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Identification of 2-(4-pyridyl)thienopyridinones as GSK-3β inhibitors

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ABSTRACT

The discovery of a novel series of 2-(4-pyridyl)thienopyridinone GSK-3β inhibitors is reported. X-ray crystallography reveals its binding mode and enables rationalization of the SAR. The initial optimization of the template for improved cellular activity and predicted CNS penetration is also presented.

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase, which exists as two isoforms (α and β) and is involved in many cell functions.[1] Its main role is the phosphorylation of glycogen synthase (GS), therefore GSK-3 is implicated in type-2 diabetes.[2] In addition GSK-3 inhibition may be beneficial for the treatment of neurodegenerative diseases, such as Alzheimers,[3] and neurological diseases such as bipolar disorders.[4] In particular lithium is indicated as the preferential treatment for bipolar disorders and the ability of this cation to inhibit GSK-3 has been proposed as a potential therapeutic mechanism of action.[5] Due to the valuable therapeutic potential, identification of GSK-3 inhibitors has become a focus of research for both academic centers and pharmaceutical companies. The availability of crystal structures of GSK-3β6 allows structure based lead optimization. GlaxoSmithKline pursues kinase inhibitor discovery through a "systems-based" research strategy. One component of this is to cross-screen compounds through a panel of kinase assays including targets of interest and selectivity screens. A GSK-3β fluorescence polarization (FP) assay ran within this panel for several years, so when novel GSK-3 inhibitors were sought the historical screening data provided a rich source of potential lead compounds. Among the molecules with GSK-3 activity were members of the thieno-pyridinone series (e.g., **1–5**), which had been prepared as part of an array targeted against protein kinases (Table 1). The respectable potency of the series and its chemical novelty as a kinase inhibitor scaffold made it an attractive template for further study.

Compounds **1**–**5** showed moderate activity towards GSK-3β in both the FP assay and a biochemical assay format.[7] Interestingly, some of the examples that were tested against the homologous kinase CDK-2 showed moderate selectivity (e.g., **2**, **3** and **5**, Table 1).[8] They were also tested in a cellular system measuring the phosphorylation of the GSK-3β substrate CRMP-2, showing weak but encouraging weak activity.[9]

The initial data package also included full-curve inhibition results against the other members of the GSK kinase screening panel. The selectivity of the thieno-pyridinone series is striking, making them excellent starting points for optimization. For example, compounds **2** and **4** show excellent selectivity against the panel (Table 2).

Cross-screening of additional analogs within the initial set of hits provided a valuable source of initial structure–activity relationships (SAR). For example, it was apparent that the pyridyl ring was important for GSK-3β activity, as evidenced by the low activity of the phenyl analogs **6–9** and the 3-pyridyl isomer **10** (FP pIC_{50} <4.8). To rationalize this data, crystal structures were obtained of three closely related compounds (Fig. 1) bound to GSK-3β: **11** (2.4 Å), **12** (2.5 Å) and **13** (2.5 Å).[10]

All three compounds showed the same binding mode within the ATP-binding pocket. Two major interactions with the GSK-3β ATP binding pocket were identified (Fig. 2) The first is a hydrogen bond

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Table 1
Enzymatic activity (GSK-3β), selectivity versus the homologous kinase (CDK-2)[8] and cellular activity (CRMP-2) for hits **1**–**5**

Compound	GSK-3β pIC50 FP[a]	GSK-3β pIC_{50} AlphaLISA[b]	CDK-2 pIC_{50}[c]	CRMP-2 pIC_{50}[d]	tPSA (Å^2)[11b]
1	7.1	7.5	n.t.	<4.0	92
2	6.7	7.5	4.7	5.8 (n = 16)	92
3	7.0	n.t.	<4[d]	<4.5	75
4	6.9	7.3	n.t.	4.8	75
5	6.3	7.2	4.8[d]	5.9 (n = 4)	58

n.t. = not tested.
[a] GSK-3β fluorescence polarization pIC_{50} (binding assay).[7a]
[b] GSK-3β Alpha LISA pIC_{50} (enzymatic activity).[7b]
[c] CDK-2 enzymatic activity assay.[8]
[d] Cellular CRMP-2 Human Phosphorolation Inhibition pIC_{50}.[9]

Table 2
Kinase panel selectivity data

Compound	**2**	**4**
GSK-3β pIC_{50} (AlphaLISA)	7.5	7.3
Number of kinases tested	51	45
Number of kinases with pIC_{50} <6.0	50	42
Aurora A	6.0	5.9

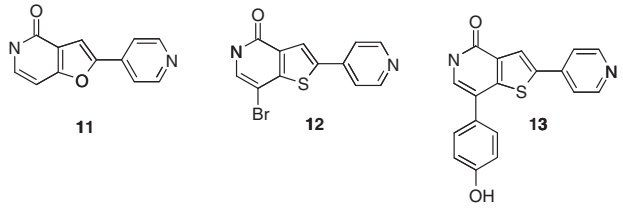

Figure 1. From left to right, **11** (GSK-3β FP pIC_{50} = 6.9)[b], **12** (GSK-3β AlphaLISA pIC_{50} = 6.8)[a], **13** (GSK-3β FP pIC_{50} = 6.9)[a].

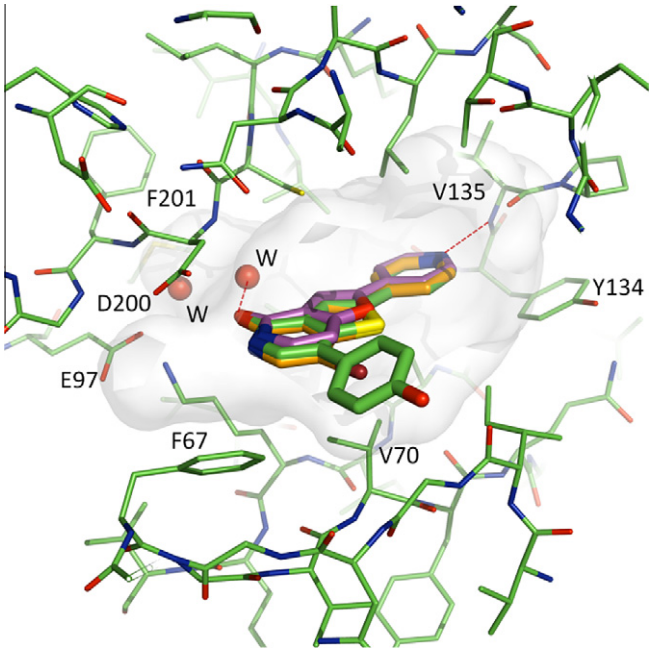

Figure 2. Superimposition of the three closely related compounds **11** (magenta), **12** (orange) and **13** (green), cocrystalized in the ATP binding pocket of the GSK-3β enzyme. Dotted lines indicated hydrogen bonds with V135 (kinase hinge region) and one of two water molecules situated in a deep interior pocket.

between the pyridyl nitrogen and the backbone NH atom of the V135 residue at the hinge region, a conserved motif in kinase inhibitors. However, the binding mode of this series is relatively unusual in that the hinge makes no other hydrogen-bonding interactions. The second hydrogen bond is formed between the pyridinone carbonyl moiety and two residual water molecules located at the back of the pocket. The water molecules form a small network and themselves hydrogen-bond to the backbone NH atoms of D200, F201, and the acidic sidechain of E97. The crystal structure is consistent with the SAR from the initial hits (Table 1), in which disruption of the hinge hydrogen bond to V135 by removing or moving the pyridyl nitrogen (**6**–**10**) leads to a large loss of potency.

The medicinal chemistry exploration of this series followed the evidence highlighted by the crystallographic data. Firstly, the 4-pyridyl hinge-binding group was exchanged for alternative *N*-containing heterocyclic-2-substituents. The synthetic approach applied for this exploration is described in Scheme 1. Knoevenagel condensation of 5-bromo-2-thiophenecarbaldehyde with malonic acid afforded the alpha–beta unsaturated carboxylic acid intermediate that was then cyclized into the pyridone via an acyl azide formation. Suzuki reaction was then applied to introduce the pyridine ring in the C-2 position. Bromination of the resulting intermediate gave the 7-bromo-thienopyridone derivative that was successfully used for a large aryls and heteroaryls exploration in the C-7 position, via Suzuki coupling. This synthetic route allowed the introduction of heterocycles in the C-2 position and aryls and heteroaryls in the C-7 position, proving to be very versatile for an exhaustive SAR expansion.

Compounds with replacement 2-hetercycles (Fig. 3) included **14**, in which a methyl substituent alpha to the pyridine nitrogen was introduced. Alternative *N*-containing heterocycles precedented as kinase hinge-binding groups such as the 7-aza-indole (**15**) and pyrazole (**16**) were also prepared. Potency and selectivity of compounds **14–16** towards GSK-3β and CDK-2 are listed in Table 3 compared with baseline compounds **1** and **5**.

This exploration confirmed that the unsubstituted 4-pyridine was the preferred heterocycle and that the introduction of a methyl group at the alpha position of the pyridine was not tolerated. This is consistent with the crystal structure (Fig. 2), which predicts that the methyl group would sterically prevent the close association between the pyridine and the hinge needed to form a hydrogen bond. Introduction of alternative heterocycles in the C-2 position (**15** and **16**) led to a slight increase in potency against GSK-3β, consistent with the donation of additional hydrogen-bonding interactions from the NH groups of these heterocycles to the hinge region. However, the hydrogen-bond accepting groups are highly conserved between most protein kinases, in view of which it is not surprising that the selectivity profile of these compounds worsened. For example, **16** gained appreciable CDK-2 activity compared to **5**. Because of this these compounds were not pursued for GSK-3β.