56 (8H, m), 1.80-1.91 (2H, m), 2.15-2.22 (2H, m), 2.74-2.87 (3H, m), 3.22-3.28 (2H, m), 4.01 (3H, s), 4.02-4.12 (1H, m), 4.82-4.90 (1H, m), 7.05 (1H, d, *J* = 5.5 Hz), 7.66 (1H, br s), 7.79 (1H, br s), 7.84 (1H, s), 8.39 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.64 (1H, s), 8.78 (1H, s).

Step 2: To a solution of 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{5-[3-methoxy-1-(piperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}pyrimidin-4-amine (95.0 mg, 0.164 mmol) in MeOH (1.0 mL) and DCM (1.0 mL) were added aq. formaldehyde (37 mass%, 0.020 mL, 0.27 mmol) and $NaBH(OAc)_3$ (55 mg, 0.26 mmol). After stirring at rt for 1 h, sat. aq. $NaHCO_3$ was added to the reaction mixture and extracted with $CHCl_3$/MeOH. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was purified

by column chromatography [amino, $CHCl_3$/MeOH = 99/1–98/2 (v/v)] to give 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{5-[3-methoxy-1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}pyrimidin-4-amine (**60a**) (81.0 mg, 0.136 mmol, 83% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.19-1.27 (2H, m), 1.49-1.56 (8H, m), 1.97-2.08 (2H, m), 2.10-2.22 (4H, m), 2.35 (3H, s), 2.79-2.87 (1H, m), 2.97-3.04 (2H, m), 3.95-4.04 (4H, m), 4.82-4.92 (1H, m), 7.00 (1H, d, *J* = 5.5 Hz), 7.80 (1H, br s), 7.86 (1H, s), 8.37 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.63 (1H, s), 8.77-8.84 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 7.17, 21.92, 31.30, 32.30, 46.07, 54.71, 56.13, 58.55, 70.75, 97.00, 98.49, 106.43, 114.00, 125.88, 127.77, 131.09, 144.45, 146.80, 151.37, 156.11, 158.70, 159.36, 160.07, 160.97. HRMS (ESI): *m/z* calcd for $C_{28}H_{36}N_9O_4S$ $(M+H)^+$ 594.2613. Found 594.2617.

2-[1-(Cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{5-[3-(difluoromethoxy)-1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}pyrimidin-4-amine (60b)



Step 1: To a solution of *tert*-butyl 4-{4-[6-({2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]pyrimidin-4-yl}amino)-4-(propan-2-yloxy)pyridin-3-yl]-3-(difluoromethoxy)-1*H*-pyrazol-1-yl}piperidine-1-carboxylate (**59b**) (2.4 g, 3.4 mmol) in DCM (20 mL) was added TFA (10 mL) at rt. After stirring at rt for 30 min, the reaction mixture was concentrated under reduced pressure. The residue was diluted with DCM and sat. aq. $NaHCO_3$. The mixture was extracted with DCM/MeOH and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was purified by column chromatography [amino, $CHCl_3$/MeOH = 99/1–98/2 (v/v)] to give 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{5-[3-(difluoromethoxy)-1-(piperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-

yl}pyrimidin-4-amine (1.75 g, 2.84 mmol, 85% yield) as a colorless amorphous solid.

^{1}H-NMR ($CDCl_3$) δ: 1.20-1.26 (2H, m), 1.50-1.56 (8H, m), 1.83-1.94 (2H, m), 2.14-2.21 (2H, m), 2.74-2.87 (3H, m), 3.23-3.29 (2H, m), 4.05-4.15 (1H, m), 4.83-4.91 (1H, m), 7.04 (1H, d, *J* = 5.5 Hz), 7.08 (1H, t, *J* = 72.3 Hz), 7.77 (1H, br s), 7.80 (1H, s), 8.15 (1H, br s), 8.42 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.58 (1H, s), 8.64 (1H, s).

Step 2: To a solution of 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{5-[3-(difluoromethoxy)-1-(piperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}pyrimidin-4-amine (1.75 g, 2.84 mmol) in MeOH (10 mL) and DCM (10 mL) were added aq. formaldehyde (37 mass%, 0.320 mL, 4.26 mmol) and $NaBH(OAc)_3$ (910 mg, 4.26 mmol). After stirring at rt for 50 min, sat. aq. $NaHCO_3$ was added to the reaction mixture and extracted with $CHCl_3$/MeOH. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After evaporating the solvent, the residue was purified by column chromatography [amino, $CHCl_3$/MeOH = 99/1–98/2 (v/v)]. The crude product was washed with Et_2O to give 2-[1-(cyclopropylsulfonyl)-1*H*-pyrazol-4-yl]-*N*-{5-[3-(difluoromethoxy)-1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl]-4-(propan-2-yloxy)pyridin-2-yl}pyrimidin-4-amine (**60b**) (1.04 g, 1.65 mmol, 58% yield) as a colorless solid.

^{1}H-NMR ($CDCl_3$) δ: 1.20-1.28 (2H, m), 1.49-1.58 (8H, m), 1.98-2.21 (6H, m), 2.35 (3H, s), 2.80-2.86 (1H, m), 2.96-3.02 (2H, m), 3.97-4.05 (1H, m), 4.82-4.90 (1H, m), 7.04-7.09 (1H, m), 7.08 (1H, t, *J* = 74.8 Hz), 7.66-7.71 (2H, m), 7.79 (1H, s), 8.42 (1H, d, *J* = 5.5 Hz), 8.45 (1H, s), 8.56 (1H, s), 8.64 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 7.16, 21.84, 31.28, 32.19, 46.01, 54.51, 59.02, 70.98, 97.24, 100.47, 106.53, 112.26, 115.52 (t, *J* = 258.2 Hz), 125.90, 128.14, 131.05, 144.44, 146.96, 152.51, 153.22 (t, *J* = 3.5 Hz), 156.34, 158.63, 159.41, 161.42. HRMS (ESI): *m/z* calcd for $C_{28}H_{34}F_2N_9O_4S$ $(M+H)^+$ 630.2424. Found 630.2434.

2. Biological evaluation

Biochemical EGFR inhibition assay

EGFR WT (669–1210) (#08-115) was purchased from Carna Biosciences. EGFR (694–1022, L858R/T790M/C797S) and EGFR (694–1022, del19/T790M/C797S) were produced and purified by Daiichi Sankyo RD Novare Co., Ltd. AlphaLISA Immunoassay Buffer (AL000F), Protein A AlphaLISA-acceptor beads (AL101M), Streptavidin Donor beads (6760002), and 384-well assay plate (6008359) were purchased from PerkinElmer Inc. Anti-phospho-tyrosine antibody (8954S) was purchased from Cell Signaling Technologies. ATP (A6559) was purchased from Sigma. HER2 substrate peptide (BML-P243) was purchased from Enzo. EDTA (347-07481) was purchased from Dojindo Laboratories. Test compounds at concentrations ranging from 0.000305 to 5 μM were added to 384-well plates using a D300e digital dispenser (Tecan). The DMSO concentration was 0.5%. A total of 2.5 μL of a mixture of HER2 substrate (100 nmol/L) and ATP (the concentration described below) was added to each well. The ATP concentration was set as 2 μM for EGFR WT and EGFR (694-1022, L858R/T790M/C797S) or 20 μM for EGFR (694-1022, del19/T790M/C797S). The enzymatic reaction was started by adding 2.5 μL of enzyme (0.2 nmol/L). The above concentrations were defined at the enzymatic reaction. The enzyme and substrates were diluted with reaction buffer [50 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 10 mM $MnCl_2$, 0.01% Tween-20, 0.1% bovine serum albumin, 1 mM dithiothreitol] and incubated at 28°C for 90 min. After the enzymatic reaction, 2.5 μL of detection buffer (AlphaLISA Immunoassay Buffer containing 0.05% anti-phospho-tyrosine, 10 μg/mL Protein A AlphaLISA-acceptor beads, 10 μg/mL Streptavidin Donor beads, and 25 mM EDTA as the final concentration) was added to each well and then incubated at room temperature for 2 h. After incubation, AlphaLISA signal was measured by EnVision Xcite (PerkinElmer). Based on the measured AlphaLISA signals, the percentage inhibition of the enzyme at each concentration of the compound was calculated (N = 4). The obtained data were analyzed using the statistical analysis software GraphPad Prism (version 6, GraphPad Software), and IC_{50} values were calculated.

Ba/F3 proliferation assay

Ba/F3 cells were purchased from RIKEN BRC. Ba/F3 cells were transduced with retroviruses or lentiviruses encoding EGFR isoforms. Ba/F3-EGFR WT, Ba/F3-EGFR del19/T790M/C797S, and

EGFR L858R/T790M/C797S cell lines were cultured based on a culture medium of 10% FBS-containing RPMI 1640 supplemented with 1.5 μg/mL puromycin. EGF at 100 ng/mL was further added to the culture solution for Ba/F3-EGFR WT. The cells were cultured in a CO_2 incubator set at 37°C with 5% CO_2. Specimens prepared by dilution were seeded into 384-well tissue culture plates with Echo555 (Labcyte Inc.) and the cells were seeded thereto at 400 cells/well for Ba/F3-EGFR WT and Ba/F3-EGFR del19/T790M/C797S and at 120 cells/well for Ba/F3-EGFR L858R/T790M/C797S (day 0). They were then cultured for an additional 3 days. On the day of compound addition (day 0) and 3 days later (day 3), ATP quantity was measured with CellTiter-Glo 2.0 (Promega Corporation) and used as an indicator of cell quantity (N = 4). In the test, puromycin-free culture medium was used. Concentrations at which cell proliferation from day 0 to day 3 was inhibited by 50% (GI_{50}) were calculated using Microsoft Excel.

***in vivo* antitumor study**

All animal studies were approved by the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. A total of 1×10^7 Ba/F3-EGFR (del19/T790M/C797S) cells suspended in DPBS were subcutaneously inoculated into female CAnN.Cg-Foxn1^{nu}/CrlCrlj mice (Charles River Laboratories, Inc.). Tumor-bearing mice were randomized into each group 10 days after tumor cell implantation. Compound **42b** (DS06652923) was suspended in an equimolar amount of 2 mol/L methanesulfonic acid and 0.5% (w/v) methylcellulose solution and administered orally for 4 days. Tumor volume was calculated as follows: 1/2 × (tumor length) × (tumor width)^2. Statistical analysis was performed using parametric Dunnett's test (SAS System release 9.2).

Western blotting

The Lysis Buffer was prepared by diluting RIPA Buffer (10×) (Cell Signaling Technology) with water at a 10-fold dilution and adding PhosSTOP (Roche) and cOmplete, Mini Protease Inhibitor Cocktail (Roche). The frozen tumor was lysed by adding Lysis Buffer on ice and then disrupted using Multi Beads Shocker (Yasui Kikai Corporation). The lysate was then centrifuged at 14,500 rpm for 15 min at 4°C, and the supernatant was collected as the tumor extract. The protein concentration of the tumor extract was measured using DC protein assay system (Bio-Rad Laboratories) and Albumin Standard (Thermo Fisher Scientific). The tumor extract was prepared at

a final concentration of 1.5 mg/mL by adding NuPAGE LDS Sample Buffer and NuPAGE Sample Reducing Agent (Thermo Fisher Scientific) and incubating at 70°C for 10 min. The prepared samples were loaded onto Perfect NT gel (DRC) at 5 μg/lane and subjected to electrophoresis in Tris/Glycine/SDS buffer (Bio-Rad Laboratories). After electrophoresis, the proteins were transferred onto a PVDF membrane (Bio-Rad Laboratories, iBlot Turb Midi PDVF Transfer Packs) using a Trans-Blot system (Bio-Rad Laboratories) for 7 min. The transferred PVDF membrane was blocked with PVDF Blocking Reagent (Toyobo) for 1 h, and then incubated overnight in a refrigerator with Phospho-EGF Receptor (Tyr1068) Antibody #2234, EGF Receptor Antibody #2232, and β-Actin (13E5) Rabbit mAb #4970 (all from Cell Signaling Technology) diluted in Can Get Signal 1 (Toyobo). After washing with TBS-T for at least 25 min, the membrane was incubated with Anti-rabbit IgG, HRP-linked Antibody #7074 (Cell Signaling Technology) diluted in Can Get Signal 2 (Toyobo) as the secondary antibody for 1 h. The membrane was then washed with TBS-T and developed using Immobilon Forte Western Substrate (Millipore Corporation). The signal was measured using ImageQuant LAS4000 (Fujifilm).

Kinase selectivity profile

Kinase inhibitory profile of compound **55a**

The kinase inhibitory profile of compound **55a** against a panel of 161 kinases measured at a concentration of the test compound of 200 nmol/L in the presence of 1 mmol/L ATP was obtained from Carna Biosciences, Inc. (https://www.carnabio.com). The evaluated kinases and their proportions of inhibition are shown below:

ABL (80.9%), ACK (-7%), ALK (8.3%), ARG (50.5%), AXL (74.5%), BLK (66.5%), BMX (14.6%), BRK (2.5%), BTK (13.4%), CSK (-3.4%), DDR1 (86.2%), DDR2 (43.4%), EGFR (33.8%), EGFR [d746-750] (98.5%), EGFR [d746-750/T790M] (96.5%), EGFR [L858R] (90.4%), EGFR [L861Q] (59.7%), EGFR [T790M] (63.3%), EGFR [T790M/L858R] (89.1%), EPHA1 (-1.2%), EPHA2 (1.2%), EPHA3 (3.4%), EPHA4 (-1.4%), EPHA5 (0%), EPHA6 (0.2%), EPHA7 (1.8%), EPHA8 (-3.9%), EPHB1 (6.4%), EPHB2 (7.6%), EPHB3 (-2.6%), EPHB4 (1.6%), FAK (-5.7%), FER (20.9%), FES (14.6%), FGFR1 (15.2%), FGFR2 (43.4%), FGFR3 (29.7%), FGFR4 (4.8%), FGR (64.1%), FLT1 (98.8%), FLT3 (104.2%), FLT4 (100.7%), FMS (67.5%), FRK (-2.3%), FYN [isoform a] (61.8%), FYN [isoform b] (50.3%), HCK (53.1%), HER2 (10.8%), HER4 (11%), IGF1R

(1.9%), INSR (4.9%), IRR (2.2%), ITK (39.3%), JAK1 (1.5%), JAK2 (14.5%), JAK3 (10.9%), KDR (96.5%), KIT (97.7%), LCK (94.1%), LTK (-3.3%), LYNa (67.4%), LYNb (65.1%), MER (83.6%), MET (5.7%), MUSK (37.1%), PDGFRα (96.8%), PDGFRβ (100%), PYK2 (22.8%), RET (94.9%), RET [G691S] (94.3%), RET [M918T] (91.6%), RET [S891A] (97.5%), RET [Y791F] (94.1%), RON (5.5%), ROS (0.7%), SRC (78.5%), SRM (2.2%), SYK (-7.4%), TEC (3.8%), TIE2 (6.4%), TNK1 (-6%), TRKA (89.2%), TRKB (62%), TRKC (74.1%), TXK (-1.5%), TYK2 (-12.1%), TYRO3 (101.6%), YES (96.3%), AKT1 (-11%), AMPKα1/β1/γ1 (44.2%), AurA (-7.5%), AurB (18.7%), AurC (-4.6%), BRSK1 (10.9%), CaMK1α (-4%), CaMK4 (2%), CDC2/CycB1 (-4.4%), CDC7/ASK (-10%), CDK2/CycA2 (-3.1%), CDK2/CycE1 (-29.9%), CDK3/CycE1 (2.3%), CDK4/CycD3 (-0.1%), CDK5/p25 (-31.7%), CDK6/CycD3 (-4.4%), CDK7/CycH/MAT1 (5.6%), CDK9/CycT1 (-8%), CHK1 (56.3%), CHK2 (5.4%), CK1α (4%), CK1ε (18.5%), CK2α1/β (-2.3%), CLK1 (81.4%), CLK2 (94.4%), DAPK1 (-2.1%), DYRK1A (-0.8%), DYRK1B (5%), Erk1 (-3.6%), Erk2 (-1.1%), Erk5 (-2.5%), GSK3α (-4.7%), GSK3β (-0.9%), HGK (100.5%), HIPK4 (9.4%), IRAK4 (99.5%), JNK1 (2.9%), JNK2 (-3.6%), JNK3 (-3.2%), MAPKAPK2 (-15.1%), MINK (102.3%), MST1 (76.9%), NEK1 (8%), NEK2 (13.4%), NEK6 (-5.5%), NEK7 (-1.4%), NEK9 (-2%), p38α (3.1%), p38β (2.5%), p38γ (-1.9%), p38δ (-2.6%), p70S6K (10.7%), PAK2 (2.6%), PBK (-4.3%), PDK1 (-5.3%), PIM1 (25.8%), PIM2 (-3.2%), PKACα (-10.3%), PKCα (29%), PKCε (53.6%), PKD2 (77%), PLK1 (1.5%), PLK3 (2.2%), QIK (1.5%), ROCK1 (-4.5%), RSK1 (17.7%), RSK3 (0.9%), RSK4 (14.5%), SGK (5.2%), SIK (-1.9%), skMLCK (28.3%), TNIK (101.6%), TSSK1 (6.7%).

Kinase inhibitory profile of compound **60b** (DS06652923)

The kinase inhibitory profile of compound **60b** (DS06652923) against a panel of 161 kinases measured at a concentration of the test compound of 200 nmol/L in the presence of 1 mmol/L ATP was obtained from Carna Biosciences, Inc. (https://www.carnabio.com). The evaluated kinases and their proportions of inhibition are shown below:

ABL (0.8%), ACK (2.1%), ALK (28.3%), ARG (1.1%), AXL (2.1%), BLK (-1.9%), BMX (-2%), BRK (-1.7%), BTK (-7.5%), CSK (4.4%), DDR1 (4.8%), DDR2 (-1.7%), EGFR (31.0%), EGFR [d746-750] (101.9%), EGFR [d746-750/T790M] (94.9%), EGFR [L858R] (93.3%), EGFR [L861Q] (67.9%), EGFR [T790M] (82.4%), EGFR [T790M/L858R] (91.7%), EPHA1 (-1%), EPHA2 (1.6%),

EPHA3 (-1.2%), EPHA4 (3.6%), EPHA5 (4.3%), EPHA6 (14.9%), EPHA7 (3.3%), EPHA8 (-10.1%), EPHB1 (1.4%), EPHB2 (0.1%), EPHB3 (0.6%), EPHB4 (-5%), FAK (2.0%), FER (42.4%), FES (18.5%), FGFR1 (-1.6%), FGFR2 (0.7%), FGFR3 (-2.8%), FGFR4 (2.9%), FGR (4.4%), FLT1 (6.6%), FLT3 (85.4%), FLT4 (6.4%), FMS (-4.9%), FRK (5.4%), FYN [isoform a] (3.5%), FYN [isoform b] (-0.8%), HCK (-15.8%), HER2 (2.0%), HER4 (19.3%), IGF1R (-0.5%), INSR (9.5%), IRR (1.2%), ITK (-0.3%), JAK1 (-2.8%), JAK2 (-4.1%), JAK3 (-0.8%), KDR (2.9%), KIT (0.4%), LCK (8.0%), LTK (0.1%), LYNa (7.3%), LYNb (1.8%), MER (1.9%), MET (2.8%), MUSK (-0.8%), PDGFRα (18.5%), PDGFRβ (32.6%), PYK2 (41.9%), RET (2.3%), RET [G691S] (5.1%), RET [M918T] (0.9%), RET [S891A] (8.1%), RET [Y791F] (7.0%), RON (-0.3%), ROS (5.7%), SRC (6.6%), SRM (-1.2%), SYK (-19.0%), TEC (-4.8%), TIE2 (-0.8%), TNK1 (4.1%), TRKA (4.8%), TRKB (7.5%), TRKC (3.7%), TXK (-0.7%), TYK2 (-9.4%), TYRO3 (97.9%), YES (18.6%), AKT1 (-5.2%), AMPKα1/β1/γ1 (2.6%), AurA (-8.7%), AurB (0%), AurC (-8.5%), BRSK1 (-6.2%), CaMK1α (-3.0%), CaMK4 (2.7%), CDC2/CycB1 (-3.2%), CDC7/ASK (-0.1%), CDK2/CycA2 (-2.6%), CDK2/CycE1 (-32.7%), CDK3/CycE1 (-1.5%), CDK4/CycD3 (2.0%), CDK5/p25 (-21.6%), CDK6/CycD3 (1.8%), CDK7/CycH/MAT1 (-0.7%), CDK9/CycT1 (3.5%), CHK1 (13.2%), CHK2 (29.4%), CK1α (11.2%), CK1ε (31.5%), CK2α1/β (0.3%), CLK1 (80.0%), CLK2 (81%), DAPK1 (0.5%), DYRK1A (3.3%), DYRK1B (14.9%), Erk1 (-3.8%), Erk2 (-1.6%), Erk5 (5.5%), GSK3α (-4.2%), GSK3β (11.7%), HGK (87.8%), HIPK4 (-1.6%), IRAK4 (64.7%), JNK1 (5.2%), JNK2 (-4.2%), JNK3 (3.8%), MAPKAPK2 (-7.9%), MINK (84.5%), MST1 (4.8%), NEK1 (2.3%), NEK2 (5.8%), NEK6 (0.7%), NEK7 (0.1%), NEK9 (1.3%), p38α (10.5%), p38β (12.2%), p38γ (2.9%), p38δ (1.9%), p70S6K (1.1%), PAK2 (-4.7%), PBK (-2%), PDK1 (-7.9%), PIM1 (29.2%), PIM2 (0.1%), PKACα (-16.2%), PKCα (6.2%), PKCε (10.8%), PKD2 (36.4%), PLK1 (6.3%), PLK3 (2.3%), QIK (-0.5%), ROCK1 (-5.1%), RSK1 (-1.5%), RSK3 (4.7%), RSK4 (4.7%), SGK (2.8%), SIK (-1.4%), skMLCK (1.7%), TNIK (94.1%), TSSK1 (6.5%).

3. X-ray crystallography

Protein expression and purification

The kinase domain of the human EGFR (amino acids 696–1022) carrying mutations T790M, C797S, L858R, E865A, E866A, and K867A was expressed from insect cells as a GST-fusion protein. The protein was purified by a three-step procedure comprising affinity capture on GSTPrep™ FF 16/10 (Cytiva), followed by cleavage of the GST-tag with thrombin, negative-affinity chromatography to remove the tag, and final size exclusion chromatography on a HiLoad® 26/600 Superdex® 75 pg column (Cytiva). For crystallization, the protein was concentrated in a final buffer containing 25 mM Tris/HCl pH 8.0, 100 mM NaCl, 2 mM DTT, and 2 mM TCEP.

Crystallization

Apo-crystals of EGFR kinase domain were grown by sitting drop vapor diffusion at 293K. Protein at 6 mg/mL was equilibrated against a reservoir solution (0.5 µL:0.5 µL) consisting of 0.46 M trisodium citrate, pH 7.0. For cryo-preservation, 20% (v/v) 2,3-butanediol was added to the crystallization drop before flash-freezing the crystals in liquid nitrogen. Crystals in complex with compound **5i** were generated by immersing apo-crystals at 293K with compound from a 100 mM stock solution in DMSO. Immersion conditions were 2.5 mM compound **5i** for 3 h, followed by 5 mM compound **5i** for 1 h.

Data collection and processing

The X-ray diffraction data were collected from complex crystals of EGFR with the ligand at the Swiss Light Source (SLS, Villigen, Switzerland) using cryogenic conditions. The crystals belong to space group *I* 2 3. Data were processed using the programs XDS and XSCALE[30].

Table S1. Data collection and processing statistics for compound **5i**

Ligand	**5i**
X-ray source	PXII/X10SA (SLS[1])
Wavelength [Å]	1.0000
Detector	PILATUS 6M
Temperature [K]	100
Space group	*I* 2 3
Cell: a; b; c [Å]	145.33; 145.33; 145.33
α; β; γ [◦]	90.0; 90.0; 90.0
Resolution [Å]	2.55 (2.80-2.55)
Unique reflections	16233 (3994)
Multiplicity	3.0 (2.9)
Completeness [%]	96.5 (97.6)
R_{sym} [%][3]	5.0 (59.1)
R_{meas} [%][4]	6.1 (72.4)
Mean(I)/sd[5]	17.62 (2.14)

[1] Swiss Light Source (SLS, Villigen, Switzerland)

[2] Values in parentheses refer to the highest-resolution bin.

[3] $$Rsym = \frac{\sum_h \sum_i^{n_h} \left| \hat{I}_h - I_{h,i} \right|}{\sum_h \sum_i^{n_h} I_{h,i}} \text{ with } \hat{I}_h = \frac{1}{n_h} \sum_i^{n_h} I_{h,i}$$

where $I_{h,i}$ is the intensity value of the ith measurement of h

[4] $$Rmeas = \frac{\sum_h \sqrt{\frac{n_h}{n_h - 1}} \sum_i^{n_h} \left| \hat{I}_h - I_{h,i} \right|}{\sum_h \sum_i^{n_h} I_{h,i}} \text{ with } \hat{I}_h = \frac{1}{n_h} \sum_i^{n_h} I_{h,i}$$

where $I_{h,i}$ is the intensity value of the ith measurement of h

[5] Calculated from independent reflections

Structure modeling and refinement

The phase information necessary to determine and analyze the structure was obtained by molecular replacement. A previously solved structure of EGFR was used as a search model. Subsequent model building and refinement were performed in accordance with standard protocols with the software packages CCP4[31] and COOT[32]. For calculation of the free R-factor, a measure to cross-validate the correctness of the final model, about 5.7% of measured reflections were excluded from the refinement procedure (see Table S2). TLS refinement (using REFMAC5[33], CCP4) was carried out, which resulted in lower R-factors and higher quality of the electron density map. The ligand parameterization and generation of the corresponding library files were carried out with CORINA[34]. The water model was built with the "Find waters" algorithm of COOT by putting water molecules in peaks of the F_o-F_c map contoured at 3.0, followed by refinement with REFMAC5 and checking all water molecules with the validation tool of COOT. The criteria for the list of suspicious waters were as follows: B-factor greater than 80 Å^2, 2 F_o-F_c map less than 1.2 σ, and distance to closest contact less than 2.3 Å or more than 3.5 Å. The suspicious water molecules and those in the ligand binding site (distance to ligand less than 10 Å) were checked manually. The Ramachandran plot of the final model shows 93.1% of all residues in the most favored region, 6.9% in the additional allowed region, and 0.0% in the generously allowed region. No residues were found in the disallowed region (Table S2). Statistics of the final structure and the refinement process are listed in Table S2.

Table S2. Refinement statistics for compound **5i**[1]

Ligand	**5i**
Resolution [Å]	102.76-2.55
Number of reflections (working/test)	15282/926
R_{cryst} [%]	19.3
R_{free}[%][2]	23.5
Total number of atoms:	
Protein	2396
Water	37
Ligand	37
Chloride	1
Deviation from ideal geometry:[3]	
Bond length [Å]	0.011
Bond angle [°]	1.48
Bonded B [Å^2][4]	3.8
Ramachandran plot:[5]	
Most favored regions [%]	93.1
Additional allowed regions [%]	6.9
Generously allowed regions [%]	0.0
Disallowed regions [%]	0.0

[1] Values as defined in REFMAC5, without sigma cut-off

[2] Test set contains 5.7% of measured reflections

[3] Root mean square deviations from geometric target values

[4] Calculated with MOLEMAN[35)]

[5] Calculated with PROCHECK[36)]

4. *In silico* study

EGFR C797S model structure preparation

A three-dimensional structural model of the EGFR T790M/C797S/L858R triple mutant (EGFR C797S model) was constructed based on the X-ray crystallographic structure of the EGFR T790M/L858R double mutant in complex with a 3-azetidinyl azaindazole compound **2** (PDB ID: 5hcz). The parent structure was altered by substituting the cysteine residue at position 797 with serine using Maestro.[37] The resultant structure of the triple mutant was then minimized using the Protein Preparation Wizard.[37]

Inhibitor docking

The three-dimensional structures of compounds **32b**, **55a**, and **60b** (DS06652923) were constructed using LigPrep.[37]These structures were then docked into the binding site of compound **2** in the EGFR C797S model using Glide SP mode.[37] The docking protocol was as follows: (i) The van der Waals radii of protein and ligand atoms were scaled to 0.9 and 0.8, respectively. (ii) Two hydrogen bond constraints were set between the protein and ligand atom pairs: [1] M793 *N*-H...HBA and [2] Q791 C=O...HBD, where HBA and HBD are Glide's atom sets for the hydrogen bond acceptor and hydrogen bond donor of the compounds, respectively. (iii) A core constraint was set to keep the atom positions of the compound's substructure, as defined by SMARTS,[38] within 1.0 Å of RMSD versus compound **2**. The SMARTS pattern used was '[cH]1[cH]ncac1[NH]c1n[cH]aaa1'. (iv) Enhanced sampling was set to four times. (v) The output option was set to allow no more than five poses per ligand. (vi) All other docking parameters were set to their default values.

Model visualization

The complex structure images of the compounds docked into the EGFR C797S model were created using PyMOL.[39]

Human protein kinase sequence alignment analysis

Our analysis of human protein kinase sequence alignment was aimed at designing EGFR-selective inhibitors. We focused on the C-3 position of the piperidine of compound **14b** and considered the amino acid residues at gk+2 (Ghose's notation[21]; L792 in EGFR), which are close to the C-3

position. We determined the amino acid frequency at the gk+2 position in 497 human protein kinases using a multiple sequence alignment (MSA) obtained from KinCoRe (http://dunbrack.fccc.edu/kincore/; MSA file URL: http://dunbrack.fccc.edu/kincore/static/downloads/alignment-files/Human-PK-alignment.aln).[40),41)] The determined amino acid frequency is shown in Table S1.

Table S3. The amino acid frequency at gk+2 positions in 497 human protein kinases[a]

Amino acid residue			Amino acid frequency (%)
Name	Symbol		gk+2 (L792 in EGFR)
Leucine	**L**		**25.35**
Serine	S		0.40
Alanine	A		1.01
Glutamate	E		0.80
Glycine	G		0.00
Valine	V		0.60
Arginine	R		2.01
Lysine	K		1.41
Threonine	T		0.00
Proline	P		0.60
Aspartate	D		0.20
Isoleucine	I		1.21
Asparagine	N		0.20
Glutamine	Q		0.40
Phenylalanine	F		18.11
Tyrosine	Y		40.64
Histidine	H		2.82
Methionine	M		1.81
Cysteine	C		1.21
Tryptophan	W		1.01
Gap	-		0.20

[a]The residue identical to that in EGFR is highlighted in bold.

From Table S3 we extracted the top three most common amino acids at gk+2 and list them in Table S4.

Table S4. The top three most common amino acid residues at gk+2 positions in 497 human protein kinases[a]

Rank	gk+2 (L792 in EGFR)		
	Residue symbol	Frequency (%)	Relative size
1	Y	40.64	Larger than L
2	**L**	25.35	Smaller than Y/F
3	F	18.11	Larger than L

[a]The 'Residue symbol' and 'Frequency (%)' columns denote the one-letter symbol of the amino acid residue and its frequency at the gk+2 position in the sequence alignment of human protein kinases, respectively. Only the three most common residues are listed in the rows. The residue identical to that in EGFR is highlighted in bold.

Ligand interaction diagrams

Ligand interaction diagrams for docked poses shown in Figure 2-2, 2-4, and 2-6 were created using Ligand Interaction Diagram[37)] and are shown in Figure S1, S2, and S3, respectively. We used the default values for threshold distances when detecting interactions.

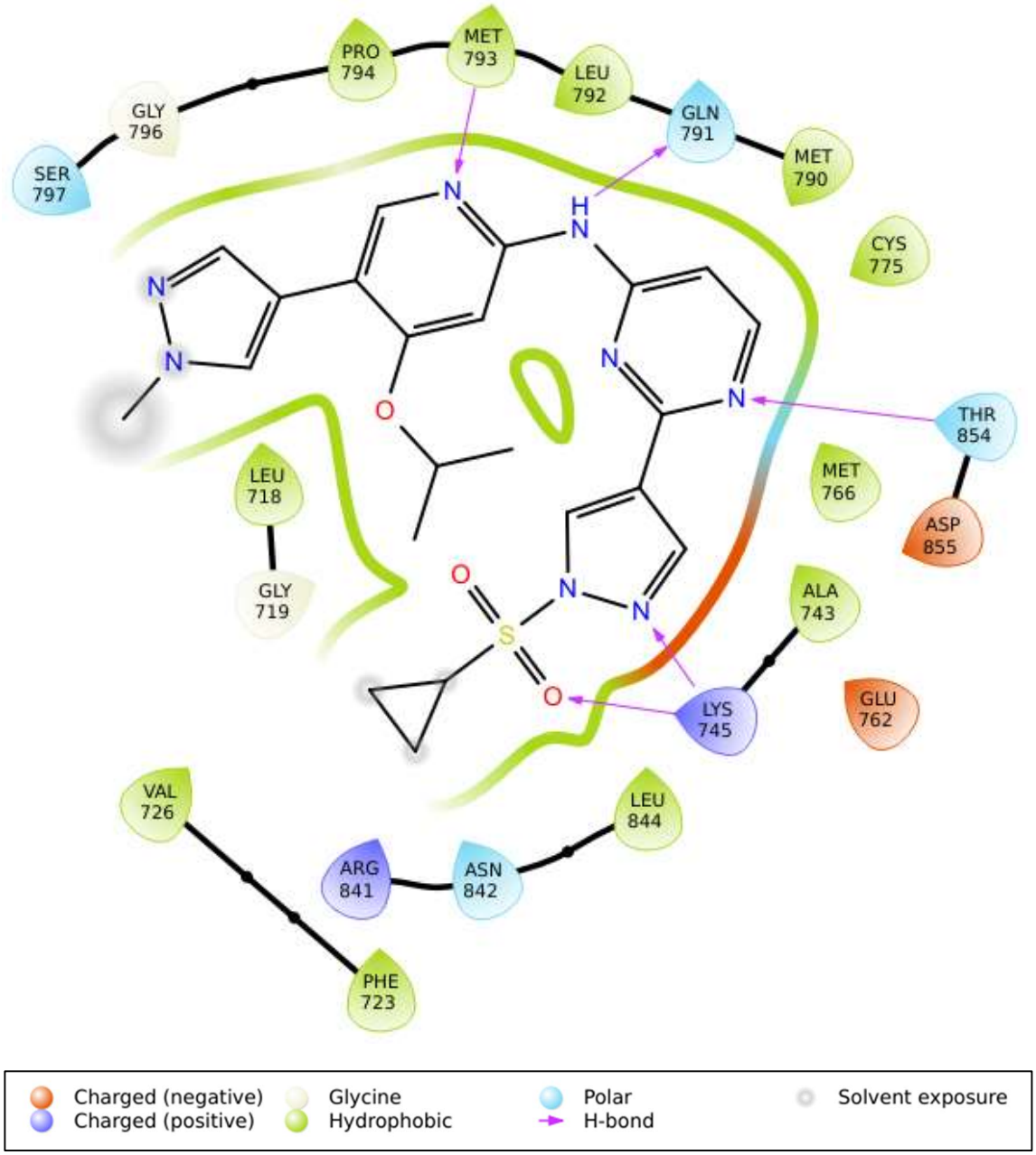


Figure S1. The interaction diagram for compound **32b** in the EGFR C797S model. The residues are represented as colored teardrop shapes, labeled with their names and numbers, and colored according to their properties. The point of the teardrop shape is oriented towards the side chain. The chain is depicted as a black line connecting residues, with residues not within the threshold distance shown as black dots. Hydrogen bonds (H-bond) and salt bridges between the residues and the compound are drawn as lines, colored by interaction type. Residues without an interaction line are involved in non-specific hydrophobic interactions with the compound. The binding pocket is indicated by a line drawn around the compound, colored based on the nearest residue. Solvent exposure is indicated on the compound atoms and by the break in the pocket line.

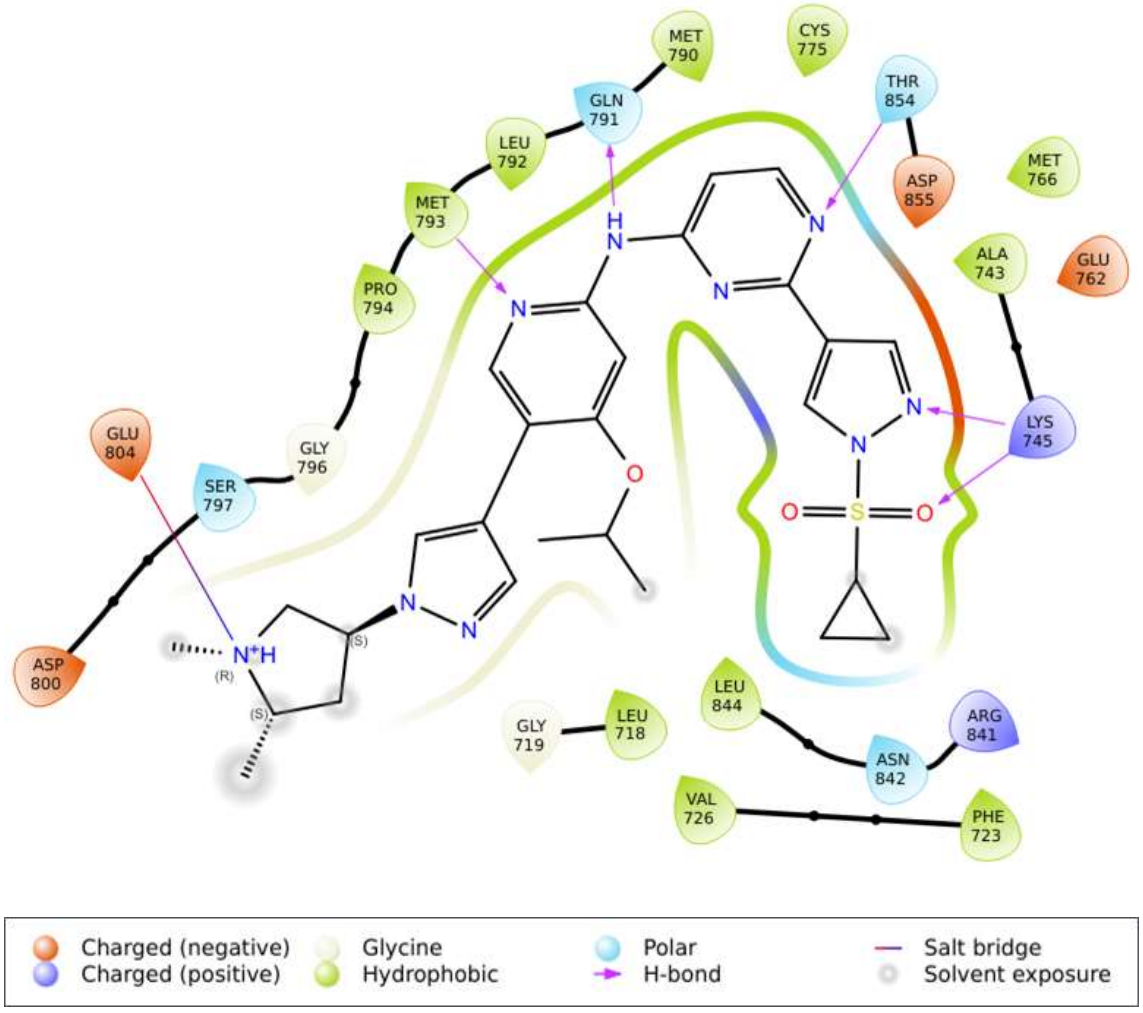


Figure S2. A diagram of the interaction for compound **55a** in the EGFR C797S model. The representation style is the same as in Figure S1.

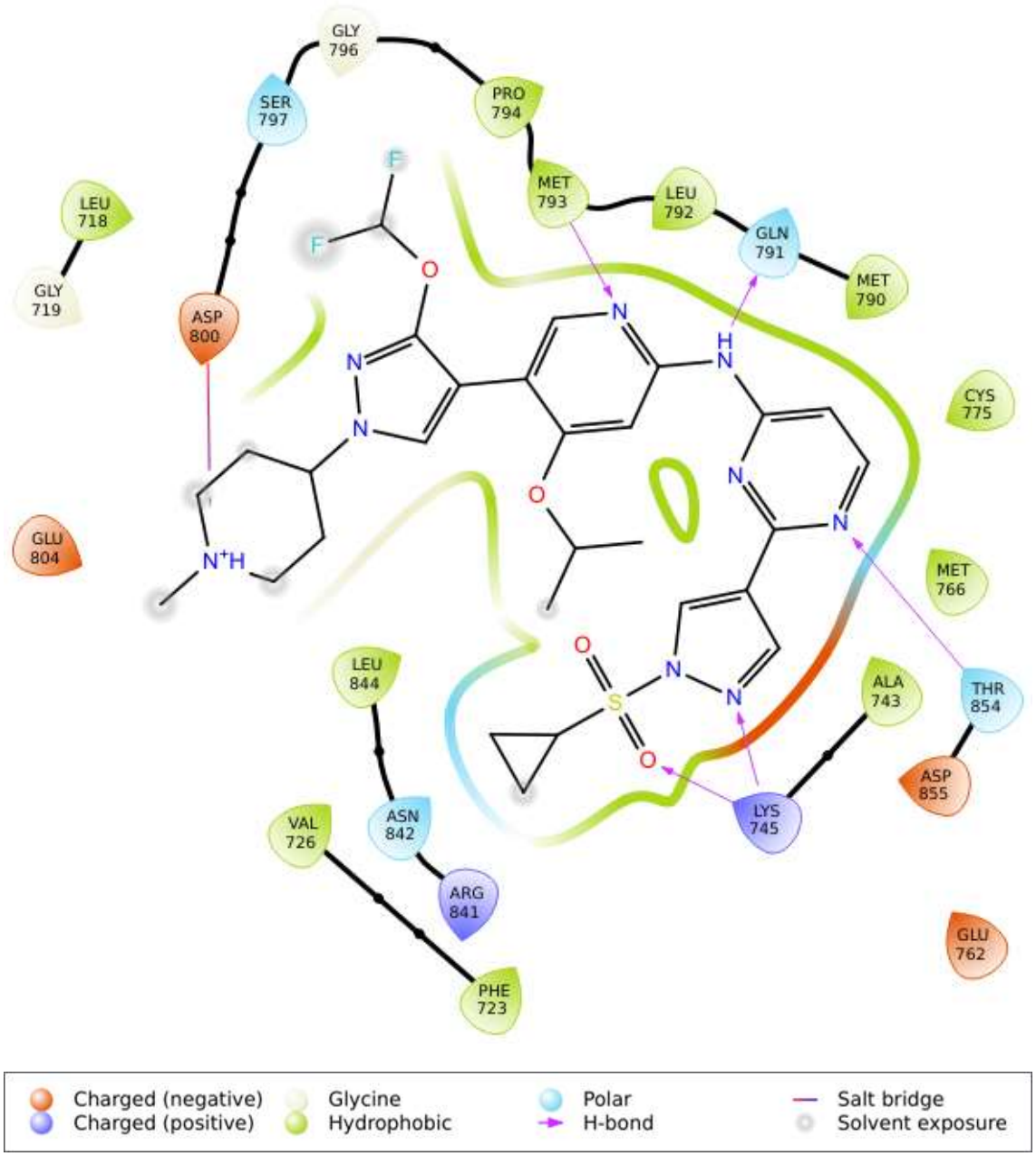


Figure S3. A diagram of the interaction for compound **60b** (DS06652923) in the EGFR C797S model. The representation style is the same as in Figure S1.

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