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Orally bioavailable Syk inhibitors with activity in a rat PK/PD model

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ABSTRACT

Design and optimization of benzo- and pyrido-thiazoles/isothiazoles are reported leading to the discovery of the potent, orally bioavailable Syk inhibitor **5**, which was found to be active in a rat PK/PD model. Compound **5** showed acceptable overall kinase selectivity. However, in addition to Syk it also inhibited Aurora kinase in enzymatic and cellular settings leading to findings in the micronucleus assay. As a consequence, compound **5** was not further pursued.

Spleen Tyrosine Kinase (Syk) is a non-receptor kinase discovered in 1991.[1] Syk is critical for the transduction of intracellular signal cascades through various immune recognition receptors, such as B cell receptor, Fc receptors, adhesion receptors, or C-type lectin receptors. Following activation Syk phosphorylates a number of substrates and participates in a multi-protein signaling complex, the 'signalosome', which leads to the activation of downstream effector pathways such as PKC, MAPK and NFκB.[2] Syk is an established drug target for autoimmune diseases because aberrant activation of immunoreceptor signaling can contribute to the initiation and progression of chronic inflammation and autoimmunity.[3]

Despite considerable efforts,[4] only few Syk inhibitors have been evaluated in clinical trials (for structures and data on clinical candidates such as compounds **1**,[5] **2**[6] and **3**[7] see Fig. 1 and Table 1). We stopped early work on Syk inhibitors sharing the binding mode of **2** because of insurmountable PK issues.[8] Furthermore, we had to abandon the promising Syk inhibitor **4** (see Fig. 1 and Table 1) due to liver findings and a hERG flag leading to an insufficient therapeutic index for autoimmune indications.[9] In rat compound **4** exhibited >100 fold higher exposure in liver and kidney compared to blood. We hypothesized that the basic, primary amino group required for potency and acceptable PK properties contributed to both the high tissue exposure (volume of distribution was V_{ss} = 16.4 L/kg) and the safety issues observed in the toxicity studies. Thus, we set out to identify structurally unrelated back up compounds devoid of an amino group.

Here we disclose the discovery of the potent, orally bioavailable Syk inhibitor **5**, which is uncharged at physiological pH and structurally unrelated to **4**. Compound **5** did not inhibit the hERG channel (IC_{50} >30 μM) and showed substantially reduced tissue exposure compared to **4**. Due to good absorption and low in vivo clearance, compound **5** exhibited favorable PK properties in rat and was active in a rat PK/PD model. Unfortunately, compound **5** could not be further pursued because in addition to Syk it also inhibited Aurora kinase in enzymatic and cellular settings leading to findings in the micronucleus assay.

A structure of the early, moderately active Boehringer Ingelheim Syk inhibitor **6**[11] bound to Syk kinase domain solved at Novartis revealed that the nitrogen in the 1-position of the 1,6-naphthyridine core and the adjacent sp^2-carbon form H-bonds with the hinge sequence of Syk (Fig. 2A, Table 2).[12] Recently, the Boehringer group has shown that the basic amine of **6** can be replaced with neutral groups, such as lactams and aromatic amides, leading to more potent analogs.[13] To mimic the binding mode of **6**, we decided to explore benzo- and pyrido-thiazole cores as novel hinge binding motifs and prepared a set of neutral pilot

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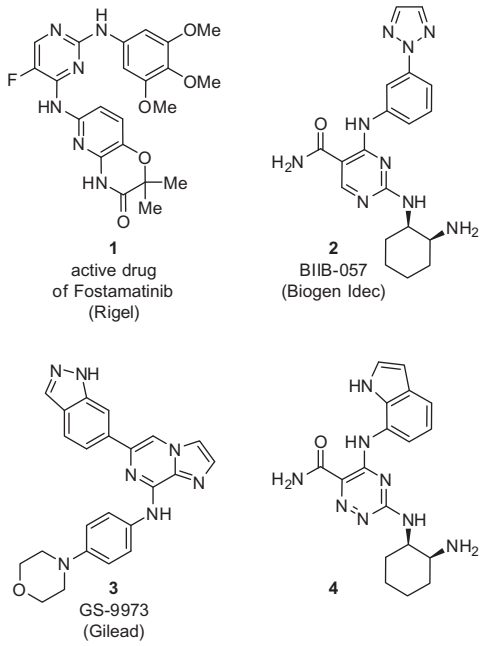

Figure 1. Structures of clinical Syk inhibitors **1**–**3** and reference compound **4**.

compounds **7**–**10** lacking the basic amine of **6** (Table 2; for the syntheses of **7**–**10** see Schemes 1–4).[14]

We were pleased to learn that these compounds are equipotent to our development candidate **4** in enzymatic, cellular and blood assays (Table 2).[10] Notably, thiazoles (**7**, **8**) and isothiazoles (**9**, **10**) were equally active. We obtained crystal structures of representative compounds **11** (thiazole; Table 2; for the synthesis see Scheme 2) and **12** (isothiazole; Table 2; for the synthesis see Scheme 3) bound to Syk kinase domain, which confirmed our binding hypothesis indicating that the novel sulfur containing thiazole and isothiazole scaffolds are bioisosteres of 1,6-naphthyridine (Fig. 2B and C). Similarly to **6**, the thiazole **11** forms two H-bonds with the hinge sequence of Syk. Isothiazole **12** cannot form the second H-bond; however, this is compensated for by a favorable interaction between sulfur and the backbone carbonyl oxygen of E449.[15]

Compounds **7**–**10** exhibited good solubility (>1 mM at pH 6.8), medium permeability (log PAMPA from −4.9 to $-5.2\cdot10^{-6}$ cm/s), medium plasma protein binding (human: 80–90%) and acceptable stability against rat liver microsomes (half-life from 34–82 min). In vivo rat PK studies revealed medium to high clearance (CL = 45–86 mL/min kg), low volumes of distribution (V_{ss} ~2 L/kg) and short mean residence times (MRT <1 h) for all three series tested (Table 2). The medium to high clearance and the short MRT were confirmed in dog PK studies.

To identify metabolic weak spots we investigated the metabolism of representative compounds from all four sulfur containing scaffolds in human and rat liver microsomes. Independent of the nature of the bicyclic core similar metabolites were observed across species mainly affecting the pyrazole (hydroxylation, N-demethylation) and the lactam substituent (hydroxylation). However, in contrast to the thiazoles, the isothiazoles formed trace quantities of glutathione conjugates indicating the formation of reactive intermediates. Their molecular masses pointed to an unexpected metabolic pathway involving addition of both GSH and H_2O. The trace quantities prevented us from isolation and structure elucidation. As a consequence, we decided not to consider the isothiazoles for the selection of potential development compounds.

However, we used the benzisothiazole core for the rapid optimization of the PK properties. Replacement of the pyrazole by aryl and 5 or 6-ring heteroaryl groups did not lead to derivatives with reduced in vivo clearance. In addition, the favorable physicochemical properties were compromised. Thus, we explored differently substituted pyrazoles and found the difluoromethyl derivative **13** to show improved PK properties (Table 2; for the synthesis see Scheme 3). However, the potency was limited. In efforts to reduce the hydroxylation of the lactam residue we introduced an isosteric cyclic carbamate (for the synthesis of the building block see

Table 1
Key in vitro data on reference compounds[10]

Compd	Syk[a] (enzyme)	Kinase[b] selectivity	Syk[a] (cell)	Syk[a] (blood)
1	68 ± 29	18 (79)	457 ± 8	3354 ± 475
2	13 ± 4	2 (77)	178 ± 8	952 ± 70
3	377 ± 90	0 (58)	878 ± 88	19,416 ± 1474
4	35 ± 4	0 (69)	99 ± 7	367 ± 27

[a] IC_{50} in nM; $n \geqslant 3$ for Syk enzyme, cell and blood assays (SEM shown).
[b] Number of kinases with IC_{50} <100 nM in addition to Syk (number of kinases tested).

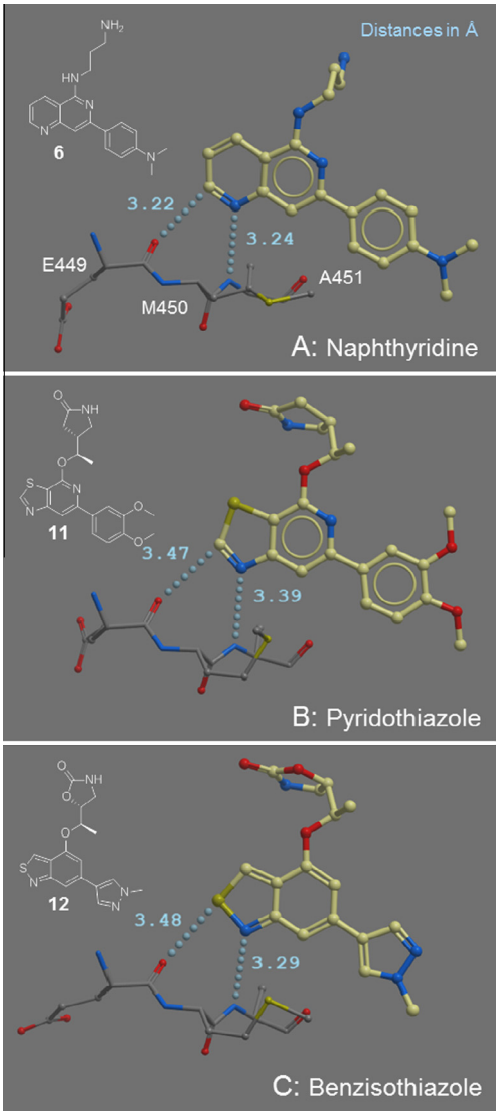

Figure 2. Crystal structures of **6**, **11** and **12** bound to Syk kinase domain (H-bonds with hinge sequence E449-M450-A451 shown).[12]