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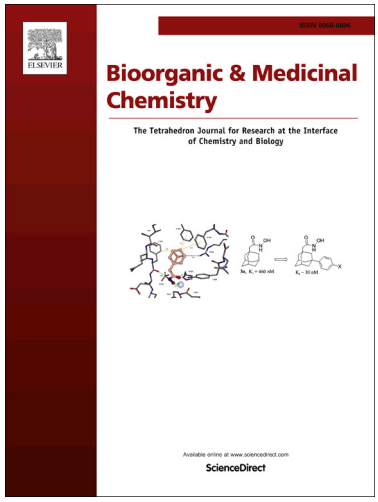

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Pyrrolo[2,3-*b*]pyridine derivatives as potent Bruton's tyrosine kinase inhibitors

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Abstract: A series of pyrrolo[*2,3-b*]pyridine-based derivatives were designed as potent Bruton's tyrosine kinase (BTK) inhibitors by using a scaffold-hopping strategy. Structure–activity relationship studies identified five compounds (**3n**, **3p**, **3q**, **3r**, and **3s**) with IC_{50} of less than 10 nM in BTK enzyme assay and five compounds (**3m**, **3n**, **3o**, **3p**, and **3t**) with IC_{50} of less than 20 nM in Ramos cell assay. As one of the most potent inhibitors, compound **3p** exhibited superior activity to that of compound **1** (RN486) and pyrrolo[2,3-*d*]pyrimidine derivative **2** in both BTK enzymatic (IC_{50} = 6.0 nM) and cellular inhibition (IC_{50} = 14 nM) assays. In addition, **3p** displayed favorable overall pharmacokinetic profiles compared with **1** and **2**.

Keywords: BTK kinase, Inhibitor, Pyrrolo[*2,3-b*]pyridine, SAR.

1. Introduction

Bruton's tyrosine kinase (BTK) belongs to the TEC family of non-receptor kinases[1,2] that is involved in multiple signaling pathways downstream of the B cell receptor and Fc receptor.[3,4] Therefore, targeting BTK is an attractive and potential therapeutic approach for the treatment of several diseases, including cancer and inflammatory diseases.[5–7]

Many small-molecule inhibitors of BTK show antitumor activity in animal models and clinical studies.[5,8] The US FDA recently approved Ibrutinib (PCI-32765) for the treatment of mantle cell lymphoma and chronic lymphocytic leukemia.[9] Several BTK inhibitors also demonstrated efficacy in animal models of rheumatoid arthritis (RA).[10–14] However, only a few of these inhibitors have entered clinical trials for RA.

(Insert Figure 1 and title for Figure 1 here)

Among the studied inhibitors, compound **1** (RN486) displayed high and selective BTK potency and appropriate drug-like properties, which useful for developing new BTK inhibitors.[11,12] The structure of compound **1** can be divided into four parts, namely, blocks A, B, C, and D.

By analyzing the binding mode of compound **1** with BTK kinase complex (PDB code, 4OTR), we found that the bidentate hydrogen bonds between block C and Met477 was crucial for maintaining BTK activity. The block C assumed pseudocyclic conformations, while the block D stacked between residues Gly480 and Leu408. Our previous studies showed that introducing pyrrolo[2,3-*d*]pyrimidine to restrain key pharmacophoric groups conformationally within the molecule lead to synthesis of the potent BTK inhibitor **2**.[15] Also, the closely related 7-azaindoles have recently received a lot of attention due to their potential biological activities and drugi-like abilities.[16-19] Accordingly, we postulated that scaffold hopping[19-22] from pyrrolo[2,3-*d*]pyrimidine to pyrrolo[2,3-*b*]pyridine might be effective in developing new BTK inhibitors as the key hydrogen bonds and hydrophobic interactions could be retained. In this paper, we report the synthesis, structure–activity relationship (SAR), and preliminary pharmacokinetic (PK) study of pyrrolo[2,3-*b*]pyridine-based BTK inhibitors **3**.

2. Result and discussion

2.1. Chemistry

Pyrrolo[*2,3-b*]pyridine derivatives (**3a–3t**), imidazo[4,5-*b*]pyridine derivative (**3u**),

and indole derivative (**3v**) were designed and synthesized (Table **1**). The assembly of halogenated fused heterocycles (block C–D) with boronic ester (block A–B)[15] was designed to generate target compounds.[23]

(Insert Scheme 1 and legend for Scheme 1 here)

Scheme **1** shows that the synthesis of brominated pyrrolo-pyridines **4b**–**4t** started from commercially available 4-bromo-1*H*-pyrrolo[2,3-*b*]pyridine (**4a**) as the same key intermediate. The sulfonylation of **4a** produced compound **5** that protects the 7-NH group and improves the reactivity of C-2 carbon-hydrogen bond. 4-Bromo-2-methyl-1H-pyrrolo[2,3-*b*]pyridine (**4b**) was prepared using C-2 methylation (LDA, MeI), followed by deprotection of **6** under NaOH. The C-2 bromination (LDA, 1,2-dibromo-1,1,2,2-tetrachloroethane) of **5** was followed by selective Suzuki coupling [$Pd(dppf)Cl_2 \cdot CH_2Cl_2$, Na_2CO_3] with a variety of arylboronic acid, which yielded 2-aryl substituted 4-bromo-1*H*-pyrrolo[2,3-b]pyridines **4c**–**4l** after deprotection. The condensation of **5** with *tert*-butyl 4-oxopiperidine-1-carboxylate resulted in intermediate **9**. The subsequent one-pot dehydration and deprotection of **9** generated 4-bromo-2-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine **4m**. Thus, **4m** was treated with acyl chloride, sulfonyl chloride or oxetan-3-one to produce **4n–4t**.

(Insert Scheme 2 and legend for Scheme 2 here)

The nitration-reduction reaction of 4-chloro-2-aminopyridine **10** followed by cyclization with 4-formyl-*N*,*N*-dimethylbenzamide yielded 4-(7-chloro-3*H*-imidazo-[4,5-*b*]pyridin-2-yl)-*N*,*N*-dimethylbenzamide **4u** (Scheme **2**)**.** Similar to the synthesis of **4p**, 4-(4-bromo-1*H*-indol-2-yl)-*N*,*N*-dimethyl-5,6-dihydropyridine-1(2*H*)-carbox-amide **4v** was ultimately produced by protection–condensation–deprotection–dehydration–acylation reactions of 4-bromo-indole **15**.

(Insert Scheme 3 and legend for Scheme 3 here)

From **4a–4v**, target compounds **3a–3v** were synthesized through direct palladium-catalyzed Suzuki coupling reaction of **4** with boronic ester **20**, followed by deprotection.[15]

2.2. In vitro BTK enzyme and cellular inhibition

The newly synthesized pyrrolo[*2,3-b*]pyridine derivatives were initially assessed for their ability to inhibit BTK enzymatic activity using a similar procedure reported in the

literature.[24] IC_{50} values are summarized in Table **1**.

(Insert Table 1 and title for Table 1 here)

Similar to the SAR of compound **2**,[15] removal of block D resulted in a significant potency loss in **3a** (R = H, IC_{50} = 562.2 nM) and **3b** (R = Me, IC_{50} = 395 nM). Thus, the hydrophobic interactions of block D with residues Gly480 and Leu408 play critical roles in maintaining BTK potency.[11] A partial recovery of the lost potency was noted with the introduction of 4-hydroxyl (**3c**), 4-methoxy (**3d**), 3-amino (**3e**), or 4-acyl (**3f**) substituted phenyl group in block D (IC_{50} of 21.3–110.3 nM). Replacing 4-acyl group of **3f** with 4-carbamyl group led to compound **3g**, which displayed further increased enzymatic activity with IC_{50} of 12.1 nM, which is comparable to that of compound **1** (IC_{50} = 13.2 nM). The introduction of piperidinyl group (**3h**) with more steric hindrance (*N*,*N*-dimethyl for **3g**) led to a 3.1-fold decrease in BTK potency. Interestingly, the replacement of the 4-carbamyl group with 4-morpholinomethyl group provided equivalent potency (**3i**, IC_{50} = 18.1 nM). The bioisosteres of 4-acetylphenyl or 4-methoxyphenyl group in block D with 5-acetylthiophen-3-yl group or 6-methoxypyridin-3-yl group led to slight decrease in BTK inhibitory activity (2.5–3.7 fold, cf. **3j** and **3f**; **3k** and **3d**). Compound **3l**, bearing 1-methyl-pyrazol-3-yl group, also displayed excellent inhibitory activity (IC_{50} = 20.6 nM), suggesting that the pyrazol ring was well tolerated in block C.

The phenyl group in block D was further replaced with unsaturated six-membered heterocyclic rings. Compound **3m** (3,6-dihydro-pyridine-4-yl) exhibited comparable potency to **3c** (4-hydroxy-phenyl), indicating that the hydrophobic non-aromatic ring is also well tolerated in block D. Based on the SAR of the aryl series (**3c–3l**), the carbonyl or sulfonyl group bearing potential hydrogen-bond acceptor was further introduced into the N-1 position of the 3,6-dihydro-pyridine ring. The results showed that compounds **3n–3s** exhibited excellent BTK inhibitory activities (IC_{50} of 6.7–10.4 nM) that are superior to that of **1**. Compounds **3n**, **3p**, **3q**, and **3r** (IC_{50} of 6.0–7.3 nM) displayed comparable BTK potency to that of pyrrolo[2,3-*d*]pyrimidine derivative **2** (IC_{50} = 7 nM).

Subsequently, the replacement of the pyrrolo[*2,3-b*]pyridine ring in block C with imidazo[4,5-*b*]pyridine (**3u**) resulted in slight potency loss (2.6-fold lower potency compared with **3g**). The bioisosteres of pyrrolo[*2,3-b*]pyridine with indole eliminated the hydrogen bonding interaction with Met477, leading to a dramatic loss of potency on BTK

(**3v**). These results suggest that the bidentate hydrogen bonding interactions with the backbone of Met477 are crucial for maintaining BTK activity.

The cellular (Ramos cell) inhibitory activity of all of the newly synthesized compounds were further investigated through a cell-based Ca^{2+} flux assay.[24] Table **1** shows that the potency in the cellular assay are in agreement with that in BTK enzymatic assay. Compounds **3l**, **3m**, **3n**, **3o**, **3p**, and **3t** (IC_{50} of 3–16 nM) displayed cellular BTK inhibitory activity that is superior to that of compound **1** (IC_{50} = 27.3 nM) and compound **2** (IC_{50} = 21.3 nM). Compounds **3m** and **3t** displayed the most potent cellular BTK activity with IC_{50} values of 3 nM (approximately 7- to 9-fold higher than that of compound **1** and **2**). Only compound **3r** demonstrated excellent BTK enzymatic inhibitory activity (IC_{50} = 7.1 nM) and low potency in the cellular assay (IC_{50} = 410 nM), probably because of the poor cellular permeability of the 1-methylsulfonyl-1,2,3,6-tetrahydropyridine-4-yl group. Overall, the SARs of cell-based and enzyme-based activities are similar.

2.3. Docking study

To better understand SAR in a structural context, docking analysis was performed using CDOCKER program of the Discovery Studio 2.5 software package. The docking orientation and interactions of compound **3p** with BTK enzyme (PDB code 4OTR) are shown in Figure **2**. Superimposed models of **3p** and **1** (RN486) revealed similar docking poses of the two compounds (Figure **2A**). Figure **2B** shows that the entire molecule of **3p** was favorably located in the BTK pocket, as expected. The N atom in the 1'-position and N–H group in the 7'-position of pyrrolo[*2,3-b*]pyridine led to the conservation of key bidentate hydrogen bonding interactions with Met477.[11,15] This has also been confirmed by the results of SAR studies that the closely related indole derivative **3v** without N atom in the 1'-position displayed no BTK inhbitory activity (IC_{50} > 5000 nM). The carbamyl group in block D formed an additional hydrogen bond with Lys406, whereas **1**, **2** and **3m** did not display any similar interaction. This interaction led 3p achieved 4-fold improved BTK potency compared with **3m**. The tetrahydropyridine ring was stacked between residues Gly480 and Leu408. The hydroxyl group in block B formed hydrogen bonds with Asp539, Lys430, and Ser538, whereas the isoquinolinone ring in block A of **3p** adopted a conformation that is opposite to that of **1**. The H-bond between 1-carbonyl group of isoquinolinone and Lys430 disappeared, but a new H-bond with water (H_2O-50)

formed. Block A still pointed into a selectivity pocket formed by the side chains of Gln412, Phe413 (both Gly-rich loops), Asp521, Asn526 (both catalytic loops), Asp539 (DFG), Leu542, Ser543, Val546, and Tyr551 (all activation loops).[11] Overall, **3p** should demonstrate a higher enzyme potency compared to **1**, which is consistent with the SAR study.

(Insert Figure 2 and title for Figure 2 here)

2.4. In vivo PK study

Compound **3p**, which showed excellent potency both in BTK enzyme and cellular assays in vitro, was selected for preliminary evaluation of PK properties in rats. Table **2** shows that when orally administered at 4 mg/kg dose, the plasma exposure (AUC) of **3p** reached 3423.0 h·ng/mL, which is 1.9–3.2-fold higher than that of **1** (RN486) and pyrrolo[*2,3-d*]pyrimidine derivative **2**. When orally administered with the same dose, the maximal plasma concentration (C_{max}) of **3p** reached 739.3 ng/mL, which is 1.8–5.5-fold higher than that of **1** (RN486) and pyrrolo[*2,3-d*]pyrimidine derivative **2.** The clearance (Cl) of **3p** was 1.2 L/h/kg, which is 2.2–2.7-fold lower than that of **1** and **2**. These results suggest that **3p** displayed favorable overall PK profiles compared with **1** and **2**.

(Insert Table 2 and title for Table 2 here)

3. Conclusion

In summary, we reported the development of pyrrolo[*2,3-b*]pyridine derivatives as a class of potent BTK inhibitors by utilizing a scaffold-hopping drug-design strategy. SAR studies were conducted and a number of compounds were identified to display high BTK inhibitory potency. Among the compounds, **3n**, **3p**, **3q**, **3r**, and **3s** exhibited IC_{50} values of less than 10 nM in BTK enzyme assay, whereas compounds **3m**, **3n**, **3o**, **3p**, and **3t** showed IC_{50} values of less than 20 nM in Ramos cell assay. The results of molecular docking indicate similar binding modes of **3p** and **1** with BTK kinase. As one of the most potent inhibitors, compound **3p** exhibited superior activity to that of compound **1** (RN486) and pyrrolo[2,3-*d*]pyrimidine derivative **2** both in BTK enzymatic (IC_{50} = 6.0 nM) and cellular inhibition (IC_{50} = 14 nM) assays. **3p** also displayed favorable overall PK profiles compared with **1** and **2**. Further evaluation of the efficacy of **3p** in animal models of RA will be conducted.

4. Experimental

4.1. Chemistry

All chemicals were purchased from commercial sources and used without further purification unless especially noted. Melting points of compounds were measured on a Melt-Temp II apparatus and uncorrected. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker BioSpin AG (Ultrashield Plus AV 400) spectrometer as deuterochloroform ($CDCl_3$) or dimethyl sulfoxide-d_6 (DMSO-d_6) solutions using tetramethylsilane (TMS) as an internal standard (δ = 0) unless noted otherwise. MS spectra were obtained on an Agilent technologies 6120 quadrupole LC/MS (ESI). High-resolution mass spectra (HRMS) were recorded on a Water Q-Tof micro mass spectrometer. The purity of the compounds was verified by the HPLC study using a mixture of solvent methanol/water or acetonitrile/water at the flow rate of 2 mL/min and peak detection at 254 nm under UV. The solvents (such as MeOH, EtOAc, EtOH, CH_2Cl_2 and others) were C.P. grade purchased from Nanjing Chemical Co. Ltd. and used without further purification. Column chromatography (CC) was carried out on silica gel (200–300 mesh, Qindao Ocean Chemical Company, China). Thin-layer chromatography (TLC) analyses were carried out on silica gel GF254 (Qindao Ocean Chemical Company, China).

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(1H-pyrrolo[2,3-b]-pyridin-4-yl)benzyl acetate (21a). A solution of **20** (120 mg, 0.31 mmol), **4a** (104 mg, 0.31 mmol) and Pd(dppf)Cl_2·CH_2Cl_2 (25 mg, 0.031 mmol) in 1,4-dioxane (10 mL) was degassed with nitrogen for 3 min followed by addition Na_2CO_3 (65 mg, 2M in water) under continuous flow of nitrogen. The reaction mixture was stirred at 100 ^{o}C for 2 h. The reaction mixture was cooled, filtered through celite, diluted with water (5 mL), and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over sodium sulfate and were concentrated in vacuo. The crude product was used in the next reaction without further purification. MS (ESI) m/z 468.2 $[M + H]^+$.

6-cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(1H-pyrrolo[2,3-b]pyridin-4-yl)-phenyl)isoquinolin-1(2H)-one (3a). A mixture of the crude product **21a** (70.2 mg, 0.15 mmol), LiOH (11 mg, 0.45 mmol), i-PrOH (5 ml) and THF (5 ml) was stirred at 50 ^{o}C for 5 h. The reaction mixture was cooled, diluted with water (15 mL), and extracted with

ethyl acetate (3 × 10 mL), the combined organic layers were dried over sodium sulfate and were concentrated in vacuo. The crude product was purified on a silica gel column using (1-10% CH_3OH/ DCM) as eluent to afford **3a** (40 mg, 62.8 %) as a white solid. Mp: 225-227 ^oC. MS (ESI) m/z 426.2 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 8.29 (d, J = 4.80 Hz, 1H), 7.60 (d, J = 8.00 Hz, 1H), 7.56 (d, J = 8.00 Hz, 1H), 7.51 (d, J = 8.00 Hz, 2H), 7.41 (d, J = 8.00 Hz, 2H), 7.26 (s, 1H), 7.18 (d, J = 8.00 Hz, 1H), 6.98 (d, J = 16.00 Hz, 1H), 6.60 (d, J = 8.00 Hz, 1H), 6.24 (s, 1H), 4.71 (d, J = 12.00 Hz, 1H), 4.23 (d, J = 16.00 Hz, 1H), 4.18 (d, J = 16.00 Hz, 1H), 2.10-2.08 (m, 1H), 1.11-1.09 (m, 2H), 0.89-0.87 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 158.29, 151.78, 151.64, 148.49, 142.25, 141.71, 140.18, 139.92, 139.64, 136.04, 135.28, 130.19, 128.62, 128.51, 126.46, 118.98, 118.27, 116.14, 110.70, 110.48, 104.05, 99.00, 57.19, 15.38, 10.72, 10.68. HPLC 97.9 %. HRMS(ESI) m/z calcd for $C_{26}H_{20}FN_3O_2$ $[M+H]^{+\cdot}$ 426.1539, found 426.1612.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-methyl-1H-pyrrolo-[2,3-b]pyridin-4-yl)benzyl acetate (21b). Using a method similar to that of **21a**, compound **21b** was synthesized as a white solid (113 mg, 54 %). MS (ESI) m/z 482.2 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 11.67 (s, 1H), 8.19 (d, J = 5.20 Hz, 1H), 7.68 (t, J = 16.00 Hz, 1H), 7.52 (d, J = 8.00 Hz, 1H), 7.49 (d, J = 8.00 Hz, 1H), 7.44 (d, J = 8.00 Hz, 1H), 7.28 (s, 1H), 7.01 (s, 1H), 6.97 (d, J = 5.20 Hz, 1H), 6.62 (d, J = 8.00 Hz, 1H), 5.93 (s, 1H), 4.82 (d, J = 16.00 Hz, 1H), 4.72 (d, J = 16.00 Hz, 1H), 2.43 (s, 3H), 2.10-2.07 (m, 1H), 2.43 (s, 3H), 1.11-1.09 (m, 2H), 0.89-0.87 (m, 2H).

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-methyl-1H-pyrrolo[2,3-b]-pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3b). Using a method similar to that of **3a**, compound **3b** was synthesized as a white solid (43 mg, 57 %). Mp: 248-250 ^oC. MS (ESI) m/z 440.2 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 11.61 (s, 1H), 8.17 (d, J = 5.20 Hz, 1H), 7.58 (t, J = 16.00 Hz, 1H), 7.49 (d, J = 8.00 Hz, 1H), 7.40 (d, J = 8.00 Hz, 2H), 7.27 (s, 1H), 7.11 (d, J = 5.20 Hz, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.60 (d, J = 8.00 Hz, 1H), 5.95 (s, 1H), 4.62 (s, 1H), 4.19-4.18 (s, 2H), 2.40 (s, 3H), 2.07-2.05 (m, 1H), 1.12-1.08 (m, 2H), 0.88-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 160.60, 158.46, 151.87, 148.71, 141.60, 140.79, 140.17, 140.07, 138.17, 137.36, 135.85, 135.19, 130.19, 128.64, 128.32, 120.14, 118.25, 116.03, 110.73, 110.51, 104.17, 96.84, 57.18, 15.37, 13.48, 10.68,

10.65. HPLC 97 %. HRMS(ESI) m/z calcd for $C_{27}H_{22}FN_3O_2$ $[M+H]^{+\cdot}$ 440.1768, found 440.1776.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(4-hydroxyphenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21c). Using a method similar to that of **21a**, compound **21c** was synthesized as a gray solid. MS (ESI) m/z 560.3 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(4-hydroxyphenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3c). Using a method similar to that of **3a**, compound **3c** was synthesized as a white solid (33 mg, 64.1 %). Mp: 182-184 ^oC. MS (ESI) m/z 518.2 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.13 (s, 1H), 9.74 (s, 1H), 8.22 (s, 1H), 7.75 (d, J = 8.00 Hz, 2H), 7.58 (t, J = 12.00 Hz, 2H), 7.43 (s, 2H), 7.27 (s, 1H), 7.16 (s, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.83 (d, J = 8.00 Hz, 2H), 6.61 (d, J = 8.00 Hz, 1H), 6.52 (s, 1H), 4.77 (s, 1H), 4.22 (s, 2H), 2.08-2.05 (m, 1H), 1.12-1.07 (m, 2H), 0.88-0.86 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 160.64, 158.36, 157.66, 151.67, 149.74, 141.82, 141.62, 140.21, 140.00, 139.36, 138.54, 135.98, 135.34, 130.28, 128.62, 128.55, 126.94, 122.42, 120.52, 118.28, 116.59, 115.65, 110.71, 110.49, 104.00, 94.20, 57.17, 15.39, 10.68, 10.65. HPLC 98.4 %. HRMS(ESI) m/z calcd for $C_{32}H_{24}FN_3O_3$ $[M+H]^{+\cdot}$ 518.1874, found 518.1879.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(4-methoxyphenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21d). Using a method similar to that of **21a**, compound **21d** was synthesized as a gray solid. MS (ESI) m/z 574.3 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(4-methoxyphenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3d). Using a method similar to that of **3a**, compound **3d** was synthesized as a white solid (38 mg, 65.7 %). Mp: 258-260 ^oC. MS (ESI) m/z 532.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.22 (s, 1H), 8.24 (d, J = 5.20 Hz, 1H), 7.88 (d, J = 8.00 Hz, 2H), 7.63-7.56 (m, 2H), 7.44 (d, J = 5.00 Hz, 2H), 7.27 (s, 1H), 7.18 (d, J = 5.00 Hz, 1H), 7.02 (d, J = 8.00 Hz, 2H), 6.97 (s, 1H), 6.61 (d, J = 5.00 Hz, 2H), 4.72 (s, 1H), 4.22 (s, 2H), 3.85 (s, 3H), 2.08-2.05 (m, 1H), 1.12-1.07 (m, 2H), 0.88-0.86 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.23, 160.64, 159.27, 158.37, 158.32, 151.69, 151.59, 149.79, 141.92, 141.83, 140.20, 139.93, 138.83, 135.98, 135.32, 130.30, 128.65, 128.59, 126.84, 123.97, 120.44, 118.28, 116.67, 114.32,

110.71, 110.49, 104.02, 94.83, 57.17, 55.19, 15.39, 10.70, 10.66. HPLC 96.4 %. HRMS(ESI) m/z calcd for $C_{33}H_{26}FN_3O_3$ $[M+H]^{+\cdot}$ 532.2031, found 532.2025.

2-(2-(3-Aminophenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-(6-cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)benzyl acetate (21e). Using a method similar to that of **21a**, compound **21e** was synthesized as a gray solid. MS (ESI) m/z 559.3 $[M + H]^+$.

2-(3-(2-(3-Aminophenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-2-(hydroxymethyl)-phenyl)-6-cyclopropyl-8-fluoroisoquinolin-1(2H)-one (3e). Using a method similar to that of **3a**, compound **3e** was synthesized as a white solid (57 mg, 60.9 %). Mp: 237-239 ^oC. MS (ESI) m/z 517.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.18 (s, 1H), 7.62 (d, J = 8.00 Hz, 1H), 7.56 (d, J = 8.00 Hz, 1H), 7.43 (d, J = 8.00 Hz, 2H), 7.27 (s, 1H), 7.17 (d, J = 5.00 Hz, 1H), 7.08 (m, 3H), 6.98 (d, J = 12.00 Hz, 1H), 6.64 (d, J = 8.00 Hz, 1H), 6.55 (m, J = 4.80 Hz, 1H), 6.55 (s, 1H), 5.16 (br s, 2H), 4.72 (br s, 1H), 4.21 (s, 2H), 2.08-2.05 (m, 1H), 1.12-1.07 (m, 2H), 0.89-0.85 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) : 163.23, 158.37, 151.71, 151.61, 149.66, 148.94, 142.15, 141.2-6.60 (d, 79, 140.20, 139.90, 139.73, 139.00, 135.98, 135.32, 131.88, 130.25, 129.35, 128.63, 128.59, 120.26, 118.29, 116.61, 114.13, 113.24, 110.71, 110.49, 104.02, 95.41, 57.10, 15.40, 10.72, 10.68. HPLC 99.2 %. HRMS(ESI) m/z calcd for $C_{32}H_{25}FN_4O_2$ $[M+H]^{+\cdot}$ 517.2034, found 517.2039.

2-(2-(4-Acetylphenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-(6-cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)benzyl acetate (21f). Using a method similar to that of **3a**, compound **3f** was synthesized as a gray solid. MS (ESI) m/z 586.2 $[M + H]^+$.

2-(3-(2-(4-Acetylphenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-2-(hydroxymethyl)-phenyl)-6-cyclopropyl-8-fluoroisoquinolin-1(2H)-one (3f). Using a method similar to that of **3a**, compound **3f** was synthesized as a white solid (45 mg, 67.3 %). Mp: 243-245 ^oC. MS (ESI) m/z 544.2 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.52 (s, 1H), 8.35 (d, J = 4.80 Hz, 1H), 8.10 (d, J = 8.00 Hz, 2H), 8.02 (d, J = 8.00 Hz, 2H), 7.65-7.58 (m, 2H), 7.44 (t, J = 12.00 Hz, 2H), 7.27 (s, 1H), 7.23 (d, J = 5.00 Hz, 1H), 6.99 (d, J = 8.00 Hz, 1H), 6.92 (s, 1H), 6.62 (d, J = 8.00 Hz, 1H), 4.70 (br s, 1H), 4.21 (s, 2H), 2.58 (s, 3H), 2.07-2.05 (m, 1H), 1.10-1.08 (m, 2H), 0.88-0.86 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) : 163.22, 160.63, 158.40, 151.75, 151.66, 150.06, 143.51, 141.79, 140.18,

140.00, 139.60, 137.25, 135.97, 135.71, 135.60, 135.24, 130.34, 128.86, 128.75, 125.31, 120.12, 118.29, 117.04, 110.72, 110.50, 104.09, 98.40, 57.14, 26.68, 15.39, 10.72, 10.69. HPLC 98.3 %. HRMS(ESI) m/z calcd for $C_{34}H_{26}FN_3O_3$ $[M+H]^{+\cdot}$ 544.2031, found 544.2027.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(4-(dimethylcarbamoyl)phenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21g). Using a method similar to that of **21a**, compound **21g** was synthesized as a gray solid. MS (ESI) m/z 615.3 $[M + H]^+$.

4-(4-(3-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-2-(hydroxymethyl)-phenyl)-1H-pyrrolo[2,3-b]pyridin-2-yl)-N,N-dimethylbenzamide (3g). Using a method similar to that of **3a**, compound **3g** was synthesized as a white solid (19 mg, 51.7 %). Mp: 252-254 ^oC. MS (ESI) m/z 573.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.40 (s, 1H), 8.32 (d, J = 4.80 Hz, 1H), 7.99 (d, J = 8.00 Hz, 2H), 7.64-7.57 (m, 2H), 7.48 (d, J = 8.00 Hz, 2H), 7.44 (d, J = 8.00 Hz, 2H), 7.26 (s, 1H), 7.22 (d, J = 5.00 Hz, 1H), 6.99 (d, J = 8.00 Hz, 1H), 6.82 (s, 1H), 6.61 (d, J = 8.00 Hz, 1H), 4.71 (br s, 1H), 4.22 (s, 2H), 2.98 (s, 6H), 2.07-2.05 (m, 1H), 1.10-1.08 (m, 2H), 0.88-0.86 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 168.50, 165.90, 163.63, 163.59, 156.95, 156.85, 155.19, 148.25, 147.07, 145.46, 144.98, 144.91, 143.06, 141.27, 141.00, 140.55, 137.52, 135.58, 133.94, 132.91, 130.39, 125.44, 123.55, 122.16, 115.99, 115.78, 109.29, 102.43, 62.43, 40.04, 20.65, 15.91, 15.88. HPLC 97.3 %. HRMS(ESI) m/z calcd for $C_{35}H_{29}FN_4O_3$ $[M+H]^{+\cdot}$ 573.2296, found 573.2314.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(4-(piperidine-1-carbonyl)phenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21h). Using a method similar to that of **3a**, compound **3g** was synthesized as a gray solid. MS (ESI) m/z 654.3 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(4-(piperidine-1-carbonyl)-phenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3h). Using a method similar to that of **3a**, compound **3g** was synthesized as a white solid (40 mg, 52.7 %). Mp: 243-245 ^oC. MS (ESI) m/z 544.2 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.38 (s, 1H), 8.31 (d, J = 4.80 Hz, 1H), 7.99 (d, J = 8.00 Hz, 2H), 7.63-7.55

(m, 2H), 7.44 (d, *J* = 8.00 Hz, 2H), 7.38 (d, *J* = 8.00 Hz, 1H), 7.36 (d, *J* = 8.00 Hz, 1H), 7.24 (d, *J* = 4.00 Hz, 1H), 6.90 (s, 1H), 6.80 (s, 1H), 6.75 (s, 1H), 6.49 (d, *J* = 8.00 Hz, 1H), 4.64 (br s, 1H), 4.21 (s, 2H), 3.82 (s, 4H), 2.07-2.05 (m, 1H), 3.82 (s, 4H), 1.63 (s, 2H), 1.53 (s, 4H), 1.10-1.08 (m, 2H), 0.88-0.86 (m, 2H). HPLC 95.9 %. HRMS(ESI) m/z calcd for $C_{38}H_{33}FN_4O_3$ $[M+H]^{+\cdot}$ 613.2609, found 613.2604.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(4-(morpholinomethyl) phenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21i). Using a method similar to that of **21a**, compound **21i** was synthesized as a gray solid. MS (ESI) m/z 643.3 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(4-(morpholinomethyl)phenyl) -1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3i). Using a method similar to that of **3a**, compound **3i** was synthesized as a white solid (35 mg, 56.3 %). Mp: 258-260 ^oC. MS (ESI) m/z 601.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.31 (s, 1H), 8.29 (d, *J* = 4.80 Hz, 1H), 7.89 (d, *J* = 8.00 Hz, 2H), 7.64-7.53 (m, 2H), 7.44 (d, *J* = 8.00 Hz, 2H), 7.38 (d, *J* = 8.00 Hz, 2H), 7.27 (s, 1H), 7.19 (d, *J* = 4.00 Hz, 1H), 6.99 (d, *J* = 8.00 Hz, 1H), 6.71 (s, 1H), 6.61 (d, *J* = 8.00 Hz, 1H), 4.75 (br s, 1H), 4.10 (s, 2H), 3.60 (s, 4H), 3.58 (s, 2H), 2.39 (s, 4H), 2.07-2.05 (m, 1H), 1.10-1.08 (m, 2H), 0.86-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.23, 160.64, 158.37, 158.32, 151.70, 151.60, 149.82, 142.46, 141.83, 140.20, 139.82, 139.24, 138.63, 137.88, 136.00, 135.31, 130.68, 130.58, 130.30, 130.16, 125.27, 120.27, 118.28, 116.76, 110.71, 110.49, 104.02, 96.04, 66.17, 62.04, 57.16, 53.14, 15.39, 10.71, 10.67. HPLC 96.4 %. HRMS(ESI) m/z calcd for $C_{37}H_{33}FN_4O_3$ $[M+H]^{+\cdot}$ 601.2609, found 601.2605.

2-(2-(5-Acetylthiophen-3-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-(6-cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)benzyl acetate (21j). Using a method similar to that of **21a**, compound **21j** was synthesized as a gray solid. MS (ESI) m/z 592.2 $[M + H]^+$.

2-(3-(2-(5-Acetylthiophen-3-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-2-(hydroxymethyl) phenyl)-6-cyclopropyl-8-fluoroisoquinolin-1(2H)-one (3j). Using a method similar to that of **3a**, compound **3j** was synthesized as a white solid (41 mg, 59.3 %). Mp: 232-234 ^oC. MS (ESI) m/z 550.2 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.16 (s, 1H), 8.36 (d, *J* = 5.20 Hz, 1H), 7.97 (d, *J* = 4.00 Hz, 1H), 7.76 (d, *J* = 4.00 Hz, 1H), 7.62 (t, *J* =

12.00 Hz, 1H), 7.56 (d, *J* = 8.00 Hz, 1H), 7.54 (d, *J* = 4.00 Hz, 1H), 7.45 (m, *J* = 8.00 Hz, 1H), 7.27 (s, 1H), 7.24 (s, 1H), 6.98 (d, *J* = 12.00 Hz, 1H), 6.72 (s, 1H), 6.61 (d, *J* = 8.00 Hz, 1H), 4.74 (s, 1H), 4.19 (s, 2H), 2.56 (s, 3H), 2.08-2.06 (m, 1H), 1.12-1.08 (m, 2H), 0.88-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.23, 160.64, 158.36, 151.70, 149.77, 143.92, 142.71, 142.30, 141.79, 140.18, 140.08, 139.39, 135.99, 135.26, 134.91, 132.16, 130.26, 128.83, 128.75, 125.70, 119.99, 118.27, 117.23, 110.72, 110.51, 104.08, 98.72, 57.12, 26.32, 15.40, 10.70, 10.67. HPLC 98.5 %. HRMS(ESI) m/z calcd for $C_{32}H_{24}FN_3O_3S$ $[M+H]^{+\cdot}$ 550.1595, found 550.1589.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(6-methoxypyridin-3-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21k). Using a method similar to that of **21a**, compound **21 k** was synthesized as a gray solid. MS (ESI) m/z 575.2 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(6-methoxypyridin-3-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3k). Using a method similar to that of **3a**, compound **3k** was synthesized as a white solid (38 mg, 52.5 %). Mp: 237-239 ^{o}C. MS (ESI) m/z 533.3 $[M + H]^+$. ^{1}H NMR(400 MHz, DMSO-d_6) : 12.36 (s, 1H), 8.76 (s, 1H), 8.29 (d, *J* = 5.20 Hz, 1H), 8.24 (d, *J* = 8.00 Hz, 1H), 7.63 (d, *J* = 5.20 Hz, 1H), 7.58 (t, *J* = 12.00 Hz, 1H), 7.44 (d, *J* = 8.00 Hz, 2H), 7.27 (s, 1H), 7.21 (s, 1H), 6.98 (d, *J* = 12.00 Hz, 1H), 6.92 (d, *J* = 8.00 Hz, 1H), 6.72 (s, 1H), 6.61 (d, *J* = 8.00 Hz, 1H), 4.73 (s, 1H), 4.22 (s, 2H), 3.90 (s, 3H), 2.08-2.06 (m, 1H), 1.12-1.08 (m, 2H), 0.88-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.19, 160.63, 158.37, 151.70, 151.60, 149.86, 143.86, 142.43, 141.84, 140.18, 139.81, 139.18, 136.36, 135.97, 135.28, 130.31, 128.68, 121.39, 120.19, 118.25, 116.84, 112.02, 111.96, 110.75, 110.49, 104.04, 95.90, 57.15, 53.36, 15.39, 10.70, 10.67. HPLC 98.2 %. HRMS(ESI) m/z calcd for $C_{32}H_{25}FN_4O_3$ $[M+H]^{+\cdot}$ 533.1910, found 533.1983.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(1-methyl-1H-pyrazol-3-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21l). Using a method similar to that of **21a**, compound **21l** was synthesized as a gray solid. MS (ESI) m/z 547.3 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(1-methyl-1H-pyrazol-3-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3l). Using a method similar

to that of **3a**, compound **3l** was synthesized as a white solid (21 mg, 48.3 %). Mp: 262-264 ^{o}C. MS (ESI) m/z 506.3 $[M + H]^+$. ^{1}H NMR(400 MHz, DMSO-d_6) : 12.09 (s, 1H), 8.20-8.19 (d, J = 5.20 Hz, 2H), 7.98 (s, 1H), 7.61 (t, J = 12.00 Hz, 1H), 7.55 (d, J = 8.00 Hz, 1H), 7.41 (d, J = 8.00 Hz, 2H), 7.27 (s, 1H), 7.15 (d, J = 5.20 Hz, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.61 (d, J = 8.00 Hz, 1H), 6.39 (s, 1H), 4.72 (s, 1H), 4.20-4.19 (s, 2H), 3.87 (s, 3H), 2.08-2.05 (m, 1H), 1.12-1.07 (m, 2H), 0.88-0.86 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.22, 160.63, 158.38, 151.71, 149.23, 141.82, 141.25, 140.19, 140.08, 138.18, 136.56, 135.92, 135.29, 132.65, 130.24, 128.61, 128.30, 120.41, 118.28, 116.52, 114.39, 110.70, 110.48, 104.02, 93.94, 57.09, 39.05, 15.39, 10.72, 10.68. HPLC 97.4 %. HRMS(ESI) m/z calcd for $C_{30}H_{24}FN_5O_2$ $[M+H]^{+\cdot}$ 506.1986, found 506.1991.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(1,2,3,6-tetrahydro-pyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21m). Using a method similar to that of **21a**, compound **21m** was synthesized as a gray solid. MS (ESI) m/z 549.2 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3m). Using a method similar to that of **3a**, compound **3m** was synthesized as a white solid (87 mg, 47.2 %). Mp: 245-247 ^{o}C. MS (ESI) m/z 601.3 $[M + H]^+$. ^{1}H NMR(400 MHz, DMSO-d_6) : 12.58 (s, 1H), 8.61 (d, J = 4.80 Hz, 2H), 8.38 (d, J = 4.80 Hz, 1H), 7.91 (d, J = 8.00 Hz, 2H), 7.56 (d, J = 4.80 Hz, 1H), 7.45 (d, J = 8.00 Hz, 2H), 7.26 (s, 1H), 7.02 (s, 1H), 6.97 (d, J = 12.00 Hz, 1H), 6.63 (d, J = 4.00 Hz, 1H), 4.70 (br s, 1H), 4.20 (s, 2H), 3.85 (m, 2H), 3.35 (m, 2H), 2.74 (m, 2H), 2.07-2.05 (m, 1H), 1.10-1.08 (m, 2H), 0.86-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.23, 160.64, 158.36, 150.21, 144.07, 141.83, 140.41, 140.19, 139.48, 138.34, 135.99, 135.55, 135.27, 130.34, 128.86, 128.72, 119.85, 119.35, 118.28, 117.16, 110.74, 110.52, 104.06, 99.34, 57.10, 41.30, 38.70, 31.25, 15.39, 10.70, 10.67. HPLC 96.7 %. HRMS(ESI) m/z calcd for $C_{31}H_{27}FN_4O_2$ $[M+H]^{+\cdot}$ 507.2024, found 507.2029.

2-(2-(1-Acetyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-(6-cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)benzyl acetate (21n). Using a method similar to that of **21a**, compound **21n** was synthesized as a gray solid. MS (ESI)

m/z 591.3 $[M + H]^+$.

2-(3-(2-(1-Acetyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-2-(hydroxymethyl)phenyl)-6-cyclopropyl-8-fluoroisoquinolin-1(2H)-one (3n). Using a method similar to that of **3a**, compound **3n** was synthesized as a white solid (37 mg, 65.8 %). Mp: 198-200 ^oC. MS (ESI) m/z 549.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.01 (s, 1H), 8.26 (d, J = 4.80 Hz, 1H), 7.58 (d, J = 8.00 Hz, 1H), 7.51 (d, J = 8.00 Hz, 1H), 7.42 (d, J = 8.00 Hz, 2H), 7.26 (s, 1H), 7.14 (d, J = 4.00 Hz, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.60 (d, J = 8.00 Hz, 1H), 6.53 (s, 1H), 6.27 (d, J = 8.00 Hz, 1H), 4.69 (br s, 1H), 4.18 (s, 2H), 4.13 (s, 2H), 3.61 (m, 2H), 2.52 (m, 2H), 2.09 (s, 3H), 2.09-2.06 (m, 1H), 1.10-1.07 (m, 2H), 0.87-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.22, 160.63, 158.35, 149.66, 142.59, 141.81, 140.19, 139.76, 139.22, 138.85, 135.96, 135.30, 130.22, 128.63, 127.17, 126.89, 121.36, 120.74, 119.69, 118.28, 116.48, 110.70, 110.48, 104.01, 96.33, 57.11, 42.38, 41.21, 37.17, 21.69, 15.39, 10.71, 10.67. HPLC 97.2 %. HRMS(ESI) m/z calcd for $C_{33}H_{29}FN_4O_3$ $[M+H]^+$ 549.2296, found 549.2290.

2-(2-(1-(Cyclopropanecarbonyl)-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-6-(6-cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)benzyl acetate (21o). Using a method similar to that of **21a**, compound **21o** was synthesized as a gray solid. MS (ESI) m/z 617.3 $[M + H]^+$.

2-(3-(2-(1-(Cyclopropanecarbonyl)-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo-[2,3-b]pyridin-4-yl)-2-(hydroxymethyl)phenyl)-6-cyclopropyl-8-fluoroisoquinolin-1(2H)-one (3o). Using a method similar to that of **3a**, compound **3o** was synthesized as a white solid (30 mg, 46.1 %). Mp: 190-192 ^oC. MS (ESI) m/z 575.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 12.01 (s, 1H), 8.26 (d, J = 4.80 Hz, 1H), 7.58 (d, J = 8.00 Hz, 1H), 7.52 (d, J = 8.00 Hz, 1H), 7.42 (d, J = 8.00 Hz, 2H), 7.26 (s, 1H), 7.15 (d, J = 4.00 Hz, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.60 (d, J = 8.00 Hz, 1H), 6.53 (s, 1H), 6.28 (s, 1H), 4.69 (br s, 1H), 4.18 (s, 2H), 3.87 (s, 2H), 3.45 (s, 2H), 2.52 (m, 2H), 2.09-2.06 (m, 2H), 1.10-1.07 (m, 2H), 0.87-0.85 (m, 2H), 0.75-0.72 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) : 171.35, 163.22, 160.63, 158.35, 158.30, 151.69, 151.60, 149.65, 142.60, 141.82, 140.19, 139.76, 139.22, 138.83, 135.97, 135.30, 130.23, 128.64, 119.69, 118.28, 116.49, 110.70, 110.48, 104.00, 96.28, 57.11, 15.39, 13.93, 11.22, 8.55. HPLC 96.5 %.

HRMS(ESI) m/z calcd for $C_{35}H_{31}FN_4O_3$ $[M+H]^{+\cdot}$ 575.2453, found 575.2448.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(1-(dimethylcarbamoyl)-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21p). Using a method similar to that of **21a**, compound **21p** was synthesized as a gray solid. MS (ESI) m/z 620.3 $[M + H]^+$.

4-(4-(3-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-2-(hydroxymethyl)-phenyl)-1H-pyrrolo[2,3-b]pyridin-2-yl)-N,N-dimethyl-5,6-dihydropyridine-1(2H)-carboxamide (3p). Using a method similar to that of **3a**, compound **3p** was synthesized as a white solid (34 mg, 50.7 %). Mp: 181-183 ^oC. MS (ESI) m/z 578.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 11.92 (s, 1H), 8.25 (d, J = 4.80 Hz, 1H), 7.59 (d, J = 8.00 Hz, 1H), 7.52 (d, J = 8.00 Hz, 1H), 7.41 (d, J = 8.00 Hz, 2H), 7.26 (s, 1H), 7.15 (d, J = 4.00 Hz, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.61 (d, J = 8.00 Hz, 1H), 6.51 (s, 1H), 6.27 (s, 1H), 4.70 (br s, 1H), 4.20 (s, 2H), 3.88 (m, 2H), 3.52 (m, 2H), 2.76 (m, 6H), 2.54 (s, 2H), 2.10-2.08 (m, 1H), 1.10-1.07 (m, 2H), 0.87-0.84 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 164.15, 163.72, 161.13, 158.90, 150.07, 143.00, 142.26, 140.69, 140.30, 139.71, 139.61, 136.45, 135.76, 130.73, 129.15, 129.07, 127.59, 122.45, 120.25, 118.79, 116.95, 111.24, 111.03, 104.57, 96.59, 57.65, 46.46, 43.58, 38.49, 25.65, 15.88, 11.14, 11.11. HPLC 97.7 %. HRMS(ESI) m/z calcd for $C_{34}H_{32}FN_5O_3$ $[M+H]^{+\cdot}$ 578.2561, found 578.2531.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(1-(morpholine-4-carbonyl)-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21q). Using a method similar to that of **21a**, compound **21q** was synthesized as a gray solid. MS (ESI) m/z 662.3 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(1-(morpholine-4-carbonyl)-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1-(2H)-one (3q). Using a method similar to that of **3a**, compound **3q** was synthesized as a white solid (28 mg, 50.1 %). Mp: 202-204 ^oC. MS (ESI) m/z 620.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 11.95 (s, 1H), 8.26 (d, J = 4.80 Hz, 1H), 7.59 (d, J = 8.00 Hz, 1H), 7.52 (d, J = 8.00 Hz, 1H), 7.42 (d, J = 8.00 Hz, 2H), 7.27 (s, 1H), 7.16 (d, J = 4.00 Hz, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.61 (d, J = 8.00 Hz, 1H), 6.52 (s, 1H), 6.27 (s,

1H), 4.67 (br s, 1H), 4.19 (s, 2H), 3.95 (m, 2H), 3.59 (m, 4H), 3.38 (m, 2H), 3.16 (m, 4H), 2.52 (m, 2H), 2.10-2.08 (m, 1H), 1.10-1.07 (m, 2H), 0.87-0.84 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.24, 162.85, 158.37, 151.68, 151.59, 149.61, 142.54, 141.80, 140.20, 139.80, 139.22, 139.03, 135.99, 135.28, 130.22, 128.61, 127.13, 121.72, 119.73, 118.30, 116.46, 110.74, 110.52, 104.02, 96.16, 65.90, 57.15, 46.85, 45.93, 42.91, 38.88, 15.39, 10.63, 10.60. HPLC 98.7 %. HRMS(ESI) m/z calcd for $C_{36}H_{34}FN_5O_4$ $[M+H]^{+\cdot}$ 620.2667, found 620.2669.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(4-(morpholinomethyl) phenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21r). Using a method similar to that of **21a**, compound **21r** was synthesized as a gray solid. MS (ESI) m/z 643.3 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(4-(morpholinomethyl)phenyl) -1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3r). Using a method similar to that of **3a**, compound **3r** was synthesized as a white solid (35 mg, 56.3 %). Mp: 258-260 ^{o}C. MS (ESI) m/z 601.3 $[M + H]^+$. ^{1}H NMR(400 MHz, DMSO-d_6) : 12.31 (s, 1H), 8.29 (d, J = 4.80 Hz, 1H), 7.89 (d, J = 8.00 Hz, 2H), 7.64-7.53 (m, 2H), 7.44 (d, J = 8.00 Hz, 2H), 7.38 (d, J = 8.00 Hz, 2H), 7.27 (s, 1H), 7.20 (d, J = 4.00 Hz, 1H), 6.99 (d, J = 8.00 Hz, 1H), 6.71 (s, 1H), 6.61 (d, J = 8.00 Hz, 1H), 4.75 (br s, 1H), 4.10 (s, 2H), 3.60 (s, 4H), 3.58 (s, 2H), 2.39 (s, 4H), 2.07-2.05 (m, 1H), 1.10-1.08 (m, 2H), 0.86-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.23, 160.64, 158.37, 158.32, 151.70, 151.60, 149.82, 142.46, 141.83, 140.20, 139.82, 139.24, 138.63, 137.88, 136.00, 135.31, 130.68, 130.58, 130.30, 130.16, 125.27, 120.27, 118.28, 116.76, 110.71, 110.49, 104.02, 96.04, 66.17, 62.04, 57.16, 53.14, 15.39, 10.71, 10.67. HPLC 96.4 %. HRMS(ESI) m/z calcd for $C_{37}H_{33}FN_4O_3$ $[M+H]^{+\cdot}$ 601.2609, found 601.2605.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(1-(N,N-dimethyl-sulfamoyl)-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl-acetate (21s). Using a method similar to that of **21a**, compound **21s** was synthesized as a gray solid. MS (ESI) m/z 656.3 $[M + H]^+$.

4-(4-(3-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-2-(hydroxymethyl)-phenyl)-1H-pyrrolo[2,3-b]pyridin-2-yl)-N,N-dimethyl-5,6-dihydropyridine-1(2H)-

sulfonamide (3s). Using a method similar to that of **3a**, compound **3s** was synthesized as a white solid (31 mg, 47.9 %). Mp: 187-189 ^{o}C. MS (ESI) m/z 614.3 $[M + H]^+$. ^{1}H NMR(400 MHz, DMSO-d_6) : 11.00 (s, 1H), 8.26 (d, J = 4.80 Hz, 1H), 7.58 (d, J = 8.00 Hz, 1H), 7.52 (d, J = 8.00 Hz, 1H), 7.41 (d, J = 8.00 Hz, 2H), 7.26 (s, 1H), 7.16 (d, J = 4.00 Hz, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.60 (d, J = 8.00 Hz, 1H), 6.53 (s, 1H), 6.28 (s, 1H), 4.70 (br s, 1H), 4.17 (s, 2H), 3.92 (m, 2H), 3.43 (m, 2H), 2.77 (s, 6H), 2.55 (m, 2H), 2.10-2.06 (m, 1H), 1.10-1.07 (m, 2H), 0.87-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.20, 160.63, 158.35, 151.70, 149.62, 142.69, 141.81, 140.19, 139.74, 139.30, 138.64, 135.96, 135.29, 130.22, 128.64, 126.74, 120.27, 119.65, 118.28, 116.51, 110.70, 110.49, 104.01, 96.45, 57.11, 45.03, 42.69, 37.79, 25.47, 15.11, 10.71, 10.67. HPLC 96.8 %. HRMS(ESI) m/z calcd for $C_{33}H_{32}FN_5O_4S$ $[M+H]^{+\cdot}$ 614.2231, found 614.2231.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(1-(oxetan-3-yl)-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzyl acetate (21t). Using a method similar to that of **21a**, compound **21t** was synthesized as a gray solid. MS (ESI) m/z 605.3 $[M + H]^+$.

6-Cyclopropyl-8-fluoro-2-(2-(hydroxymethyl)-3-(2-(1-(oxetan-3-yl)-1,2,3,6-tetrahydropyridin-4-yl)-1H-pyrrolo[2,3-b]pyridin-4-yl)phenyl)isoquinolin-1(2H)-one (3t). Using a method similar to that of **3a**, compound **3t** was synthesized as a white solid (37 mg, 56.8 %). Mp: 245-247 ^{o}C. MS (ESI) m/z 563.3 $[M + H]^+$. ^{1}H NMR(400 MHz, DMSO-d_6) : 11.90 (s, 1H), 8.25 (d, J = 4.80 Hz, 1H), 7.59 (d, J = 8.00 Hz, 1H), 7.52 (d, J = 8.00 Hz, 1H), 7.42 (d, J = 8.00 Hz, 2H), 7.27 (s, 1H), 7.15 (d, J = 4.00 Hz, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.61 (d, J = 8.00 Hz, 1H), 6.51 (s, 1H), 6.23 (s, 1H), 4.69 (br s, 1H), 4.60-4.57 (m, 2H), 4.52-4.49 (m, 2H), 4.19 (m, 2H), 3.58-3.55 (m, 1H), 3.45 (m, 4H), 3.04 (m, 2H), 2.11-2.08 (m, 2H), 1.10-1.07 (m, 2H), 0.87-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 163.24, 160.64, 158.32, 149.60, 142.37, 141.80, 140.21, 139.84, 139.31, 139.07, 135.99, 135.30, 130.22, 128.63, 128.57, 126.65, 121.87, 119.79, 118.30, 116.40, 110.73, 110.52, 104.02, 95.80, 74.53, 58.24, 57.15, 48.77, 45.53, 38.86, 15.39, 10.65, 10.62. HPLC 96.4 %. HRMS(ESI) m/z calcd for $C_{34}H_{31}FN_4O_3$ $[M+H]^{+\cdot}$ 563.2453, found 563.2448.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(4-(dimethylcarbamoyl)phenyl)-3H-imidazo[4,5-b]pyridin-7-yl)benzyl acetate (21u). Using a method similar to that of **21a**, compound **21u** was synthesized as a gray solid. MS (ESI) m/z 616.3 $[M + H]^+$.

4-(7-(3-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-2-(hydroxymethyl)-phenyl)-3H-imidazo[4,5-b]pyridin-2-yl)-N,N-dimethylbenzamide (3u). Using a method similar to that of **3a**, compound **3u** was synthesized as a white solid (42 mg, 55.6 %). Mp: 219-221 ^oC. MS (ESI) m/z 574.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 14.10 (s, 1H), 8.47 (d, J = 4.80 Hz, 1H), 8.21 (d, J = 8.00 Hz, 1H), 7.67 (m, 7H), 7.41 (d, J = 4.80 Hz, 1H), 7.28 (s, 1H), 7.00 (d, J = 12.00 Hz, 1H), 6.64 (d, J = 8.00 Hz, 1H), 5.56 (br s, 1H), 4.24-4.16 (m, 2H), 3.01 (s, 3H), 2.93 (s, 3H), 2.08 (m, 1H), 1.10-1.07 (m, 2H), 0.87-0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : 169.30, 163.60, 160.61, 158.38, 154.76, 151.79, 149.27, 141.61, 140.18, 138.44, 138.17, 137.67, 136.57, 135.40, 131.59, 130.68, 129.77, 129.32, 128.89, 128.53, 128.41, 127.70, 126.62, 119.44, 118.34, 110.75, 104.00, 57.38, 34.72, 15.40, 10.71, 10.67. HPLC 95.2 %. HRMS(ESI) m/z calcd for $C_{34}H_{28}FN_5O_3$ $[M+H]^{+\cdot}$ 574.2248, found 574.2245.

2-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-6-(2-(1-(dimethylcarbamoyl)-1,2,3,6-tetrahydropyridin-4-yl)-1H-indol-4-yl)benzyl acetate (21v). Using a method similar to that of **21a**, compound **21v** was synthesized as a gray solid. MS (ESI) m/z 618.3 $[M + H]^+$.

4-(4-(3-(6-Cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)-2-(hydroxymethyl)-phenyl)-1H-indol-2-yl)-N,N-dimethyl-5,6-dihydropyridine-1(2H)-carboxamide (3v). Using a method similar to that of **21a**, compound **21v** was synthesized as a white solid (18 mg, 42.6 %). Mp: 238-240 ^oC. MS (ESI) m/z 577.3 $[M + H]^+$. ^1H NMR(400 MHz, DMSO-d_6) : 11.34 (s, 1H), 7.54 (t, J = 16.00 Hz, 1H), 7.46 (m, 2H), 7.35 (m, 2H), 7.26 (s, 1H), 7.17 (t, J = 16.00 Hz, 1H), 7.09 (s, 1H), 6.98 (d, J = 12.00 Hz, 1H), 6.59 (d, J = 8.00 Hz, 1H), 6.34 (s, 1H), 6.21 (s, 1H), 4.60 (br s, 1H), 4.18 (m, 2H), 3.88 (m, 2H), 3.30 (m, 2H), 2.77 (s, 6H), 2.48 (m, 2H), 2.09-2.07 (m, 1H), 1.10-1.07 (m, 2H), 0.87-0.85 (m, 2H). HPLC 95.7 %. HRMS(ESI) m/z calcd for $C_{35}H_{33}FN_4O_3$ $[M+H]^{+\cdot}$ 577.2535, found 577.2539.

4.2. BTK enzymatic assay

The HTRF kinase assay (components supplied as kit by Cisbio) was chosen for BTK enzyme assays. It uses time resolved fluorescence resonance energy transfer (TR-FRET) to detect production of a phosphorylated substrate. A peptide substrate is labeled with a biotin that can bind to XL665 labeled streptavidin, and the anti-phosphoresidue antibody is labeled with Eu^{+}. Upon phosphorylation of the substrate, the antibody binds to phosphorylated substrate that enables TR-FRET detection in homogenous assay format. All the reagents used for the BTK kinase assays including their resources are BTK kinase (Invitrogen), HTRF kinEASE-TK kit (Cisbio Bioassays), ATP(Sigma), DTT(Sunshine), $MgCl_2$ and $MnCl_2$ (Sigma). The assay buffer was composed of 50 mM HEPES (pH 7.0), 5 mM $MgCl_2$, 5 mM DTT, 0.1 % NaN_3, 0.1 % BSA and 0.1 mM orthovanadate. The HTRF assays were preformed according to the manual in the kit. All reagents were dispensed into each well plate according to the orders as follow: (1) BTK enzyme: 0.5 ng/μL, 4 μL; (2) Each compound as well as control: 0.008-50 mM; (3) Reagent: 22.4 μM ATP and 0.15 μM substrate, 2 μL; (4) Incubation: Ambient, at 25 ^{o}C, 5 min; (5) Reagent: antibody and XL-665, 8 μL. Then following 1 hour incubation at room temperature fluorescence was measured on the PHERAStar FS microplate reader (BMG Lab Technologies). Signal was expressed in terms of HTRF ratio (fluorescence intensity at 665 nm/fluorescence intensity at 620 nm).

4.3. Cellular (Ramos cell) activity in a Ca^{2+} flux assay.

Ramos cells (CRL-1596; American Type Culture Collection, Manassas, VA) were seeded in an assay plate, 20,000 cells per well, in phenol red-free RPMI 1640 medium (Gibco, Life Technologies) containing 2 % heatinactivated fetal bovine serum (Gibco, Life Technologies), and loaded with a calcium dye in Fluo-4 Direct™ Calcium Assay Kits for 40 min (Invitrogen). Next, cells were treated with either compounds or vehicle for 20 min in the dark before the plate was transferred to a PHERStar FS (BMG, Germany). Immediately after the transfer and a 10s recording of baseline fluorescence, cells were stimulated with a purified goat anti-human IgM antibody (10 μg/mL) (SouthernBiotech, USA) for 8 min, during which fluorescence signal was monitored and recorded per 10 s. Difference between the signal and that at baseline, designated adjusted relative fluorescence unit, was fitted with GraphPad Prism version 5.00 (GraphPad

Software Inc., San Diego, CA) to determine IgM-induced calcium influx and the IC_{50} values.

4.4. Molecular modeling

The protein-ligand complex crystal structure of ethyl analog of RN486 bound to BTK (PDB code 4OTR) was chosen as the template. The molecular docking procedure was referred to CDOCKER protocol within Discovery Studio 2.5. The enzyme preparation and the hydrogen atoms adding was performed in the prepared process. The whole BTK enzyme was defined as a receptor and the site sphere was selected on the basis of the binding location of **1** (RN486). **1** and the irrelevant water molecule were moved and compound **3p** was placed. After completion of docking, 10 poses were scored and selected on the basis of the calculated CDOCKER energy.

4.5 Pharmacokinetic Profiles of compound 3p in SD rats

Compound **3p** were administered to 3 male SD rats (weight ranging from 180 g to 240 g) at doses of 4 mg/kg for po administration. The dosing volume was 5 mL/kg. After administration, blood samples were collected at the point including 2 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, and 24 h for analyses, the collected blood samples were centrifuged at 4000 rpm for 5 min at 4 ^{o}C, and then analyzed after protein precipitation. LC/MS/MS analysis of compound **3p** was performed under optimized conditions to obtain the best sensitivity and selectivity of the analyte in selected reaction monitoring mode (SRM) containing an internal standard. Plasma concentration-time data were measured by a noncompartmental approach using the software WinNonlin Enterprise, version 5.2 (Pharsight Co.,Mountain View, CA).

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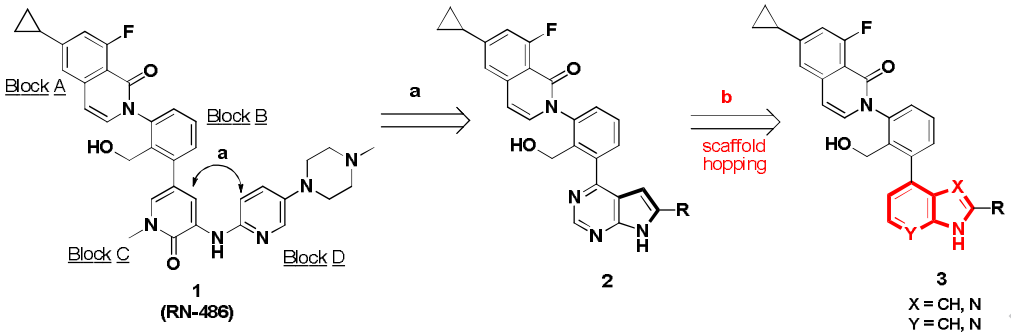


Figure 1. Design of the target compounds (**3**)

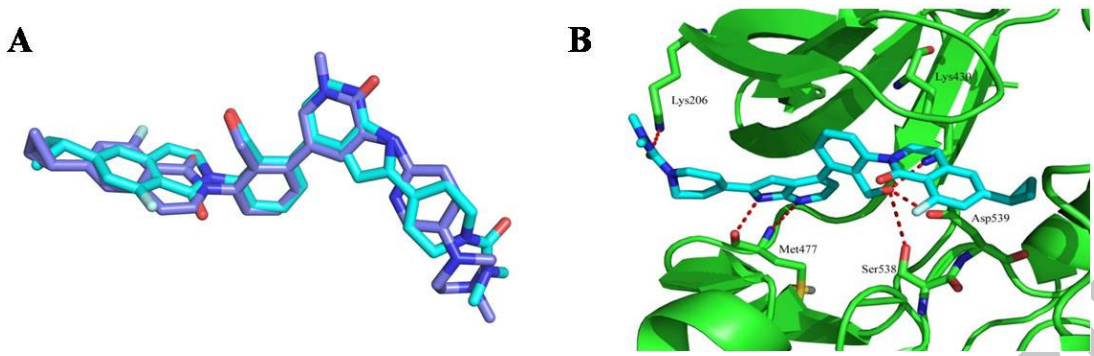
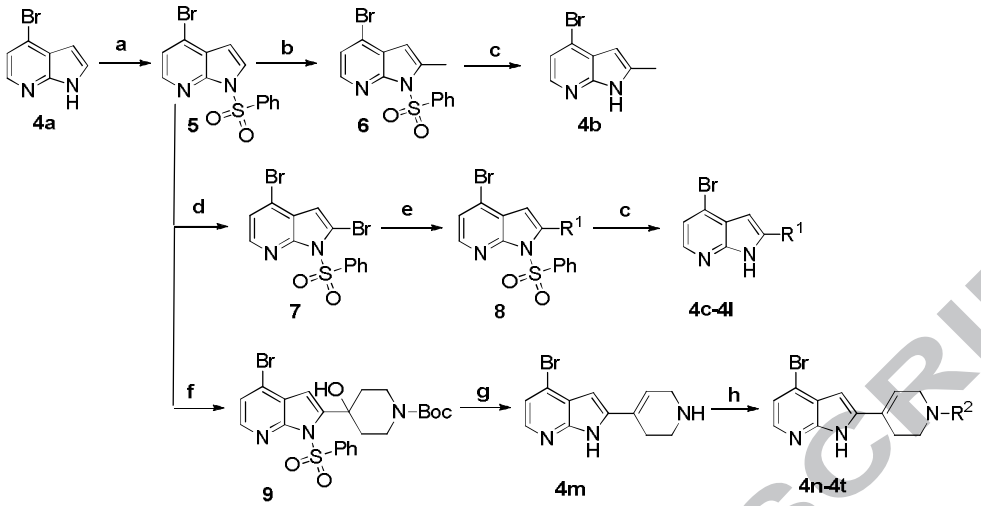
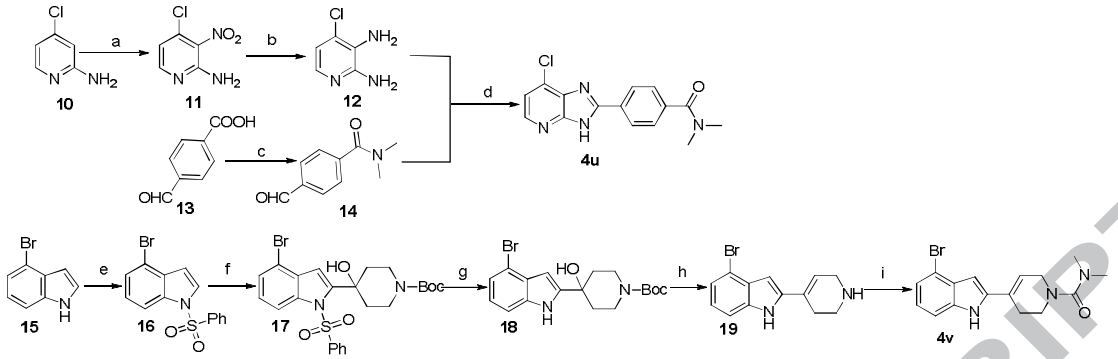


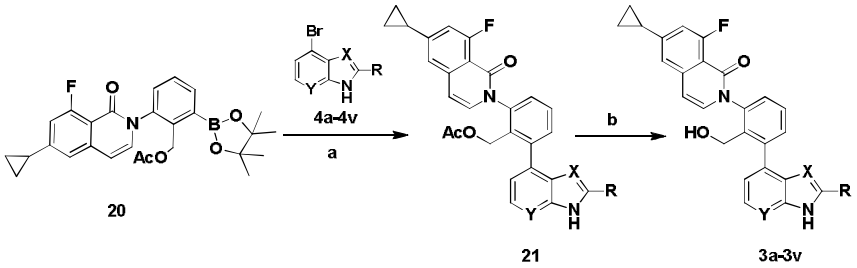
Figure 2. Docking mode of compound **3p** with BTK. (a) Superposed docking poses of **3p** (cyan) and **1** (ice blue). (b) Docking poses of **3p** (cyan) with BTK pocket, PDB ID: 4OTR.



Scheme 1. Reagents and conditions: (a) benzenesulfonyl chloride, $(CH_3)_3COK$, THF, rt, 4 h; (b) CH_3I, LDA, anhydrous THF, -78 °C, 3 h; (c) NaOH, CH_3OH, THF, rt, 2 h; (d) 1,2-dibromo-1,1,2,2-tetrachloroethane, LDA, anhydrous THF, -78 °C, 3 h; (e) Pd(dppf) $Cl_2 \cdot CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, 80 °C, 2-3 h; (f) *tert*-butyl 4-oxopiperidine-1-carboxylate**,** LDA, anhydrous THF, -78 °C, 3 h; (g) CF_3COOH, con H_2SO_4, rt, 12 h; (h) acetyl chloride for **4n**, cyclopropanecarbonyl chloride for **4o**, dimethylcarbamic chloride for **4p**, morpholine-4-carbonyl chloride for **4q**, methanesulfonyl chloride for **4r**, dimethylsulfamoyl chloride for **4s**, DIPEA, anhydrous DMSO, 0 °C to rt, 2-12 h, oxetan-3-one for **4t**, $NaBH_3CN$, $ZnCl_2$, MeOH, 50 °C, 3 h.

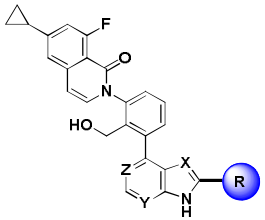


Scheme 2. Reagents and conditions: (a) Fuming nitric acid, conc H_2SO_4, 0 ^oC to rt, 3 h; (b) $SnCl_2 \cdot 2H_2O$, HCl, CH_3CH_2OH, 50 ^oC, 3 h; (c) $SOCl_2$, dimethylamine hydrochloride, DIPEA, THF, 0 ^oC to rt, 5 h; (d) nitrobenzene, 150 ^oC, 12 h; (e) benzenesulfonyl chloride, $(CH_3)_3COK$, THF, rt, 4 h; (f) *tert*-butyl 4-oxopiperidine-1-carboxylate**,** LDA, anhydrous THF, -78 ^oC, 3 h; (g) NaOH, CH_3OH, THF, rt, 2 h; (h) 37% HCl, rt, 12 h; (i) dimethylcarbamic chloride, DIPEA, anhydrous DMSO, 0 ^oC to rt, 3 h.



Scheme 3. Reagents and conditions: (a) Pd(dppf) $Cl_2 \cdot CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, 80 ^oC, 2-3 h; (b) LiOH, THF, *i*-PrOH, 50 ^oC, 3-7 h.

Table 1. Enzymatic and cellular inhibition for compounds **3**



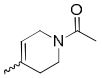
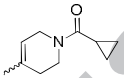
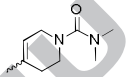
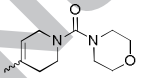
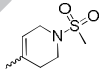
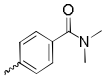
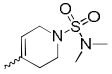
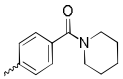
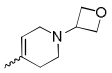
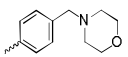
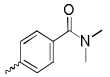
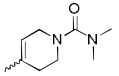
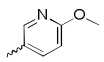
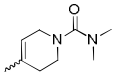
Compds	R	X	Y	Z	BTK IC_{50} (nM)	Ramos Cell Ca^{2+} Flux IC_{50} (nM)	Compds	R	X	Y	Z	BTK IC_{50} (nM)	Ramos Cell Ca^{2+} Flux IC_{50} (nM)
3a	H	CH	N	CH	562.2	350	**3m**		CH	N	CH	28.3	3
3b	Me	CH	N	CH	395	213	**3n**		CH	N	CH	6.7	12
3c		CH	N	CH	39.8	110	**3o**		CH	N	CH	10.4	16
3d		CH	N	CH	27.3	56.3	**3p**		CH	N	CH	6.0	14
3e		CH	N	CH	110.3	2410	**3q**		CH	N	CH	7.3	42
3f		CH	N	CH	21.3	32	**3r**		CH	N	CH	7.1	410
3g		CH	N	CH	12.1	94.2	**3s**		CH	N	CH	9.7	27
3h		CH	N	CH	38	52	**3t**		CH	N	CH	31.4	3
3i		CH	N	CH	18.1	69	**3u**		N	N	CH	33.3	92.7
3j		CH	N	CH	78.1	69	**3v**		CH	CH	CH	>5000	>5000
3k		CH	N	CH	68.3	196	**2**		CH	N	N	7	21.6
3l		CH	N	CH	20.6	9.5	**1** (RN486)					13.2	27.3

Table **2**. PK properties study for compound **3p** in SD rat[a]

parameter	**1**	**2**	**3p**
p.o. dose (mg/kg)	4	4	4
C_{max}(ng/mL), p.o.	134.1	421.8	739.3
T_{max}(h) , p.o.	4.0	0.8	1.9
AUC_{last}(h*ng/mL), p.o.	1053.5	1793.3	3423.0
Cl (L/h/kg) , p.o.	3.2	2.6	1.2
$t_{1/2}$ (h), p.o.	4.4	5.4	8.9

[a] Compound **3p** (as well as **1** & **2**) was formulated using 5%DMA+5% solutol

Graphical Abstract

Pyrrolo[2,3-*b*]pyridine derivatives as potent Bruton's tyrosine kinase inhibitors

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