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Development of highly selective casein kinase 1δ/1ε (CK1δ/ε) inhibitors with potent antiproliferative properties

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ABSTRACT

The development of a series of potent and highly selective casein kinase 1δ/ε (CK1δ/ε) inhibitors is described. Starting from a purine scaffold inhibitor (SR-653234) identified by high throughput screening, we developed a series of potent and highly kinase selective inhibitors, including SR-2890 and SR-3029, which have $IC_{50} \leqslant 50$ nM versus CK1δ. The two lead compounds have ⩽100 nM EC_{50} values in MTT assays against the human A375 melanoma cell line and have physical, in vitro and in vivo PK properties suitable for use in proof of principle animal xenograft studies against human cancer cell lines.

The casein kinase 1 (CK1) family of serine/threonine-specific kinases is comprised of seven members (α, β1, δ, ε, γ1, γ2 and γ3); each isoform has a preference for pre-phosphorylated substrates.[1] CK1 kinases regulate diverse processes including Wnt signaling,[2,3] membrane trafficking,[4] the actin cytoskeleton,[5] the DNA damage response,[6] and circadian rhythms.[7] Importantly, aberrant CK1δ and CK1ε activity is implicated in human pathologies, including neurodegenerative diseases, sleep disorders and cancer. CK1 kinases are ubiquitously expressed in the central nervous system and CK1δ is thought to play roles in dopamine signaling, neurotransmitter release and the phosphorylation of neurotransmitter receptors.[8,9] Further, CK1δ expression is elevated in Alzheimer's disease tissue and CK1δ phosphorylates tau, which initiates microtubule destabilization and neuronal cell death.[5,10] These kinases may also play roles in cleavage of the amyloid precursor protein (APP),[9] as CK1 inhibitors disrupt APP cleavage and a constitutively active form of CK1ε augments APP peptide production.[9,11] Finally, the up-regulation of CK1 isoforms in Alzheimer's patients makes CK1 an attractive target for the treatment of Alzheimer's disease.[9]

Casein kinases 1δ and 1ε are highly expressed in some cancers and appear to control tumor cell growth, apoptosis, metabolism and differentiation.[10,12] For example, forced expression of kinase-impaired mutants of CK1δ blocks SV40-induced cell transformation and mammary carcinogenesis in vivo.[13] Further, CK1ε is required for the survival of breast cancer subtypes that rely on aberrant β-catenin activity, and active, myristoylated CK1ε is sufficient to provoke transformation via stabilization of β-catenin and activation of Wnt transcription targets.[14] CK1δ/ε-directed stabilization of β-catenin may occur via CK1δ/ε-directed phosphorylation of lipoprotein receptor-related protein 5/6 (Lrp5/6) and/or dishevelled (dvl/dsh).[15–18] CK1δ and CK1ε also play roles in ovarian cancer[19] and pancreatic adenocarcinoma.[20]

These important biological roles have stimulated considerable effort to develop CK1δ/ε inhibitors.[10,21–24] Included among the many small molecule inhibitors of CK1δ that have been reported are CKI-7,[25] D4476,[26,27] IC261,[28] (R)-DRF053,[22] Bischof-5[24] (compound **5** in Ref. 24) and PF-670462 (see Fig. 1).[29,30] CKI-7 is a 6 μM CK1 inhibitor, but does not readily pass cell membranes.[25,26] IC261, D4476 and (R)-DRF053 are cell-permeable yet have limitations. Specifically, D4476 is a 0.3 μM CK1 inhibitor in vitro,[26] has low activity (20–50 μM) in cell-based assays,[27,29] and also inhibits p38α, raising concerns regarding off-target effects.[10,26,27] Further,

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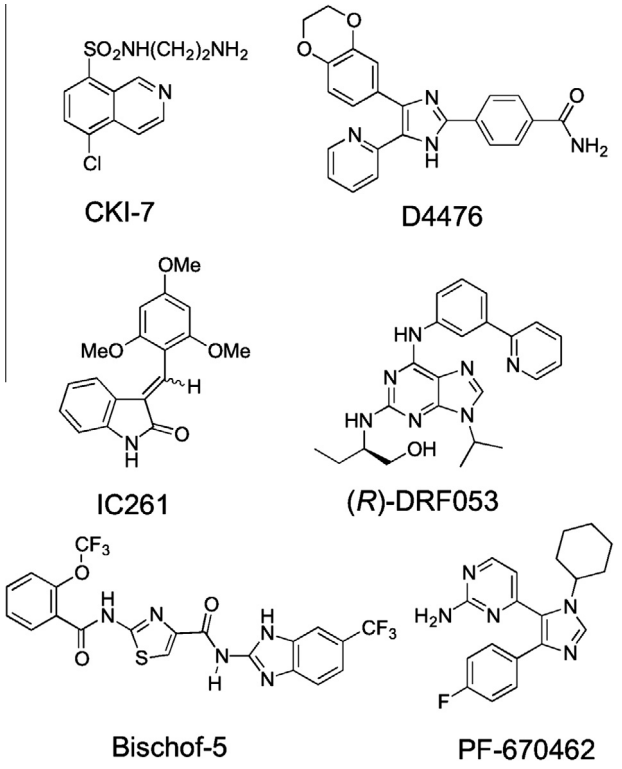
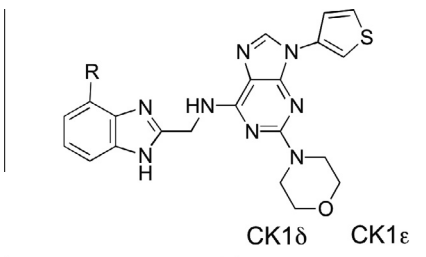


Figure 1. Representative CK1δ/ε inhibitors.

the IC_{50} of IC261 is only 1 μM for CK1 inhibition in vitro and 25 μM in cells,[10] and there are off target effects as IC261 binds to tubulin and inhibits microtubule polymerization.[28] Moreover, (R)-DRF053 is a potent, dual CK1/CDK inhibitor (14 nM vs CK1), yet only exhibits weak (EC_{50} 17.2 μM) antiproliferative activity against human neuroblastoma SH-SY5Y cells. Bischof-5 is yet another potent (48 nM) CK1δ inhibitor, but is also weakly active in cells, likely due to poor cell penetration.[29] Finally, PF-670462 is a 14 nM inhibitor of CK1δ in vitro and was initially reported to be highly selective, at least among the 45 kinases tested.[29] Subsequent studies showed that PF-670462 also potently inhibits p38 and EGFR.[30] Both PF-670462 and PF-4800567 (Pfizer's CK1ε inhibitor)[30] lack anti-cancer activity.[28]

A high-throughput screening (HTS) campaign under the auspices of the MLPCN program at Scripps Florida, targeting inhibitors of Wee1 degradation,[31] identified SR-653234 as a promising hit. Extensive mechanistic and biochemical profiling studies demonstrated that SR-653234 and especially its analog SR-1277 (Fig. 2) are highly selective CK1δ/ε inhibitors and that CK1δ plays a crucial role in regulating the activity of Wee1 at the G2/M cell cycle



	CK1δ	CK1ε
SR-653234 (R = H)	160 nM	540 nM
SR-1277 (R = NO_2)	49 nM	260 nM

Figure 2. CK1δ/ε inhibition data for SR-1277 and SR-653234.

interface.[11] These efforts led to SR-1277 being designated as Probe ML177 in the MLPCN system.[32] However, SR-1277 has poor solubility, sub-optimal PK properties and metabolic liabilities due to the thiophene unit and especially the aryl nitro substituent.[33,34] Therefore, we have performed and report herein additional SAR studies that led to the identification of several analogs (including SR-2890 and SR-3029) that are appropriate for progression into murine xenograft studies against human cancers.

We adopted the general procedure published by Schultz for synthesis of analogs of SR-653234 and SR-1277.[35–37] As depicted in Figure 3 for the synthesis of SR-653234, the *N*-thienyl intermediate **2** was accessed via a Chan–Lam coupling reaction of commercially available dichloropurine **1** and 3-thienylboronic acid.[36,38,39] A one-pot double nucleophilic substitution sequence then converted intermediate **2** into the targeted CK1δ/ε inhibitor. The regioselectivity of the latter sequence is excellent, with the first amine nucleophile adding to C(6) of the purine scaffold as has been demonstrated previously.[35–37]

The substituted 2-(aminomethyl)benzimidazoles (**6**) used in this study that are not commercially available were synthesized as summarized in Figure 4. Thus, a substituted phenylenediamine **3** (prepared by reduction of the corresponding *ortho*-nitroaniline,[40] if not commercially available) was coupled to *N*-Boc-glycine using EDC and HOBt as the coupling reagents to give a mixture of **4a** and **4b**. The mixture of these two amides was heated at 80 °C in acetic acid to effect cyclization to the *N*-Boc protected benzimidazole **5**. Finally, the Boc group was removed by treatment of **5** with a mixture of HCl (12 N in water) and dioxane at room temperature overnight. The product **6** was obtained as the HCl salt by precipitation from diethyl ether. This three-step procedure usually did not require any chromatographic purification steps, and provided the substituted benzimidazoles **6** (with a range of substituents corresponding to those in the inhibitors presented in Tables 1–3) having acceptable purity for use directly in the synthesis of the targeted CK1δ/ε inhibitors according to the procedure summarized in Figure 3.

Using this chemistry, we synthesized a series of analogs of SR-653234 with a range of substituents in the benzimidazole ring to probe the effect of this substitution on inhibitor activity. Substitution of the benzimidazole ring in either position 4 (R^1) or position 5 (R^2) led to an increase of CK1δ inhibition compared to the unsubstituted parent compound SR-653234 (Table 1). A trifluoromethyl group at R^1 modestly enhanced CK1δ inhibition (compare entries 1 and 2) while nitro and methanesulfonyl substituents at this position led to significantly more active analogs SR-1277 and SR-2805 (entries 7 and 11). Improvements of CK1δ inhibitor activity were also achieved by incorporating a range of substituents at R^2. Substitution with a trifluoromethyl group (SR-1273, entry 3), a nitro group (SR-1278, entry 8), a cyano group (SR-1276, entry 6), a methoxy group (SR-1279, entry 9) or a methanesulfonyl group (SR-2797, entry 10) led to significant improvement of CK1δ inhibitor activity.

A thiophene substituent, especially when not substituted at positions 2 and/or 4, is generally considered to be a liability in view of the potential for production of highly reactive metabolites.[41,42] To avoid this potential problem, we sought other groups that could be used at the purine 9-position (R^3) without significant loss of CK1δ inhibitory activity (Table 2).[43] Replacement of the thiophene ring of SR-653234 by a cyclopentyl group led to a more potent inhibitor, SR-2149 (entry 1). Although the furan-containing analogs SR-2850 and SR-2007 had excellent potency, the furan ring is also a known metabolic liability, especially when not substituted at positions 2 and 4.[43] On the other hand, several inhibitors bearing fluoro-substituted phenyl rings at position R^3 had very interesting properties. As depicted by the results in entries 4–7 of Table 2, the position of the fluorine substituent dramatically influenced the