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Discovery of potent and selective inhibitors of calmodulin-dependent kinase II (CaMKII)

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ABSTRACT

We hereby disclose the discovery of inhibitors of CaMKII (**7h** and **7i**) that are highly potent in rat ventricular myocytes, selective against hERG and other off-target kinases, while possessing good CaMKII tissue isoform selectivity (cardiac γ/δ vs. neuronal α/β). *In vitro* and *in vivo* ADME/PK studies demonstrated the suitability of these CaMKII inhibitors for PO (**7h** rat F = 73%) and IV pharmacological studies.

Calmodulin-dependent protein kinase II (CaMKII) is a serine-threonine kinase activated by the Ca^{2+}/calmodulin complex binding to its regulatory domain.[1] It has been shown to be a major regulator of intracellular Ca^{2+} levels in many cell types, including cardiomyocytes. L-type Ca^{2+} channels, Na^{+} channels ($Na_v1.5$), and Ryanodine receptors are among its substrates.[2,3] A Pubmed search for CaMKII and heart disease revealed 449 citations, and the number keeps growing every year.[4] Knock-out mouse studies and pharmacological inhibition of CaMKII resulted in protection against structural heart disease and arrhythmias.[5]

The field of CaMKII research is dominated by use of KN-92/KN-93 as the main small-molecule functional inhibitor tools in spite of their poorly understood mechanism of action.[6] Small molecules reported to date possess biochemical potency but lack either selectivity[7,8] or cellular potency[9,10] that limits their use as pharmacological tools.

Because of our long-standing interest in cardiovascular drug discovery and more specifically in cardiac channelopathies,[11–14] we set out to discover our own tool compounds with the potential to address important underserved medical needs.

We began our project with a high-throughput screen (HTS) of our small-molecule library using biochemical inhibition assay[15] for CaMKIIδ, the heart's most abundant CaMKII isoform, that resulted in a family of hits each containing an imidazo[1,2-*b*]pyridazine central core. Compound **1** (Table 1) was the most potent compound in that family, and was used as a starting point for the initial hit expansion.

An X-ray structure of CamKIIδ co-crystallized with **1** revealed the inhibitor was bound in the ATP pocket (Fig. 1). The imidazo[1,2-*b*]pyridazine core (B/C rings) is a single hinge binder to Val93-NH, with the 2-thiophene (A ring) forming a stacking interaction with the gatekeeper residue, Phe90. The asymmetric unit cell contains two CaMKIIδ/compound **1** complexes. In the chain A complex, the basic amine attached at the *meta* position of the phenyl D ring is directed out of the pocket toward solvent. In the chain B complex, the basic amine is directed into the pocket toward Asn141 and Asp157.

Hit to lead optimization began with the amine portion of the molecule as it was perfectly positioned for focused library synthesis. We determined that a basic amine is required as alkyl- and

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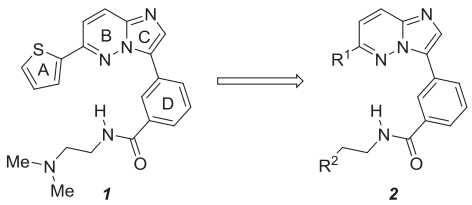
Table 1
HTS hit and the initial hit expansion.



	R^1	R^2	IC_{50}, nM
1			37
2a	2-Th	Et	4436
2b	2-Th	OH	454
2c	2-Th	NEt_2	5.8
2d	2-Th	N*i*-Pr_2	41
2e	2-Th	*N*-Morpholinyl	474
2f	2-Th	*N*-Pyrrolidinyl	191
2g	2-Th	*N*-Piperazinyl	325
2h	3-Th	NMe_2	65
2i		NMe_2	57
2j	Ph	NMe_2	245
2k	Ph	NEt_2	44

hydroxyl-replacements are substantially less active (**2a**–**b**). The replacement of dimethyl- with a diethyl-substituent led to 6-fold boost in potency (**2c**). Further bulking up the amine (**2d**) has proven unproductive. Cyclization attempts (**2e**–**g**) led to precipitous drop in potency.

Next, we attempted to replace 2-thiophene with another aryl or heteroaryl substituent, as well as some non-cyclic alkyl and amino-substituents. We found out that only the most lipophilic 5- and 6-membered heterocycles were tolerated, with 2-thienyl being the optimal substituent. 3-Thienyl (**2h**) and 1*H*-pyrrol-1-yl (**2i**) substituents were found to be slightly less potent (~2-fold). The unsubstituted phenyl (**2j**–**k**) was the most potent among 6-member substituents. It is worth mentioning that the 1*H*-pyrrol-1-yl substituent with its nitrogen electron pair masked by aromatic conjugation was the only nitrogen-containing heterocyclic Ring A of any significance to this project.

Using the 2-thienyl A-ring and the optimized *N*,*N*-diethylethylenediamine amide as placeholders we turned our attention to B/C and D ring systems. In search of potential imidazo[1,2-*b*]pyridazine B/C ring replacements we came across only one potentially useful replacement – pyrazolo[1,5-*a*]pyrimidine – an isomeric structure that is different only in the location of the bridge-head nitrogen (Table 2).

Compound **3a** was more potent than **2c** by approximately 1.5-fold. Introduction of nitrogen into the D-ring (**3b**, 2-pyridyl vs. phenyl) provided an additional 2-fold potency boost. Testing the original imidazo[1,2-*b*]pyridazine B/C scaffold in conjunction with the new 2-pyridyl D-ring as well as other electron-deficient heterocycles has proven difficult from the synthetic point of view. This, along with a slight potency edge, has influenced our choice of the pyrazolo[1,5-*a*]pyrimidine B/C ring system as the scaffold for the future optimization.

As the X-ray structure of CaMKIIδ with **1** revealed two conformations of the D-ring and basic amine, we speculated that the replacement of the phenyl D-ring's C—H bond in the *ortho*-position with the pyridine's nitrogen heavily favoured the conformation which directed the amine into the pocket, providing a 2-fold boost in potency. This conformational manipulation could also be

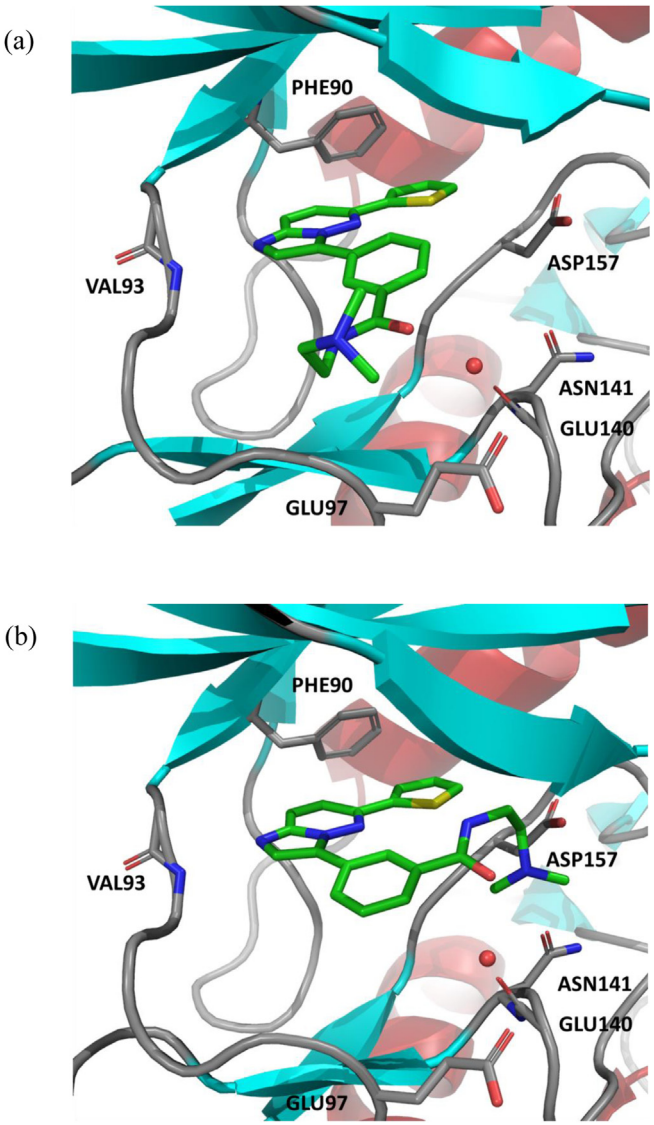


Fig. 1. X-ray structure of CaMKIIδ co-crystallized with compound **1**. The asymmetric unit contains two protein/ligand complexes with different conformations of the inhibitor. Chain A (a) has the inhibitor amine directed out toward solvent. Chain B (b) has the amine directed into the ATP pocket. PDB Accession code 6AYW.

achieved by expanding the D-ring to a bicyclic. Importantly, a key driver of potency in these bicyclics was the maintenance of near planarity between the B/C and D rings. This was determined by the interaction between the D-ring and the C-ring's 2-H. *N*-methylbenzimidazole (**3c**) and imidazo[1,2-*a*]pyridine (**3d**), which place the lone pair of a nitrogen in proximity to the C-ring's 2-H, were relatively potent and determined by modelling to be nearly planar. In contrast, imidazo[1,2-*a*]pyridine (**3e**), which introduces a clash between a C—H and the C-ring's 2-H, was ~100-fold less potent. This confirmed the importance of planarity and helped solidify 2-Py as the D-ring of choice.

We previously established that basic amine is important for potency, and wanted to further explore whether ethylene diamine fragment could be further optimized (Table 3).

Methyl scan of the linker (**4a**–**e**) revealed that one substitution (R^1 = (*S*)-Me, **4b**) provided a nice boost in potency (~18-fold) while all other substitutions were found to be equipotent at best. We hypothesized that (*S*)-Me group helps remove the free energy penalty needed to pay for pre-organizing the molecule in *gauche* conformation necessary for the ionic interaction of the basic amine