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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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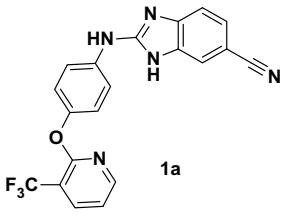


CK1γ IC_{50}: 0.14 μm
CK1α IC_{50}: 12.1 μm
CK1δ IC_{50}: 5.8 μm
GSK3β IC_{50}: 30.8 μm
PO_4-LRP6 IC_{50}: 6.6 μm

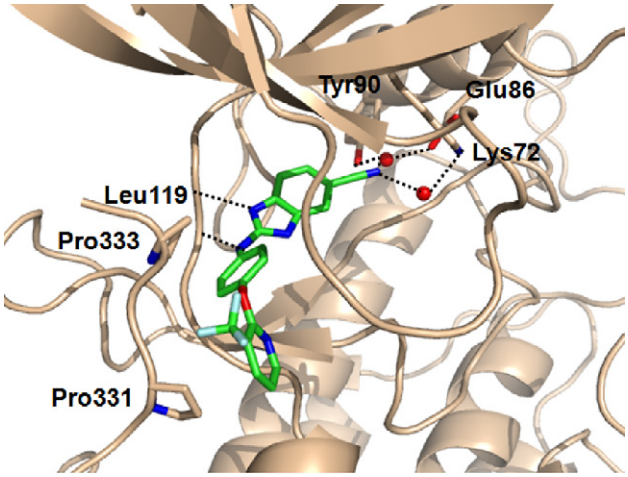
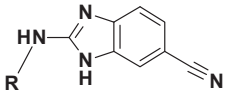


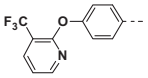
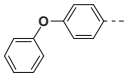
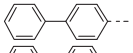
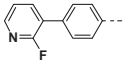
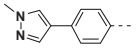
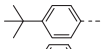
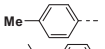
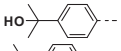
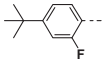
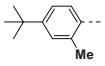
Figure 1. Selective CK1γ inhibitor **1a** and co-crystal structure with CK1γ3.

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.

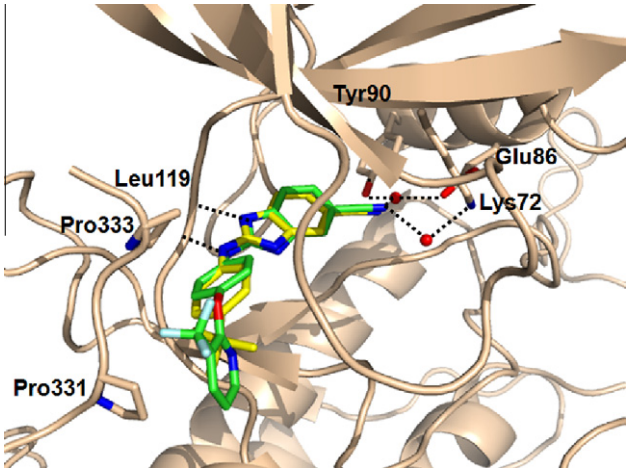


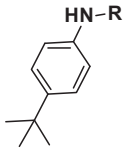
Figure 2. Overlay of the co-crystal structure of **1a** (green) and **1f** (yellow) with CK1γ3.

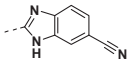
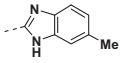
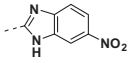
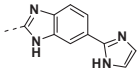
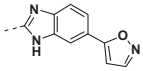
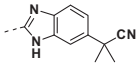
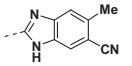
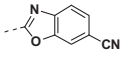
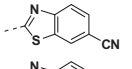
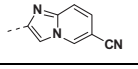
of molecules.[16] Replacing the trifluoromethylpyridine in **1a** (LE = 0.32, LipE = 1.54) with a phenyl ring (**1b**) resulted in a slight loss in both enzyme and cell activity. Further replacement of the diaryl ether with a diaryl moiety (**1c**–**1e**) led to improved enzyme and cell potency relative to **1a**, while maintaining good selectivity over CK1α, CK1δ and GSK3β. Among these inhibitors, **1d** with the lowest *c* Log P (3.70) demonstrated improved ligand efficiency (LE = 0.42) and lipophilic efficiency (LipE = 3.64) compared to **1a**. Finally, the diaryl-group was replaced by a *para*-substituted phenyl ring. Although compounds with a *tert*-butyl group (**1f**) or a methyl group (**1g**) in *para*-position showed similar Ck1γ potency as **1c**–**1e** and improved ligand efficiency, the lower LipE (2.02 for **1f**, 3.04 for **1g**) indicated that the gain in potency was not attractive relative to lipophilicity cost. Co-crystal structure of **1f** with CK1γ3 revealed that the *tert*-butyl phenyl moiety was engaged in hydrophobic interaction with Pro331 and Pro333 (Fig. 2).

Modulation of the physicochemical properties of **1f** by replacing one of the methyl groups with a hydroxyl group led to compound **1h** (*c* Log *P* = 3.36) demonstrating similar enzyme potency as **1f** and good selectivity over CK1α, CK1δ and GSK3β. More importantly, compound **1h** exhibited significantly improved lipophilic efficiency (LipE = 4.39) relative to **1f** and good cellular potency (IC_{50} = 0.70 μM). Substituents at the *ortho* position of the aniline, which would affect the 'face-to-face' hydrophobic interaction with Pro333 by distorting the orientation of the aniline, were expected to be detrimental to CK1 isoform selectivity and GSK3β selectivity. Introduction of a fluorine at this position (**1i**) resulted in a three-fold decrease of CK1γ potency relative to **1f** while maintaining similar potency on CK1α, CK1δ and GSK3β. A larger methyl group at this position (**1j**) further decreased CK1γ potency (IC_{50} = 0.86 μM).

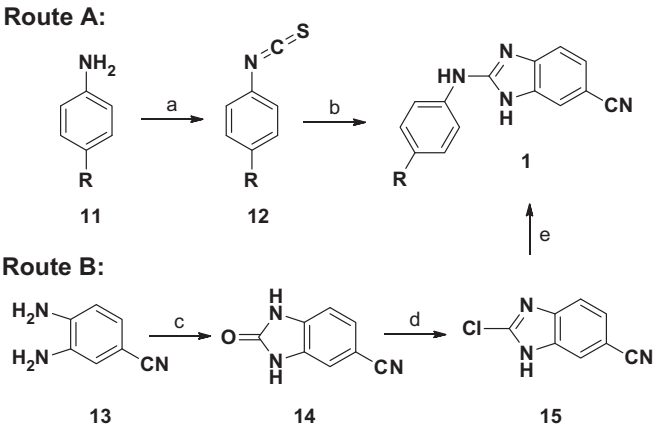
An additional SAR study was conducted by synthesizing compounds derived from **1f** with focus on the modification of the cyanobenzimidazole core (Table 2). Replacing the cyano group with a methyl group (**2**) eroded the activity and highlighted the importance of the water mediated hydrogen bond interaction between the cyano group and the side chains of Lys72, Glu86 and Tyr90. Further attempts to replace the cyano group and/or mimic its role, such as nitro **3**, imidazole **4**, isoxazole **5**, or isobutyronitrile **6**, all resulted in significant loss of CK1γ activity. Introduction of a methyl group next to the cyano group resulted in a five-fold less active compound (**7**) than **1f**. This is most likely due to a distortion of the conformation of the benzimidazole avoiding a steric clash of the methyl group with the gatekeeper Leu116 of CK1γ3. The

Table 2
Modifications of the cyanobenzimidazole core.



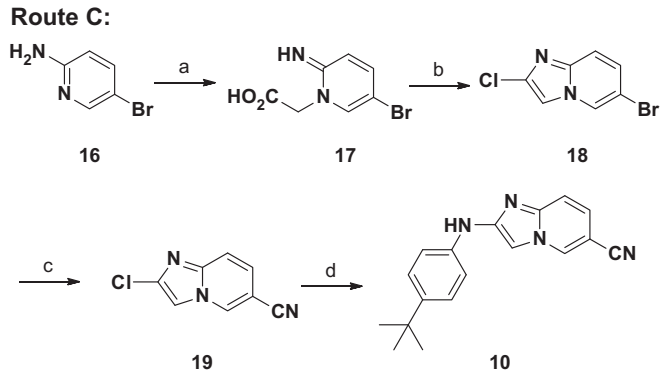
Compd	R	CK1γ[a] IC50 (μM)	CK1δ[a] IC50 (μM)	PO4-LRP[b] IC50 (μM)	Syn. route[c]
1f		0.029	2.62	1.51	A
2		5.80	Und.	Und.	B
3		0.50	2.88	2.34	A
4		8.52	Und.	Und.	A
5		0.42	Und.	Und.	A
6		0.53	Und.	Und.	A
7		0.14	3.97	13.0	A
8		0.18	2.37	14.9	B
9		0.039	1.66	3.83	B
10		0.033	3.17	9.10	C[d]

Und. = undefined.
[a] Inhibition of kinase activity (Lance).
[b] Cell assay measuring phosphorylation of LRP6 in HEK293 cell.
[c] See Scheme 1 for detailed synthetic routes.
[d] See Scheme 2 for detailed synthesis.



Scheme 1. Synthesis of benzimidazoles (**1–7**). Reagents and conditions: (a) thiophosgene, K_2CO_3, $CHCl_3$, room temperature; (b) 3,4-diamino benzonitrile, EDC, THF, 70 °C, 35–50% for two steps; (c) 1,1′-carbonyldiimidazole, CH_2Cl_2, room temperature; (d) $POCl_3$, DMF, 110 °C; (e) RNH_2, $MeSO_3H$, CH_3CN, microwave, 140 °C, 39–62%.

benzoxazole analog **8** was six-fold less active than **1f** but there was only a slight loss in CK1γ activity for the benzothiazole compound **9**. Imidazo[1,2–a]pyridine compound **10**, a regioisomer



Scheme 2. Synthesis of Imidazo[1,2-*a*]pyridine compound **10**. Reagents and conditions: (a) chloroacetic acid, TEA, CH_3CN, 85 °C, 53%; (b) $POCl_3$, CH_3CN, 85 °C, 60%; (c) $Pd(PPh_3)_4$, $Zn(CN)_2$, 90 °C, DMF, 77%; (d) $Pd_2(dba)_3$, Me_4t-BuXPhos, Cs_2CO_3, 150 °C, t-BuOH, 10%.

of benzimidazole **1f**, exhibited similar CK1γ activity but significantly decreased cell potency.

The isoform selective CK1γ inhibitor **1h** emerged from the SAR studies with promising cellular potency and lipophilic efficiency and was therefore selected for further characterization. Compound **1h** showed no inhibitory activity against an internal panel of 48 kinases (>50 POC at 1 μM) [9] and was stable in both rat and human liver microsomes (Cl_{int} <14 μL/min/mg). When dosed intravenously in rat (0.5 mg/kg) compound **1h** demonstrated moderate clearance (1.3 L/h/kg) and half-life (1.5 h).[17] However, high clearance (14.4 L/h/kg) and short half-life (0.6 h) were observed when dosed intravenously in mouse (1.5 mg/kg).

The benzimidazole derivatives **1–7** were synthesized using two different approaches shown in Scheme 1. Route A starts with the synthesis of arylisothiocyanates **12** prepared from the corresponding anilines **11** by reaction with thiophosgene in the presence of potassium carbonate. In a one-pot transformation, the crude arylisothiocyanates **12** were subsequently treated with 3,4-diaminobenzonitrile (**13**) in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) to deliver the desired benzimidazole derivatives **1–7** in yields ranging from 35% to 50% for two steps.[18] An alternative synthetic route B started with the conversion of 3,4-diaminobenzonitrile (**13**) to benzimidazolone **14** by using 1,1′-carbonyldiimidazole as carbonylating reagent. Chlorination of the benzimidazolone **14** with phosphorous oxychloride provided the necessary 2-choloro-1*H*-benzimidzaole intermediate **15**.[19] Finally, microwave assisted coupling of 2-choloro-1*H*-benzimidzaole **15** with desired anilines in the presence of methanesulfonic acid gave benzimidazole derivatives **1** in 39–62% yield. Amination of 2-chloro-6-cyanobenzoxazole and 2-chloro-6-cyanobenzothiazole with 4-(*tert*-butyl)aniline provided benzoxazole **8** and benzothiazole **9**, respectively (similar to route B for the synthesis of benzimidazoles).

The synthesis of imidazo[1,2-a]pyridine compound **10** is illustrated in Scheme 2. Reaction of 2-amino-5-bromo-pyridine **16** with chloroacetic acid in the presence of triethylamine provided iminopyridine acetic acid **17** in good yield. Conversion of **17** to desired 2-chloro-6-bromo-imidazolpyridine (**18**) was accomplished by treatment with phosphorous oxychloride in refluxing acetonitrile.[20] Selective Pd-catalyzed cynation of intermediate **18** afforded 2-chloro-6-cyano-imidazolpyridine **19**. Amination of **19** using palladium-catalyzed coupling with 4-(*tert*-butyl)aniline provided desired compound **10** in 10% unoptimized yield.

In summary, a class of 2-phenylamino-6-cyano-1*H*-benzimidazoles was discovered as potent CK1γ inhibitors with excellent broad kinase and CK1 isoform selectivity starting from a high-throughput screen. SAR efforts delivered compound **1h**, which displayed good cellular potency and modest pharmacokinetic properties in rodents. The structural and SAR information obtained from this study should provide useful insights into the future design of other isoform selective CK1γ inhibitors with requisite pharmacokinetic properties for pharmacodynamic studies.

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