

Selective Antiplatelet Action Through Targeted Covalent Inhibition of Pyk2 at a Paralog-Specific Cysteine

Kayla Williams

University of Sydney

Abstract

Platelet-driven thrombosis underlies major cardiovascular disorders including ischemic stroke and myocardial infarction.¹ Although antiplatelet agents are widely used to prevent thrombus formation, their clinical utility is often limited by an increased risk of bleeding. The non-receptor tyrosine kinase Pyk2 (FAK2) has emerged as an attractive therapeutic target because genetic and pharmacological studies suggest that inhibition of Pyk2 suppresses thrombosis while largely preserving physiological haemostasis.^{2,3} However, the development of selective Pyk2 inhibitors has been hindered by the high degree of homology shared with its paralogue, FAK1.⁴

Here, we report the structure-guided development of the first covalent Pyk2 inhibitor that exploits a unique cysteine residue (Cys545) located adjacent to the ATP-binding pocket and absent in FAK1. Starting from the reversible inhibitor PF-4594755, we incorporated electrophilic warheads and rationally designed linker architectures to enable selective covalent engagement of Cys545. Screening of a focused library identified compound 2-1 as a potent lead, with intracellular target engagement confirmed using washout-based NanoBRET assays. Mutation of Cys545 abolished irreversible inhibition, validating the proposed covalent mechanism. Chemoproteomic profiling using an alkyne-tagged analogue further demonstrated excellent target selectivity across the cellular proteome.

Functionally, compound 2-1 produced sustained inhibition of platelet activation, strongly suppressing thromboxane-dependent aggregation while having only modest effects on ADP- and thrombin-mediated responses. The compound reduced thrombus formation under physiologically relevant arterial and venous flow conditions and significantly enhanced recombinant tissue plasminogen activator-mediated thrombolysis in a murine thrombosis model without increasing bleeding. These findings establish Cys545 as a tractable paralog-selective cysteine for covalent kinase inhibitor design and highlight targeted covalent inhibition of Pyk2 as a promising strategy for the development of next-generation antithrombotic therapies with improved safety profiles.

[1] M. Koupenova, et al, "Thrombosis and Platelets: An Update", *Eur. Heart J.* 2017, 38, 785-791.

[2] I. Canobbio, et al, "Impaired Thrombin-Induced Platelet Activation and Thrombus Formation in Mice Lacking the Ca²⁺-Dependent Tyrosine Kinase Pyk2", *Blood* 2013, 121, 648-657.

[3] G. F. Guidetti, et al, "Focal Adhesion Kinases in Platelet Function and Thrombosis", *Arterioscler. Thromb. Vasc. Biol.* 2019, 39, 857-868.

[4] C. A. Lipinski, et al, "Differential Role of Proline-Rich Tyrosine Kinase 2 and Focal Adhesion Kinase in Determining Glioblastoma Migration and Proliferation", *Mol. Cancer Res.* 2003, 1, 323-332.

Biography

Dr Kayla Williams, is an Associate Lecturer in the School of Pharmacy at the University of Sydney. She completed her PhD in Chemistry under the supervision of Dr Xuyu Liu, where her research focused on precision medicine approaches for the modulation of a variety of target proteins. Her research lies at the interface of medicinal chemistry and chemical biology, with particular interests in the design of selective small-molecule modulators, covalent inhibitors, and targeted protein degradation strategies, including PROTACs. Her work aims to develop innovative therapeutic approaches to better understand and selectively manipulate disease-relevant proteins. In addition to her research, Kayla teaches medicinal chemistry and pharmaceutical sciences at the University of Sydney.

