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2-Phenylamino-6-cyano-1*H*-benzimidazole-based isoform selective casein kinase 1 gamma (CK1γ) inhibitors

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ABSTRACT

Screening of the Amgen compound library led to the identification of 2-phenylamino-6-cyano-1*H*-benzimidazole **1a** as a potent CK1 gamma inhibitor with excellent kinase selectivity and unprecedented CK1 isoform selectivity. Further structure-based optimization of this series resulted in the discovery of **1h** which possessed good enzymatic and cellular potency, excellent CK1 isoform and kinase selectivity, and acceptable pharmacokinetic properties.

The casein kinase 1 (CK1) family of highly conserved serine/threonine protein kinases has six human isoforms (α, δ, ε, γ1, γ2 and γ3) that regulate several cellular growth and survival processes including Wnt signaling, cell cycle control, DNA repair, and apoptosis.[1] Several studies suggest that CK1 plays an important role in oncogenesis resulting from the deregulation of these cellular processes. CK1α is an essential component of the Wnt/β-catenin signaling pathway. It binds to axin, and phosphorylates β-catenin, ultimately leading to the degradation of β-catenin.[2] CK1δ and CK1ε, two closely related CK1 isoforms, have been shown to be necessary for proper activation of Wnt/β-catenin signaling[3] and have been implicated in the progression of colon, pancreatic, and breast cancer.[4] CK1γ is also a key regulator of the Wnt/β-catenin signaling pathway, coupling Wnt receptor activation to cytoplasmic signal transduction.[5] CK1γ phosphorylates the cytoplasmic domain of the Wnt co-receptor LRP5 and LRP6 upon Wnt ligand binding which leads to axin recruitment to the membrane and ultimately to the inhibition of β-catenin degradation and Wnt pathway activation. In addition, CK1γ2 has recently been discovered to inhibit TGF-β function through phosphorylation and subsequent degradation of activated SMAD3.[6]

Potent and specific small molecule modulators of CK1 family members are crucial tools to tease out their redundant versus distinct roles in vivo and to improve our current therapeutic strategies that target CK1 in human cancer. Pyrvinium, an FDA-approved anti-helminthic drug, was recently identified as a very potent and specific activator of CK1α that inhibits the Wnt/β-catenin pathway and decreases cell viability.[7] On the other hand, PF-670462, which inhibits CK1δ/ε with great potency and selectivity, displayed only modest effect on cancer cell survival despite being a potent inhibitor of Wnt signaling.[8] Potent and selective inhibitors of CK1γ, however, have not been reported to date. Herein, we present the discovery and optimization of benzimidazole compounds as potent and selective CK1γ inhibitors.

The benzimidazole compound **1a** (Fig. 1) was identified as a potent inhibitor of CK1γ from a high-throughput screen of the Amgen compound library. Compound **1a** showed good CK1γ potency (IC_{50} = 0.14 μM) and no inhibitory activity against an Ambit panel of 399 kinases (>50 POC at 1 μM)[9], including glycogen synthase kinase 3β (GSK3β) which downregulates the Wnt signaling pathway.[10] More importantly, compound **1a** demonstrated excellent selectivity over other CK1 isoforms such as CK1α and CK1δ. However, compound **1a** showed only modest potency in the LRP6 phosphorylation cell assay[11] and poor pharmaceutical properties (high metabolic clearance and low solubility).

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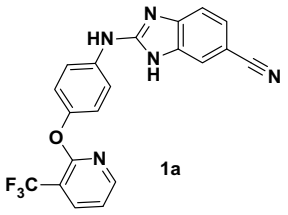


CK1γ IC_{50}: 0.14 μm
CK1α IC_{50}: 12.1 μm
CK1δ IC_{50}: 5.8 μm
GSK3β IC_{50}: 30.8 μm
PO_4-LRP6 IC_{50}: 6.6 μm

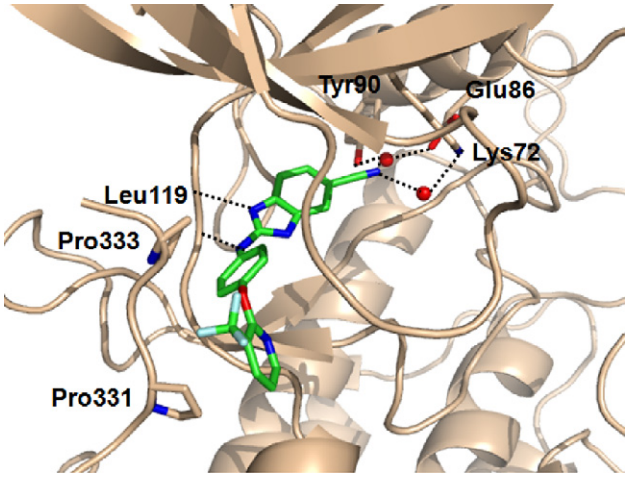
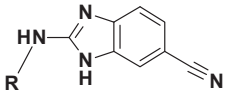


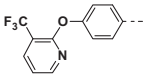
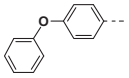
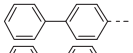
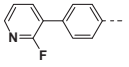
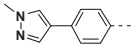
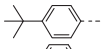
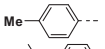
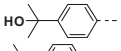
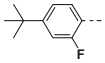
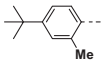
Figure 1. Selective CK1γ inhibitor **1a** and co-crystal structure with CK1γ3.

In order to understand the origin of its potency and selectivity, a co-crystal structure of **1a** with CK1γ3 was obtained. As shown in Figure 1,[12] the cyano benzimidazole moiety of **1a** binds deeply into the ATP binding pocket of CK1γ3 with the cyano group involved in a hydrogen bond interaction with the side chain of Lys72 through one water molecule and with the side chains of Glu86 and Tyr90 through another water molecule. The 2-amino-benzimidazole portion is hydrogen bonded to the main chain nitrogen and carbonyl oxygen of Leu119 from the linker of CK1γ3. The phenyl group is involved in a 'face-to-face' hydrophobic interaction with Pro333 while the pyridyl group is involved in an 'edge-to-face' hydrophobic interaction with Pro331. Both prolines are part of the tail in CK1γ3 immediately C-terminal to the kinase domain that comes close to the ATP binding site and are exclusively/uniquely conserved in the CK1γ isoforms (CK1γ1, CK1γ2, and CK1γ3). They cannot be found in other CK1 isoforms and kinases. Thus, it could be argued that the observed selectivity of compound **1a** against other CK1 isoforms and kinases could stem from its hydrophobic interaction with these two prolines.

The binding mode of **1a** with CK1γ3 led us to the initiate structure–activity relationship (SAR) studies focusing initially on the variation of the aniline moiety in order to improve both enzyme and cell potency while maintaining good CK1 isoform selectivity and GSK3β selectivity (Table 1).[13] With improved pharmaceutical properties also as a goal, the strategy was to target smaller and less lipophilic molecules. Ligand efficiency (LE)[14] and lipophilic efficiency (LipE)[15] were tracked to monitor the binding effectiveness

Table 1
Left-hand-side SAR of 2-phenylamino-6-cyano-1*H*-benzimidazoles



Compd	R	*c* Log *P*[a]	CK1γ[b] IC_{50} (μM)	LE[c]	LipE[d]	CK1α[b] IC_{50} (μM)	CK1δ[b] IC_{50} (μM)	GSK3β[e] IC_{50} (μM)	PO_4-LRP6[f] IC_{50} (μM)	Synthetic route[g]
1a		5.32	0.14	0.32	1.54	12.1	5.77	30.8	6.56	A
1b		5.78	0.27	0.36	0.79	8.82	3.17	ND	Und.	A
1c		5.57	0.099	0.40	1.43	5.49	2.18	30.8	4.89	A
1d		4.33	0.026	0.41	3.25	4.67	1.63	24.2	1.17	A
1e		3.70	0.046	0.42	3.64	3.54	1.08	Und.	1.45	A
1f		5.51	0.029	0.47	2.02	7.58	2.62	44.5	1.51	A
1g		4.19	0.060	0.52	3.04	19.7	3.61	30.1	4.54	A
1h		3.36	0.018	0.48	4.39	9.18	2.32	60.0	0.70	A
1i		5.69	0.079	0.42	1.41	Und.	2.18	Und.	10.1	B
1j		6.01	0.86	0.36	0.06	Und.	4.37	Und.	ND	B

ND = not determined.
Und. = undefined.
[a] Calculated logarithm of octanol/water distribution coefficient.
[b] Inhibition of kinase activity (Lance).
[c] LE (ligand efficiency) = −1.36 log K_i/N (*N* = number of non H atoms).
[d] LipE = pIC_{50} − *c* Log *P*.
[e] Inhibition of kinase activity (Alpha-Screen).
[f] Cell assay measuring phosphorylation of LRP6 in HEK293 cell.
[g] See Scheme 1 for detailed synthetic routes.