

Novel Screening Assay and Discovery of Potent Inhibitors of H-PGDS

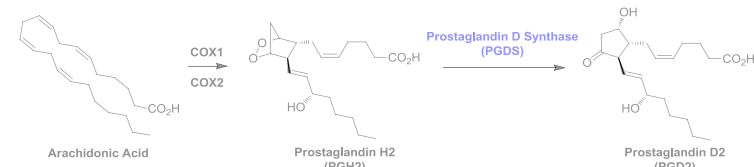
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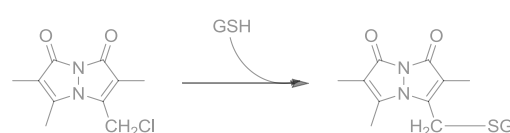
ABSTRACT

Prostaglandin D₂ (PGD₂) synthesis is catalyzed by hematopoietic-type PGD-synthase (H-PGDS) in mast cells and is released in large quantities during allergic and asthmatic anaphylaxis. PGD₂ formed by H-PGDS also plays a detrimental pro-inflammatory role in Duchene's Muscular Dystrophy. H-PGDS is therefore a key target for development of selective, potent inhibitors for therapeutic use against these diseases. We describe here the discovery of potent inhibitors of H-PGDS which were primarily identified using a new FP-Based Binding Assay. In this assay, an H-PGDS inhibitor-fluorescein conjugate serves as a specific fluorescent probe for the enzyme. Displacement of the probe by an introduced test compound leads to a decrease in the fluorescence polarization (FP) state of the probe, providing a direct signal for binding to the active site. Novel, rationally designed H-PGDS binders were prepared and found to exhibit good potency using this assay (**KMN-698-305**, Compound **1c**; IC₅₀ = 79 nM). Potent binders were further evaluated for H-PGDS inhibition and hits confirmed using an enzymatic assay with quantification of PGD₂ formation.

INTRODUCTION

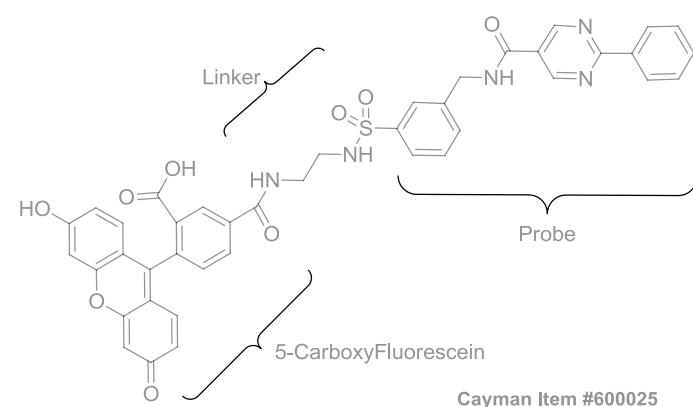


H-PGDS catalyzes the isomerization of PGH₂, the highly-unstable product of COX-1/COX-2 metabolism of arachidonic acid, to PGD₂. The functional activity of H-PGDS can be quantified by measuring production of PGD₂ using a competitive immunoassay. However, this method is problematic because of the inherent instability of PGH₂ and difficulty in automating for HTS.



H-PGDS also exhibits glutathione S-transferase (GST) activity, which is sometimes exploited for HTS. GSTs & glutathione peroxidases are important enzymes involved in a wide variety of cellular processes (ex. detoxifying enzymes). The primary disadvantage of employing the GST assay as a screening tool is that it could potentially select for molecules that also inhibit other cellular GSTs. The Fluorescence Polarization assay described here overcomes these assay limitations by eliminating the need for PGH₂ while still probing the PGH₂ binding site of the enzyme. This enabled the rational design, synthesis, and screening of novel binding molecules that acted as potent inhibitors of the enzyme.

FIGURE 1. DETECTION ANALYTE USED IN H-PGDS FPBA.



RESULTS

5-Carboxyfluorescein was chemically linked to a known H-PGDS inhibitor to create a sensitive detection analyte for use in the study (Figure 1).

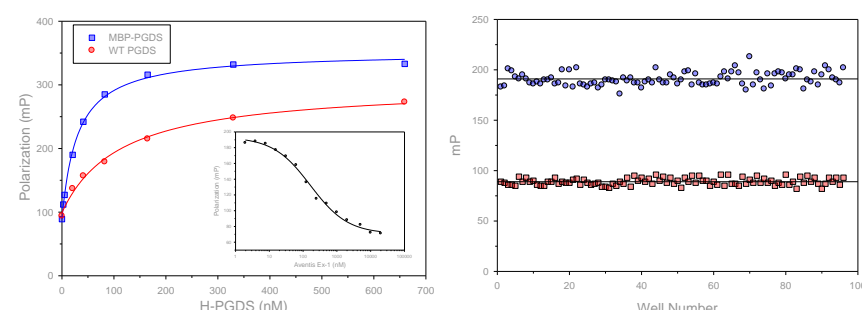


Figure 2: A) Maltose-Binding-Protein (MBP)-fused human recombinant H-PGDS showed a significantly enhanced ΔmP range compared to H-PGDS alone. Inset shows a typical probe displacement curve (AV-Ex-1). B) The FPBA is a robust assay characterized by a Z' value of 0.74 in a 96-well format.

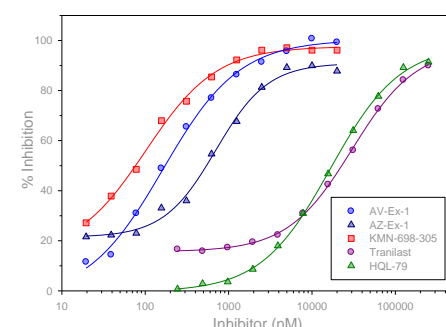


Figure 3: Probe Displacement by Select Inhibitors Represented as Percent Inhibition. Several previously described H-PGDS inhibitors were evaluated in the FPBA. The IC₅₀ values for AV-Ex-1,¹ AZ-Ex-1,² Tranilast,³ and HQL-79⁴ were determined to be 125 nM, 250 nM, 35 nM and 15 μM, respectively. The Cayman compound, **KMN-658-305** (Compound **1c**), exhibited an IC₅₀ of 79 nM.

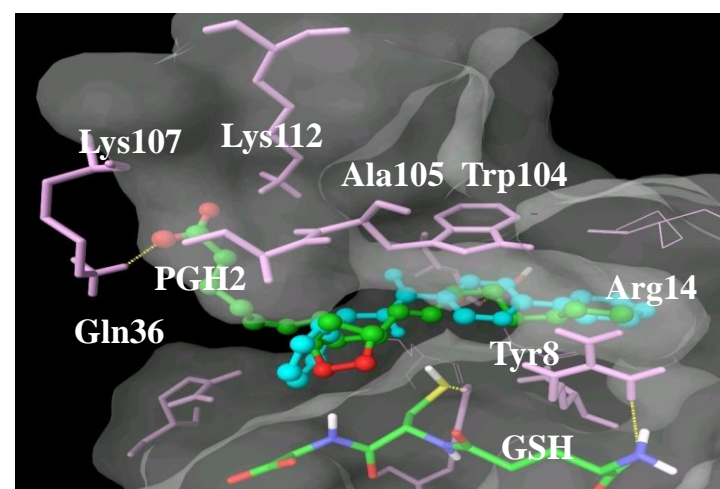
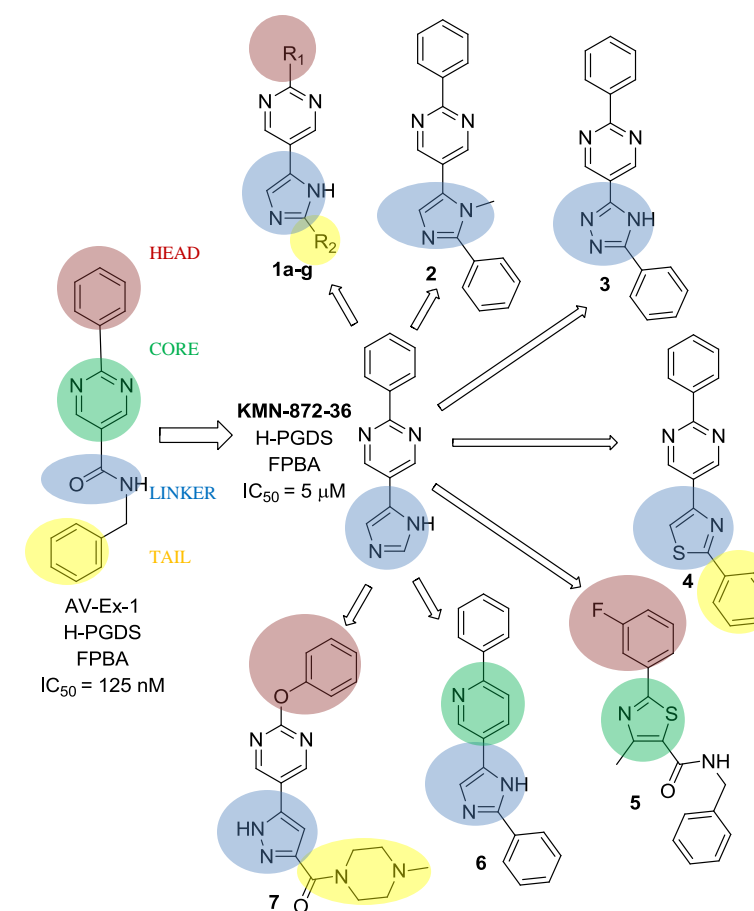


Figure 4: A model overlay of PGH₂ (green) and a known inhibitor (cyan) docked in the H-PGDS active site. Shown in pink are the binding pocket residues. Key Ligand-Protein interactions are π-π stacking of the pyrimidine ring with Trp104, H-bond interaction of the amide N with GSH, and H-bond interaction of the pyrimidine N to a water molecule (not shown) in the active site.

MEDICINAL CHEMISTRY

SCHEME 1. Design Rational for Amide Replacement Analogs



As shown in Scheme 1, multiple 2-phenylpyrimidin-5-yl amide replacement analogs of a known H-PGDS inhibitor were targeted for synthesis. A diverse set of compounds incorporating variations in the head, core, linker and tail were chemically synthesized (representative synthesis of **1a** shown in Scheme 2). Other chemistry provided a structurally diverse set of analogs for SAR analysis. Compounds were evaluated for H-PGDS inhibition (see Table 1) and **1c** discovered as the most potent (FPBA IC₅₀ = 79 nM, **KMN-698-305**). The compounds were further evaluated using an *in vitro* enzymatic functional assay (PGD₂ production quantified using Cayman EIA Kit **Cat.#512021**) This and other unreported data along with molecular modeling studies (Figure 4) proved useful in defining H-PGDS active site SAR.

SCHEME 2. SYNTHESIS OF 2-SUBSTITUTED IMIDAZOPYRIMIDINE TARGETS

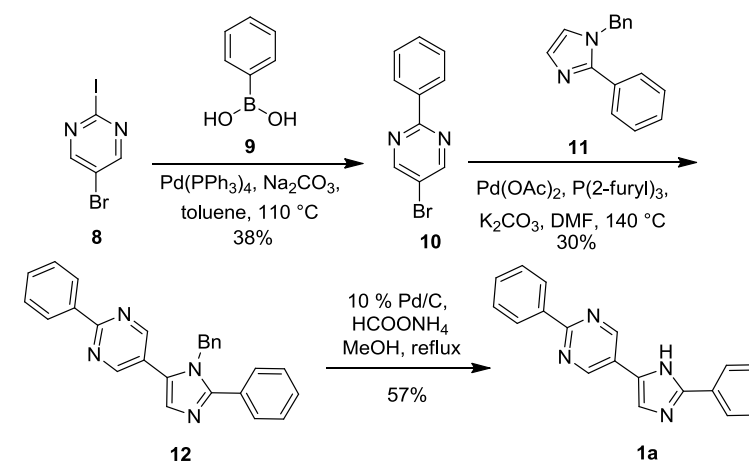


TABLE 1. EVALUATION OF NEW H-PGDS INHIBITORS

R ₁	R ₂	Compounds 2-7 (See Scheme 1)	FPBA IC ₅₀ (μM)	Inhibitor EIA IC ₅₀ (μM)
1a			0.25	0.012
1b			0.188	0.004
1c			0.079	0.021
1d			0.156	0.041
1e			0.125	0.075
1f			0.08	0.006
1g			0.625	0.37
2			0.625	0.25
3			1	0.084
4			1	0.15
5			0.90	0.26
6			0.48	0.097
7			0.65	0.42

CONCLUSIONS

•A single-step homogenous HTS assay for H-PGDS was developed and successfully utilized to evaluate a series of novel inhibitors.⁵

•Displacement assay uses a fluorescence conjugate of a strong H-PGDS binder is fast (30 minutes), gives reproducible results over broad conc. range, compares well with other known assays, and is now commercially available.⁶

•Structurally diverse novel series of multiheteroaryl compounds were rationally designed and synthesized as potent HPGDS inhibitors.⁷

•The FP-based assay for H-PGDS provided a novel method to screen for active site-directed binding molecules that also function as enzyme inhibitors.

REFERENCES

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- 5) Methods for Assaying Compounds or Agents for Ability to Displace Potent Ligands of Hematopoietic Prostaglandin D Synthase. PCT WO2009/140364
- 6) Cayman FP-based Inhibitor Screening Assay Kit **Item #600007**; www.CaymanChem.com
- 7) Multiheteroaryl Compounds as Inhibitors of H-PGDS and their use for Treating Prostaglandin D2 Mediated Diseases. PCT WO2010/033977