Structural and Mutational Investigation of Human Hematopoietic Prostaglandin D_2 Synthase and Inhibitors

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KEY FINDING

Open *versus* closed pocket: Two conformations for inhibitor-bound H-PGDS were identified for the first time.

ABSTRACT

Human hematopoietic prostaglandin D_2 synthase (H-PGDS) plays a role in both allergic and inflammatory diseases such as asthmatic anaphylaxis and mastocytosis, making it a potential target for selective inhibitor development. Herein we describe our continued investigation of the structural characteristics and distinct binding mechanisms of H-PGDS with inhibitors. This study aims to evaluate a SAR series of inhibitors containing amide or imidazole linkers in parallel with mutational analysis of H-PGDS through thermal shift assay (TSA), X-ray crystallography, and surface plasmon resonance (SPR) experiments. Additionally, identification of H-PGDS key interactions and conformational changes related to the reduced glutathione (GSH) cofactor in the active site as well as the first crystal structure of H-PGDS with the natural substrate provide further insight into the design of human H-PGDS inhibitors.

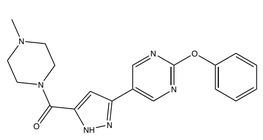
OBJECTIVE

To investigate two structural conformations (open *versus* closed pocket), we performed a series of mutational studies using K50A, Q36A, and Q36A:K50A mutants through:

- Glutathione S-transferase (GST) activity assay
- Thermal shift assay (TSA)
- Single-cycle kinetics surface plasmon resonance (SPR)
- Crystallographic studies with the natural substrate and proposed inhibitors with and without glutathione (GSH)

All methods are described in detail in the manuscript (in preparation).

COMPOUNDS REFERENCED:



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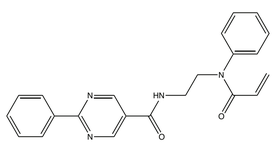

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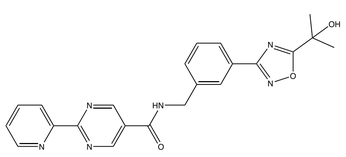

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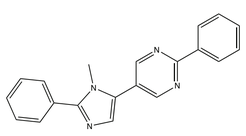

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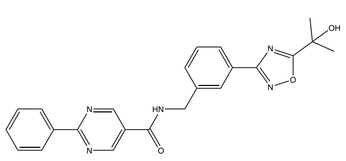

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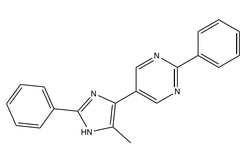

KMN-872-42

KMN-010034

RESULTS

GST Activity Assay Suggests Different Mode of Inhibition for Imidazole *versus* Amide Series

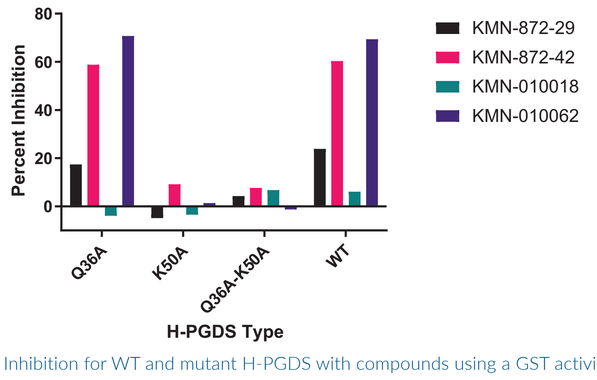

FIGURE 1 – Inhibition for WT and mutant H-PGDS with compounds using a GST activity assay.

TSA Suggests Different Mode of Inhibition for Imidazole *versus* Amide Series

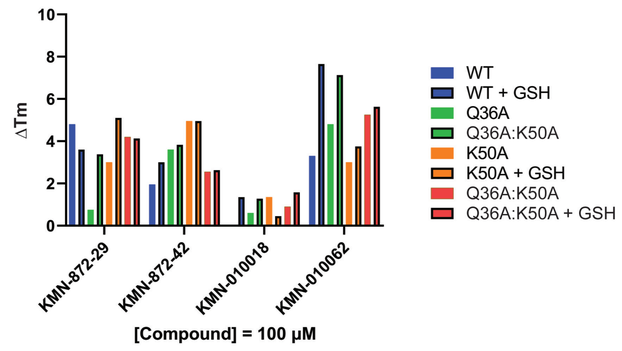

FIGURE 2 – Change in melting temperature (ΔTm) observed in TSA with compounds against WT and mutant H-PGDS (± GSH).

- Based on the GST enzyme assay results, KMN-010062 resulted in 80% inhibition followed by KMN-872-42 (60% inhibition). However, KMN-872-29 and KMN-010018 had below 30% and 20% inhibition, respectively.
- The K50A and Q36A:K50A mutants lost both activity and ligand-binding ability (no response was observed in the assay with any of the compounds). Based on the TSA results, WT and Q36A behaved the same with all four compounds: ΔTm shifted positive when GSH was present for KMN-010062.
- These results suggest specific interactions (perhaps hydrogen bonding interactions) between the cofactor and amide group stabilize the complex.
- No major change in ΔTm was observed ± GSH for K50A and Q36A:K50A.

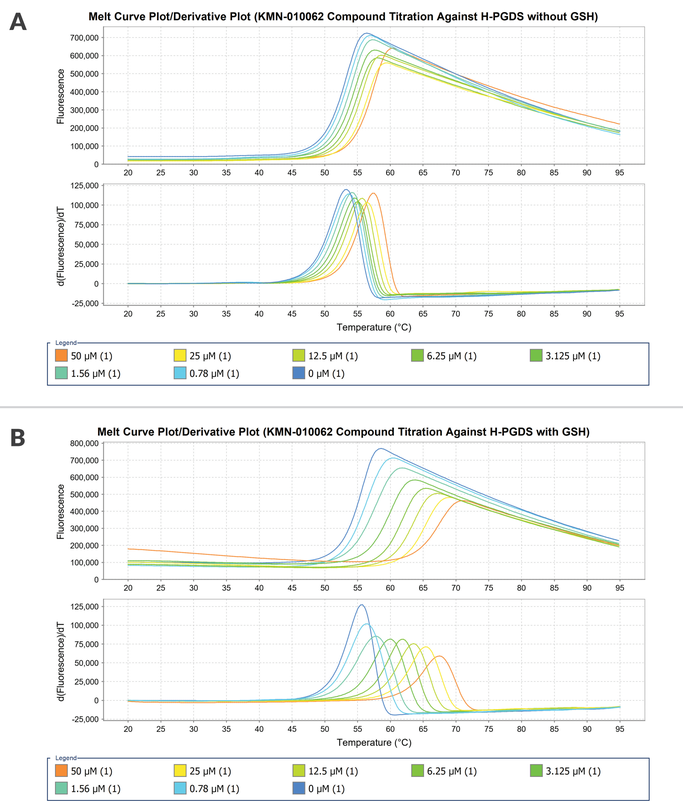

FIGURE 3 – (A) TSA KMN-010062 compound titration against WT H-PGDS without GSH. **(B)** TSA KMN-010062 compound titration against WT H-PGDS with GSH.

	With 2 mM (*4 mM) GSH			
	KMN-010023	KMN-010024	KMN-010034	KMN-010062
Protein	K_d (nM), T ($1/k_{off}$)	K_d (nM), T ($1/k_{off}$)	K_d (nM), T ($1/k_{off}$)	K_d (nM), T ($1/k_{off}$)
WT	0.91, 85.47	0.80, 204.08	1.91, 45.45	1.64, 41.66*
Q36A H-PGDS	1.00, 161.29	0.83, 357.14	0.81, 120.48	1.39, 125.0*
K50A H-PGDS	18.9, 31.25	-	14.5, 23.35	26.54, 47.61*

	With 300 µM GSH			
	KMN-010023	KMN-010024	KMN-010034	KMN-010062
Protein	K_d (nM), T ($1/k_{off}$)	K_d (nM), T ($1/k_{off}$)	K_d (nM), T ($1/k_{off}$)	K_d (nM), T ($1/k_{off}$)
WT	3.72, 24.11	1.14, 45.64	7.23, 24.39	7.09, 15.15
Q36A H-PGDS	1.25, 114.1	1.32, 100.0	2.3, 43.47	5.74, 21.74
K50A H-PGDS	37.34, 22.24	75.80, 7.69	49.15, 7.19	415.5, 6.13

TABLE 1 – Single-cycle kinetics for compounds against WT and mutant H-PGDS with 2 mM GSH **(top)** or 300 µM GSH **(bottom)**. For select experiments, 4 mM GSH was used, as indicated by the asterisk (*). KMN-010023 and KMN-010024 contain amide linkers, KMN-010034 contains an imidazole linker, and KMN-010062 contains an amide linker/Michael acceptor.

- Nearly 3X faster inhibitor k_{off} for WT and Q36A in the absence of GSH.
- For K50A, faster k_{off} for inhibitors were mostly observed compared to WT and Q36A.
- Our data suggests GSH as a cofactor could have a direct effect on the dissociation rate of ligand binding.

Structural Data Support Different Binding Modes for Two Specific Analogs

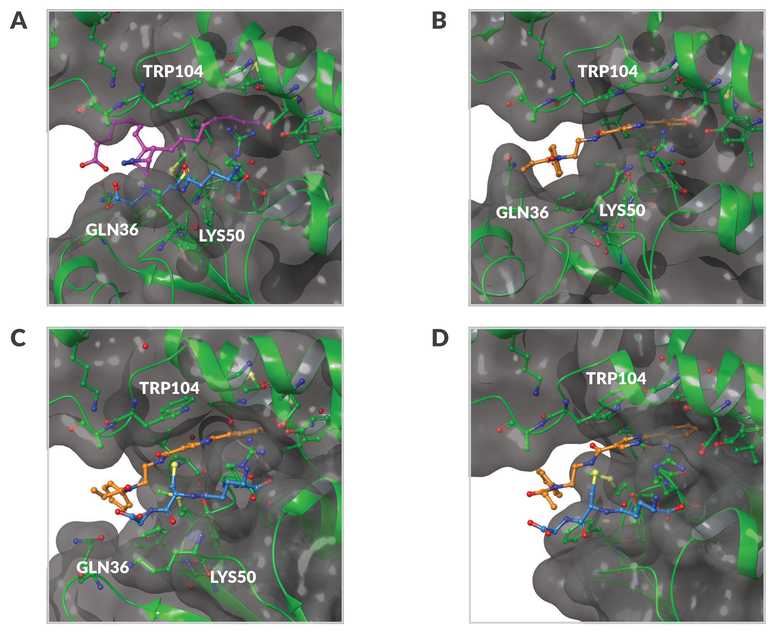

FIGURE 4 – H-PGDS structures are depicted in cartoon fashion with binding site residues and ligands as sticks. Important residues are labeled in white. **(A)** X-ray co-crystallographic structure of the natural substrate (purple)/GSH (blue)/WT H-PGDS (green). **(B)** X-ray co-crystallographic structure of KMN-010062 (orange)/WT H-PGDS (green). **(C)** X-ray co-crystallographic structure of KMN-010062 (orange)/GSH (blue)/WT H-PGDS (green). **(D)** X-ray co-crystallographic structure of KMN-010062 (orange)/GSH (blue)/K50A mutant H-PGDS (green). **(E)** Overlay of KMN-010034 (salmon)/WT H-PGDS (pink) and KMN-010062 (purple)/GSH (grey)/WT H-PGDS (teal) with highlighted conformational changes.

Complexes in **Figure 4A** and **4C** were crystallized with GSH and show Gln36 and Lys50 pointing out, providing an open (helix-2 out) binding pocket. In contrast, the protein complex in **Figure 4B** was crystallized without GSH and shows Gln36 and Lys50 flipped in, providing a closed (helix-2 in) binding pocket. This suggests the importance of helix-2 translocation **(Figure 4E)**. Additionally, the mutant structures have been co-crystallized with KMN-010062 **(Figure 4D)** and KMN-010018 (data not shown). For the crystal structure of the K50A/KMN-010062, the helix-2 is truncated, however the GSH binds similarly to that of the WT/KMN-010062 structure. This highlights the helix-2 conformational change that has been observed.

CONCLUSIONS

- We found that imidazole in place of amide linkers between the pyrimidine core and the tail group maintains high potencies while suffering from poor physiochemical properties and pharmacokinetics.
- SPR results for WT and Q36A suggest faster k_{off} when less GSH is present. For K50A, faster k_{off} for inhibitors were mostly observed compared to WT and Q36A.
- The K50A/KMN-010062 crystal structure suggests that the inhibitor may also form a covalent bond with GSH.
- K50A mutant can cause this enzyme to lose its activity and binding ability (no conformational change for Lys50 side chain).
- We obtained the co-crystal structure of H-PGDS bound with the natural substrate.
- Taken together, these data suggest two structure conformations for H-PGDS upon binding to inhibitors: open *versus* closed pocket. Further studies are needed to identify its physiological relevance.

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RESOURCES

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