# IMINORIBITOL-MODIFIED 3,4-DIARYL-ISOXAZOLES AS POTENT INHIBITORS of CASEIN KINASES (CK) 1δ/ε

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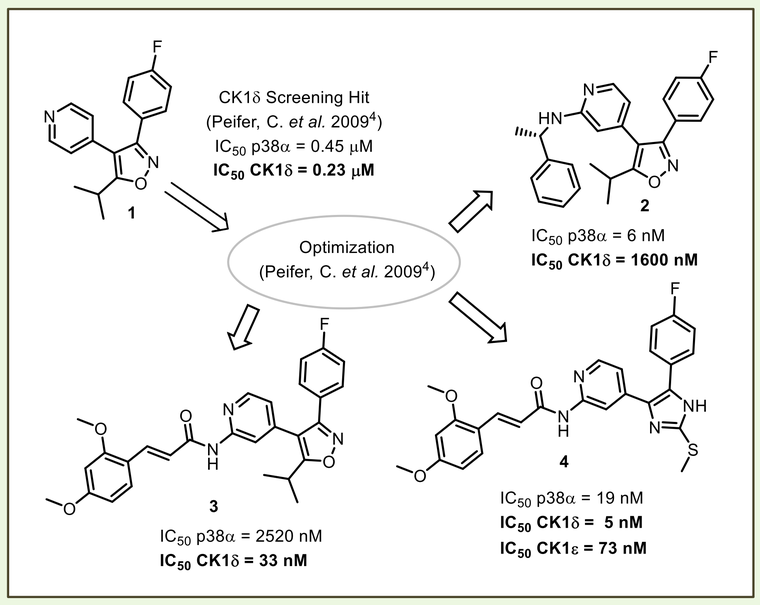
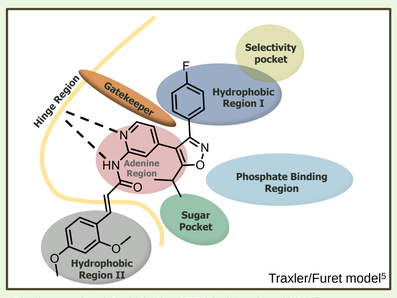
## Introduction

Casein kinases (CK) are serine-threonine protein kinases that are conserved in eukaryotic organisms.[1] They phosphorylate a high number of substrates and play important roles in the regulation of multiple cellular processes, such as cell division, apoptosis, membrane transport, immune response, inflammation, DNA repair, circadian rhythm, Wnt signalling, etc.[1] Deregulation and dysfunction of CK-isoforms including CK1δ/ε have been associated with proliferative disorders, such as cancer and neurodegenerative diseases (Alzheimer's or Parkinson's disease), as well as sleeping disorders.[1-2] Therefore, inhibition of CK1-isoforms has become an attractive target for therapeutic application.

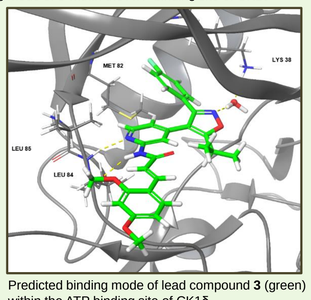
Highly desirable are isoform-selective kinase inhibitors because these will lead to reduced toxicity and improved efficacy, but creating such inhibitors is very challenging as kinase active sites are very similar. However, building inhibitors from scaffolds with inherent chirality may confer otherwise unachievable binding selectivity toward the highly homologous kinase domains.[3]

Here we present the modification of the previously reported 3,4-diaryl-isoxazole-based CK1 inhibitor **3**[4] with enantiopure iminoribitol scaffolds to enhance its activity and selectivity. Guided by molecular modelling the pharmacophore of the lead inhibitor **3** was extended to the more hydrophilic areas of the ATP binding site to enable selective interactions.



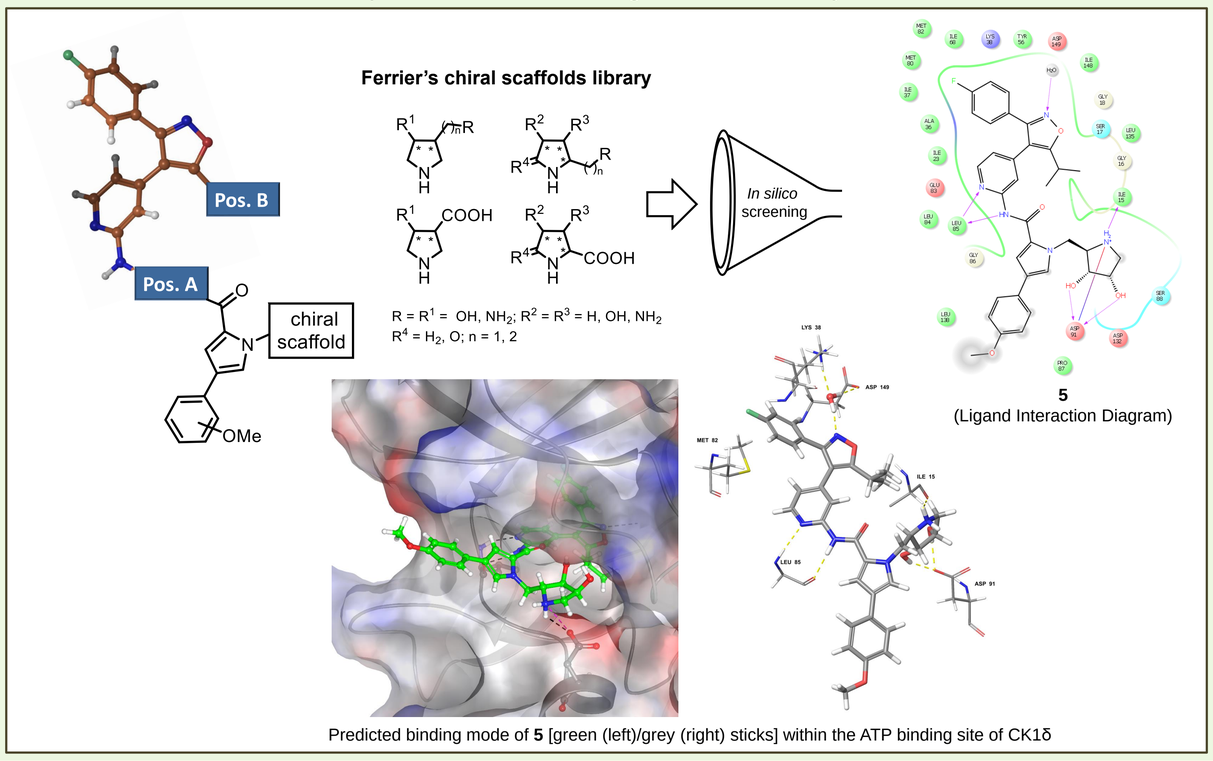

Binding mode of **3** within the ATP binding site of CK1δ




Predicted binding mode of lead compound **3** (green) within the ATP binding site of CK1δ

## Computational Inhibitor Design

To further optimize the diary-isoxazole pharmacophore towards selective binding interactions in the ribose pocket we used a molecular modelling-based design approach. Thus, chiral pyrrolidine scaffolds, prepared at the Ferrier Research Institute (FRI), were either attached directly to Pos. B, maintaining the (*E*)-3-(2,4-dimethoxyphenyl)acryl amide side chain for interaction with the hydrophobic region II, or to Pos. A *via* an aryl-substituted pyrrole building block resembling the original side chain. *In silico* calculations then identified compound **5** as the first target of choice for synthesis.

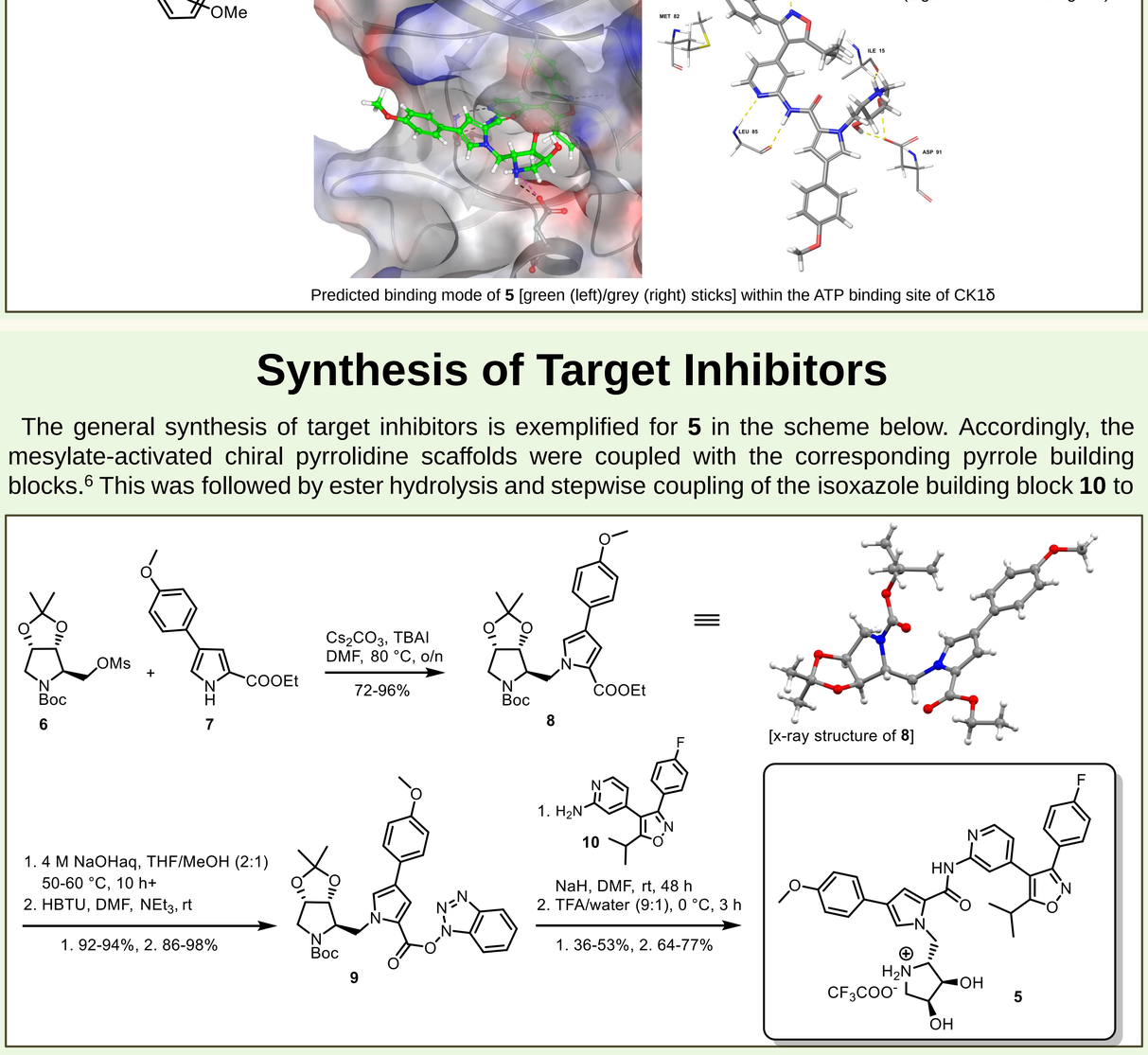



Predicted binding mode of **5** [green (left)/grey (right) sticks] within the ATP binding site of CK1δ

## Synthesis of Target Inhibitors

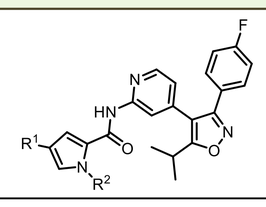
The general synthesis of target inhibitors is exemplified for **5** in the scheme below. Accordingly, the mesylate-activated chiral pyrrolidine scaffolds were coupled with the corresponding pyrrole building blocks.[6] This was followed by ester hydrolysis and stepwise coupling of the isoxazole building block **10** to the resulting carboxylic acids *via* their HOBt active esters. Finally, protecting groups were cleaved under acidic reaction conditions to yield the desired inhibitors (**5**/**14**-**15**).[6]

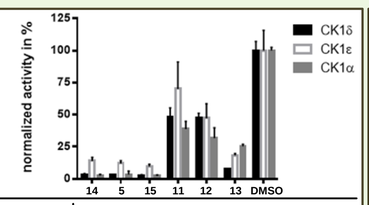
Inhibitor **13** with omitted methoxyphenyl substituent was prepared in analogous fashion except that methyl 1*H*-pyrrole-2-carboxylate was used as the starting material. Inhibitors in which the iminosugar unit was replaced with a methyl group (**11**-**12**) were prepared by initial methylation of the pyrrole building blocks under standard reaction conditions followed by subsequent ester hydrolysis and coupling with **10** as outlined. The required enantiomerically pure iminoribitol building blocks were prepared in 10 steps from D- or L-methionine employing established procedures.[6]

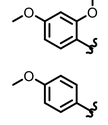
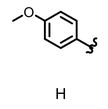
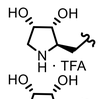
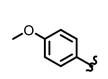
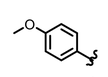
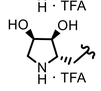
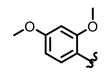
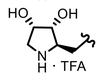


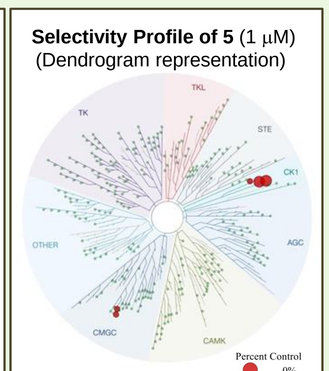

## Biological Testing

Biological evaluation of **5**/**11-15** revealed **5** and **14** as the most potent inhibitors against CK1δ/ε with $IC_{50}$ values in the nanomolar range.[6] Although consistent with our initial *in silico* predictions, the chirality of the pyrrolidine scaffolds did not impact the test results – no preference towards affinity and/or CK1 isoform-selectivity was observed. Omitting the methoxyphenyl substituent or replacing the pyrrolidine unit with a methyl reduced or ablated compounds' activities. Selectivity profiling of compound **5** in a panel of 320 kinases at a concentration of 1 μM showed that its activity was relatively specific for CK1.[6]






| | R1 | R2 | CK1δ Residual Activity [%] at 10 μM ± SD | CK1δ $IC_{50}$ [μM] ± SD | CK1ε Residual Activity [%] at 10 μM ± SD | CK1ε $IC_{50}$ [μM] ± SD |
|---|---|---|---|---|---|---|
| **11** |  | Me | 48 ± 7 | n.d. | 70 ± 21 | n.d. |
| **12** |  | Me | 48 ± 4 | n.d. | 47 ± 11 | n.d. |
| **13** | H |  | 7.7 ± 0.2 | 0.45 ± 0.07 | 18 ± 1 | 1.5 ± 0.3 |
| **5** |  |  | 2.9 ± 0.3 | 0.038 ± 0.003 | 12 ± 2 | 0.065 ± 0.012 |
| **14** |  |  | 3.4 ± 0.7 | 0.047 ± 0.04 | 14 ± 3 | 0.063 ± 0.009 |
| **15** |  |  | 2.8 ± 0.5 | 0.037 ± 0.004 | 9.7 ± 1.6 | 0.1 ± 0.014 |

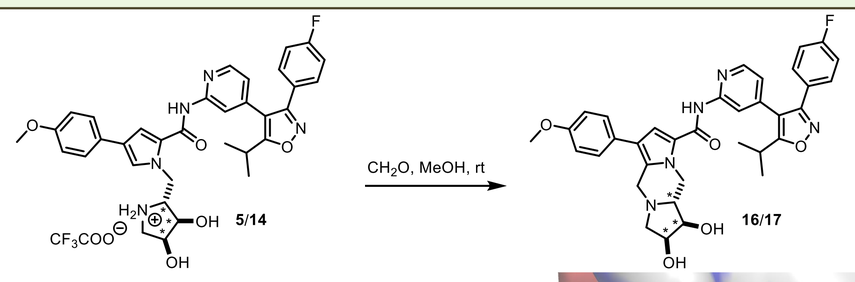



| Kinase | Residual Activity [%] |
|---|---|
| CK1δ | 1 |
| CK1ε | 4 |
| JNK3 | 15 |
| CK1α | 16 |
| p38α | 17 |
| JNK2 | 40 |

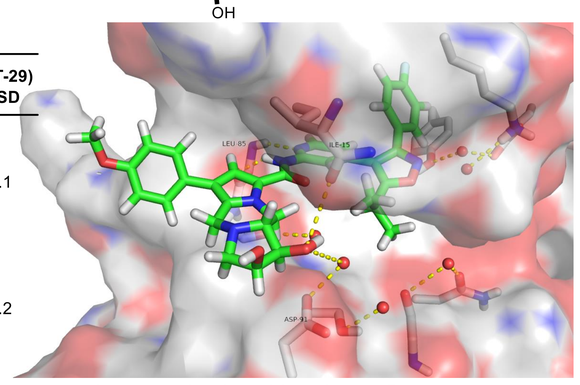
Selectivity Score of **5**: 0.02

## X-Ray Analysis of Ligand-CK1δ Co-Crystals

To our surprise, X-ray crystallographic analysis of ligand-CK1δ co-crystals, to corroborate the predicted binding mode of our target inhibitors **5** and **14**, uncovered the presence of new ligands (**16** and **17**).[6] These new ligands were formed by spontaneous Pictet-Spengler cyclization[7] during co-crystallisation experiments with traces of formaldehyde. Further experiments identified PEG[8] as the likely source of formaldehyde. The two ligands (**16** and **17**) were re-synthesised and tested for their biological activities against CK1δ/ε. Both exhibited nanomolar activity in *in-vitro* kinase assays and were found to be more potent than the originally designed inhibitors **5** and **14**.




| Ligand | $IC_{50}$ (CK1δ) [μM] ± SD | $IC_{50}$ (CK1ε) [μM] ± SD | $EC_{50}$ (MCF-7) [μM] ± SD | $EC_{50}$ (HT-29) [μM] ± SD |
|---|---|---|---|---|
| **16** | 0.011 ± 0.001 | 0.056 ± 0.005 | 1.3 ± 0.2 | 1.1 ± 0.1 |
| **17** | 0.024 ± 0.004 | 0.196 ± 0.026 | 1.9 ± 0.3 | 1.4 ± 0.2 |




Co-crystal structure of **16** (green) bound to CK1δ

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