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Discovery of **TAK-659** an orally available investigational inhibitor of Spleen Tyrosine Kinase (SYK)

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ABSTRACT

Spleen Tyrosine Kinase (SYK) is a non-receptor cytoplasmic tyrosine kinase that is primarily expressed in hematopoietic cells. SYK is a key mediator for a variety of inflammatory cells, including B cells, mast cells, macrophages and neutrophils and therefore, an attractive approach for treatment of both inflammatory diseases and oncology indications. Using in house co-crystal structure information, and structure-based drug design, we designed and optimized a novel series of heteroaromatic pyrrolidinone SYK inhibitors resulting in the selection of the development candidate **TAK-659**. **TAK-659** is currently undergoing Phase I clinical trials for advanced solid tumor and lymphoma malignancies, a Phase Ib study in advanced solid tumors in combination with nivolumab, and PhIb/II trials for relapsed/refractory AML.

Spleen Tyrosine Kinase (SYK) is a 72 kD non-receptor cytoplasmic tyrosine kinase that is primarily expressed in hematopoietic cells. SYK is a key mediator for a variety of inflammatory cells and immunology signaling pathways, including B cell receptor (BCR) in B cells, FcγR in lymphoid and myeloid cells, and FcεRI in mast cells, basophils, and neutrophils.[1a–f] Recent studies have implicated abnormal SYK activation in several hematopoietic malignancies including chronic lymphocytic leukemia (CLL), peripheral T-cell lymphoma (PTCL), acute myeloid leukemia (AML) and diffuse large B-cell lymphoma (DLBCL).[2a–c] Research has also identified an oncogenic collaboration between SYK and FMS-like tyrosine kinase 3 (FLT3) in AML. In addition, activating mutations within the FLT3 gene on chromosome 13q12 have been detected in approximately 30% of AML patients and accounted for one of the most frequently identified genetic alterations in AML.[3a,b] Internal tandem duplication (ITD) of the FLT3 gene occurs at high frequency in AML and SYK, is critical for the development of FLT3-ITD-induced myeloid neoplasia since it is more highly activated in primary human FLT3-ITD-positive AML than its FLT3 wild-type (WT) AML counterparts, and moreover transactivates FLT3 by direct phosphorylation.[4a–e] Hence, SYK inhibition provides a desirable mechanistic intervention for potential treatment of inflammatory diseases and oncology indications.

In recent years, a number of SYK inhibitors have advanced to clinical trials for inflammatory disease indications, such as asthma, arthritis and allergic diseases.[5] Most have been terminated in Phase I/II studies due to various reasons. An example is fostamatinib (**R–788**). Fostamatinib showed promising Phase I results in patients with rheumatoid arthritis, although little efficacy was observed in Phase II studies.[6a,b] When dosed at 150 mg BID, patients were experiencing adverse events, such as diarrhea, upper

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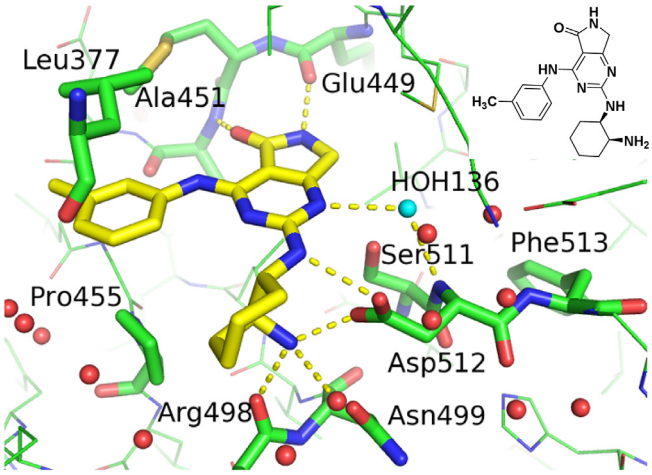
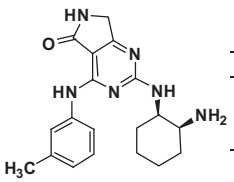


Fig. 1. Co-crystal structure of compound **1** in the SYK active site.



SYK IC$_{50}$	13 nM
Cell[1] EC$_{50}$	35 nM
Kinase	**Fold-Selectivity**
JAK3	19
ZAP70	21
VEGFR2	250
CYP (2D6)	7.9 μM
cLogP	3.6
LE[2]	0.41
LipE[3]	4.3
E$_h$(HLM)[10]	0.25
hERG	41%@10μM

[1]Cellular BLNK phosphorylation inhibition
[2]Ligand efficiency (LE): 1.4(-logIC$_{50}$)/N
[3]Lipophilic Efficiency (LipE) = LipE = pIC$_{50}$ - LogP
Human liver microsomes extraction ration: E$_h$(HLM)

Fig. 2. Chemical and physical properties of compound **1**.

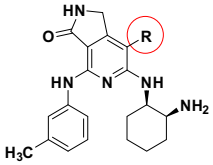
respiratory tract infections, neutropenia, elevated alanine transaminase (ALT), and hypertension. Fostamatinib is a non-selective SYK inhibitor, and therefore the observed undesirable side effects were hypothesized to be caused by off-target inhibition.[7a,b] In the past, generation of selective SYK inhibitors has been challenging,[5,7c,d] nonetheless as shown in this article, we succeeded in the design of highly selective SYK compounds by utilizing structure-based drug design (SBDD). Herein we report the discovery of **TAK-659**, a potent and selective SYK inhibitor that is currently undergoing Phase Ib/II evaluations in adult patients with solid tumors and hematologic malignancies.

Supported by high-throughput protein crystallization technology and applying SBDD strategy, we had designed a novel series of dihydropyrrolopyrimidinone SYK inhibitors, which led to the discovery of our first lead compound, compound **1**. A co-crystal structure of compound **1** in the SYK active site[8a] was obtained (Fig. 1). Compound **1** binds to the hinge region of SYK which adopts the active DFG-in (Phe513) conformation. The lactam core makes two direct hydrogen bonds (HBs) with the hinge through Ala451 and Glu449. Multiple interactions exist between the primary and secondary amines of the cyclohexyl ring with the polar region comprised of Arg498, Asn499, and Asp512. One of the pyrimidine ring nitrogen atoms forms a water mediated HB (HOH136; cyan) with Ser511. Leu377 and Pro455 provide a lipophilic pocket for the *m*-methyl aniline ring of compound **1**.

While compound **1** (see Scheme 1 in Supplemental Data for synthesis)[9a,10] is an efficient and potent inhibitor of SYK (IC$_{50}$ = 13 nM; LE = 0.41; LipE = 4.3), its cellular potency as measured by inhibition of phosphorylation of BLNK, a direct cellular substrate of SYK,[10] and selectivity toward JAK3 were not optimal. It also has hERG and CYP liabilities (Fig. 2). Our goals for lead optimization were to improve cellular potency and JAK3 selectivity, reduce inhibitions toward CYPs and hERG, and replace the aniline group, which is a known toxicophore that can potentially form reactive metabolites.

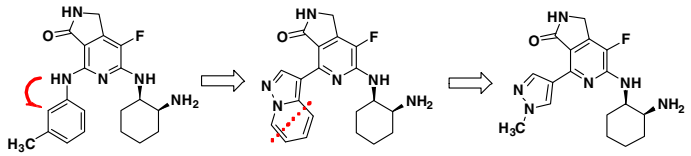
One way to improve potency and selectivity was to disrupt the water-mediated HB between the pyrimidine nitrogen and Ser511. Since water HOH136 was not a structural water, replacing it should be entropically beneficial to enhance potency. From SAR development (Table 1, and Scheme 2 in Supplemental Data for syntheses of compound **2a**–**f**),[10] displacement of the water with different **R** groups increased both enzyme (3 to 26-fold) and cellular potency (7 to > 12-fold), while kinase selectivity was compromised. Most of these compounds inhibited CYPs, with the exception of compound **2b**. Although compound **2b** showed improved enzyme and cellular potency, was selective against JAK3 (170-fold selective compared to SYK potency) and had no CYPs liability, further study showed that it inhibited hERG. To mitigate the hERG liability and remove the problematic aniline group, a number of aniline mimetics were synthesized. As shown in Table 2, the cyclized

Table 1
Structure-activity relationship of **R** substituents on compound **1**. Cell EC$_{50}$ represents cellular p-BLNK inhibition in RA1/GFP-BLNK cells.

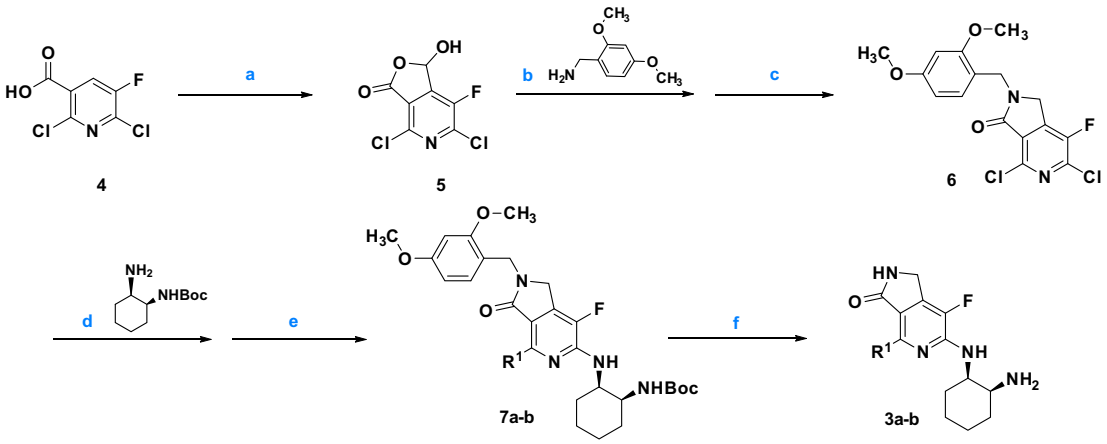


	R	2a H	2b F	2c Cl	2d Me	2e CN	2f 
SYK IC$_{50}$ (nM)		4.7	1.1	0.8	3.1	0.5	0.9
Cell EC$_{50}$ (nM)		5	9	NT	NT	< 3	4
Fold-Selectivity against individual SYK potency							
JAK3		70	170	53	18	3.3	10
ZAP70		23	29	19	9	3.5	0.5
VEGFR2		260	590	NT	NT	1,800	220
CYPs (>10 μM)		No	Yes	No	No	No	No
cLogP		4.1	4.3	4.7	4.6	3.9	4.1
LipE		4.2	4.7	4.4	3.9	5.4	5.0

Table 2
Lead optimization and discovery of **TAK-659**.



	2b	**3a**	**3b** (TAK–659)
SYK IC_{50} (nM)	1.1	2.3	3.2
Cell EC_{50} (nM)	9	9	9.8
	Fold-Selectivity against individual SYK potency		
JAK3	170	260	36
ZAP70	29	170	23
VEGFR2	590	2800	42
CYPs (>10 μM)	✓	✓	✓
cLogP	4.3	2.0	0.9
LipE	4.7	6.7	7.5
hERG (10μM)	47%	NT	<10%



Scheme 3. Reagents and conditions: **(a)** LDA, −78 °C, DMF/THF, 1 h, 80%; **(b)** (2,4-dimethoxyphenyl)methanamine, $NaBH_3CN$, AcOH, MeOH, rt, 4 h, 74%; **(c)** EDC, HOBt, DMF, 2 h, 90%; **(d)** *tert*-butyl (l*S*,2*R*)-2-amino-cyclohexylcarbamate, DIEA, ACN, 100 °C, 2–4 days, 40%; **(e)** for R^1 = **3a**: pyrazolo[1,5-*a*]pyridin-3-ylboronic acid; for R^1 = **3b**: (1-methyl-1H-pyrazol-4-yl)boronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, DME-H_2O, 100 °C, o/n, 68%; **(f)** neat TFA, 60 °C, 2 h, 79%.

pyrazolopyridine compound **3a**, retained both enzyme and cellular potency, possessed improved kinase selectivity, and did not inhibit CYPs. Upon further optimization by lowering molecular weight and cLogP, and improving LipE, compound **3b**, with methylpyrazole, was identified. Compound **3b** had a good *in vitro* profile, did not have CYP or hERG liabilities, and had become our drug candidate **TAK-659**

Syntheses of compound **3a** and **TAK-659** were performed according to the method depicted in Scheme 3. 2,6-Dichloro-5-fluoronicotinic acid was lithiated via treatment with LDA in THF at −78 °C. Quenching with DMF gave hemiacetal intermediate **5**, which underwent reductive amination through reaction with (2,4-dimethoxyphenyl)methanamine and $NaBH_3CN$. The resulting amino acid (not shown) was cyclized via amide coupling to form the lactam ring. Displacement of the chloro group of intermediate **6** with commercially available *tert*-butyl (l*S*, 2*R*)-2-amino-cyclo-hexyl-carbamate[9b] was followed by Suzuki coupling with pyrazolo [1, 5-a] pyridin-3-ylboronic acid or (1-methyl-1H-pyrazol-4-yl) boronic acid, respectively. Final Boc deprotection of **7** afforded compounds **3a** and **TAK-659**.

Shown in Fig. 3 is a co-crystal structure of **TAK-659** in the SYK active site that was solved at 1.93 Å.[8b] **TAK-659** maintains all key interactions with the SYK active site. For example, the HBs between the lactam core and the hinge through Ala451 and Glu449, the methyl pyrazole ring occupying the lipophilic region

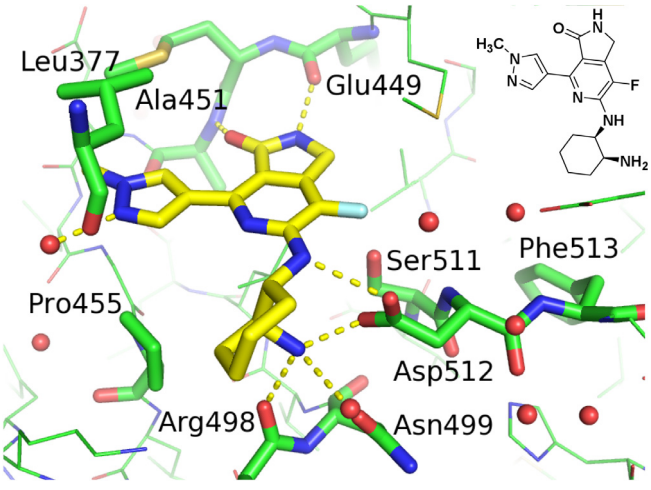


Fig. 3. Co-crystal structure of compound **TAK-659** in the SYK active site. C^5-F displaced the HOH136 normally found bound between the core N and the backbone of Asp512.

with one of the nitrogens forming an HB with a water molecule, multiple interactions between the primary and secondary amines of the cyclohexyl ring with the polar region comprised of Arg498,

Asn499, and Asp512, and the C^5-F substituent disrupting the water-mediated network by displacing HOH136 (cyan).

280 kinases screened in house and at Invitrogen/Life Technologies showed that **TAK-659** was selective against most other kinases, but potent toward both SYK and FLT3 (Tables 3–6 in Supplemental Data).[10] In a cell proliferation assay, **TAK-659** showed inhibition toward a SYK-dependent cell line (OCI-LY10).[11] The result demonstrated that the sensitivity to **TAK-659** was associated with mutations impacting SYK activity in B cell lymphomas, whereas **TAK-659** was not cytotoxic for adherent primary or solid tumor cell lines. In cell viability assays (Fig. 4 in Supplemental Data),[10] **TAK-659** was shown to be sensitive toward FLT3-ITD dependent cell lines, MV4-11 and MOLM-13 while the WT FLT3 RS4-11 (ALL cell line) and RA1 (Burkitt's Lymphoma cell line) were not sensitive toward **TAK-659**. To evaluate SYK inhibition *in vivo* in a pre-clinical immunology model, **TAK-659** was tested against an *in vivo* C57BL/6 mouse whole blood assay (Fig. 5 in Supplemental Data).[10] The purpose of this assay was to evaluate CD86 (cluster of differentiation 86) expression after primary B-cell activation which was dependent on SYK activity. The result showed that **TAK-659** blocked anti-IgD (immune-globulin D antibody) stimulated CD86 expression in mouse peripheral B cells *in vivo*.

Assessment of tumor growth inhibition in the OCI-LY10 xenograft and DLBCL PHTX-95L (primary human tumor graft from DLBCL patient) mouse models, **TAK-659** demonstrated potent tumor growth inhibition (TGI) after 20 days of treatment.[11] In the FLT3-dependent MV4-11 xenograft model, **TAK-659** showed tumor regression at 60 mg/kg daily after 20 days of dosing (Fig. 6 in Supplemental Data).[10]

In conclusion, we have utilized high-throughput protein crystallography and SBDD to design a novel series of SYK inhibitors, which has facilitated in the discovery and optimization of a potent and selective dihydropyrrolopyrimidinone lead compound **1**. Displacement of water in the SYK active site enhances potency and selectivity against JAK3, and replace-ment of the aniline and lowering of cLogP reduces hERG and reactive metabolite risk, which has led to the discovery of **TAK-659**. **TAK-659** inhibits cellular proliferation in SYK-dependent DLBCL and FLT3-dependent AML cell lines, and shows notable TGI in SYK- and FLT3-dependent xenograft models. Clinical trials of **TAK-659** for the treatment of advanced solid tumors and lymphoma malignancies are ongoing.[12,13]

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.10.087.

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9. (a) Full experimental procedures for all syntheses are contained within the following patent: Arikawa Y, Jones B, Lam B, et al. Patent No.: WO/2011/079051 A1. US20110152273 A1, 2011. (b) Optically pure *tert*-butyl (1S,2R)-2-amino-cyclohexyl-carbamate is commercially available from Aldrich and TCI-US, etc.
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13. Takeda global site disclosed **TAK-659** entering Phase Ib/II clinical trial for solid tumors and hematological malignancies. May, 2014. Also obtained latest results from www.clinicaltrials.gov.