Discovery of Potent Multiheterocycle H-PGDS Inhibitors

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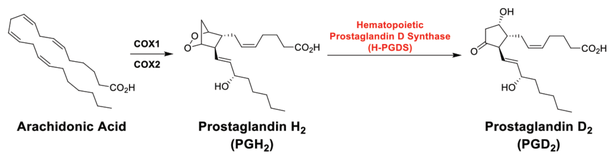
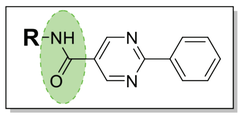
ABSTRACT

Hematopoietic-type Prostaglandin D Synthase (H-PGDS) is responsible for the enzymatic production of the pro-inflammatory mediator prostaglandin D_2 (PGD_2). H-PGDS is expressed in muscle fibers from patients with Duchenne Muscular Dystrophy (DMD) and polymyositis, and has been reported to play a role in the etiology of the muscle necrosis associated with these conditions.[1] Targeting H-PGDS with small molecule inhibitors is therefore a reasonable approach to the potential discovery and development of therapies for treatment of DMD and other inflammation-related disorders. Herein we describe the discovery of potent H-PGDS inhibitors through rational and structure-based drug design, which were identified using Cayman's new H-PGDS Fluorescence Polarization Binding Assay (FPBA) and functional assays.

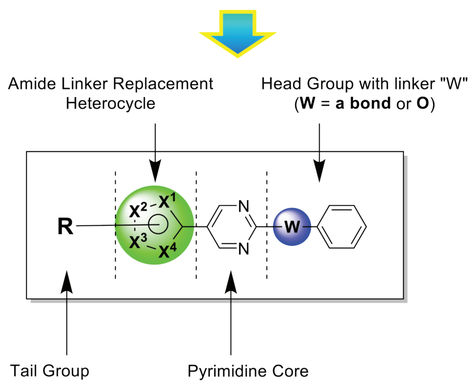


INTRODUCTION

H-PGDS is a glutathione-dependent enzyme that catalyzes the isomerization of prostaglandin H_2 (PGH_2), the highly-unstable product of COX-1/COX-2 metabolism of arachidonic acid, to PGD_2. Herein we report novel small molecule ligands that bind to the substrate binding site and thus inhibit PGD_2 production. As illustrated below, new H-PGDS inhibitors address potential amide metabolic (hydrolytic) liabilities of published compound series by Sanofi-Aventis[2] and Pfizer[3] by replacing the Amide Linker moiety between the Pyrimidine Core and the Tail Group with hydrolytically-stable five-membered heterocycle ring isosteres such as imidazolyl and pyrazolyl moieties. In addition, the novel series begins to investigate SAR with regard to the insertion of an oxygen spacer between the Head Group and the Core Heterocycle.[4]

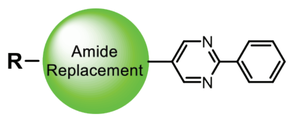


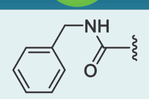
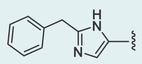
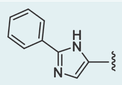
Sanofi-Aventis and **Pfizer** H-PGDS Inhibitors



Novel **Cayman Chemical** H-PGDS Inhibitors

Table 1: Amide Replacement SAR



Compound No.	R– Amide Replacement	FP Binding IC_{50} (μM)	Functional/ Enzyme IC_{50} (μM)	Cellular IC_{50} (μM)
Sanofi-Aventis 1		0.11	0.12	0.07
KMN-010003		0.19	0.31	0.25
KMN-010009		0.10	0.13	0.04

DISCUSSION

Amide Replacement SAR (TABLE 1)

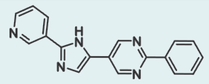
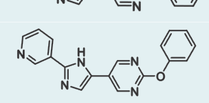
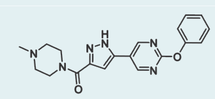
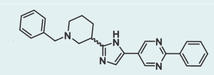
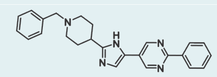
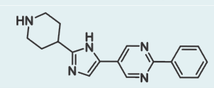
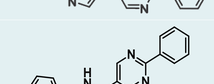
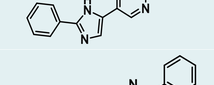
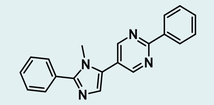
Replacement of the Amide Linker with 4-imidazolyl (**KMN-010001-010003, KMN-010009-010011**) led to compounds essentially equipotent with **Sanofi-Aventis 1**. Replacement with 2-imidazolyl (**KMN-010012**) results in a slight decrease in, but still robust, activity.

Replacement of the Amide Linker with thiazole and oxazole rings (**KMN-010013-010015**) resulted in significant loss of functional and cellular activity.

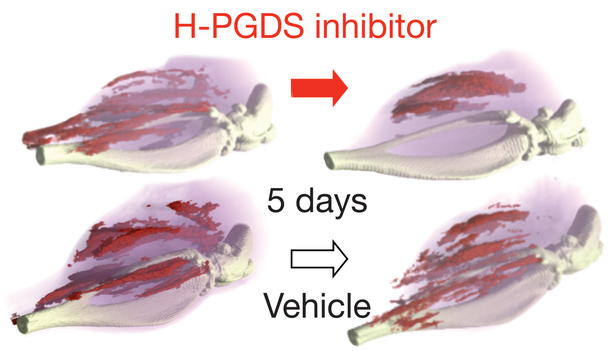
Head and Tail Group SAR (TABLE 2)

A Trp104 π-π stacking 'clothespin' locks all inhibitors in a fixed orientation, with head and tail groups adjusting to fit by extending into the putative glutathione binding pocket. (See *FIGURE 1* illustrating the binding of piperidine analog **KMN-010019)**, and those bearing either aromatic (pyridine) or saturated (piperidine, piperazine) heterocycles comprising a basic nitrogen that exist as the corresponding cationic ammonium in physiological pH generally appear to retain nanomolar potencies (**KMN-010002, KMN-010017, KMN-010018, KMN-010004, KMN-010019, KMN-010020**).

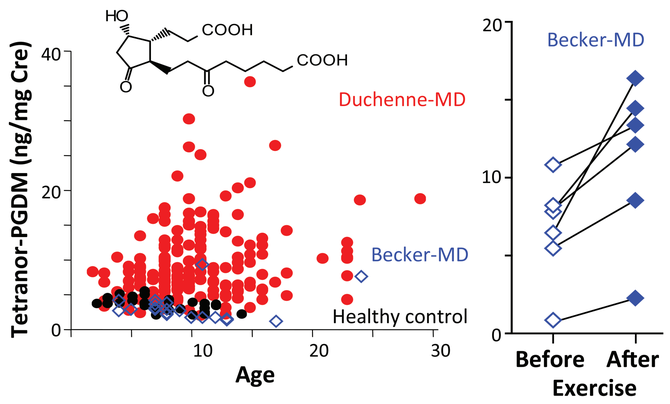
Table 2: Head and Tail Group SAR

Compound No.	Compound Structure	FP Binding IC_{50} (μM)	Functional/ Enzyme IC_{50} (μM)	Cellular IC_{50} (μM)
KMN-010002		0.10	0.06	0.03
KMN-010017		0.38	0.62	0.23
KMN-010018		0.68	0.48	0.35
KMN-010004		0.11	0.20	0.63
KMN-010019		0.07	0.06	0.42
KMN-010020		0.58	0.56	1.3
KMN-698-261		0.10	0.13	0.08
KMN-872-29		0.36	1.5	0.24
KMN-872-42		0.37	0.61	0.67

Reduction of necrotic area in *mdx* mice



Tetranor-PGDM in muscular dystrophy patients



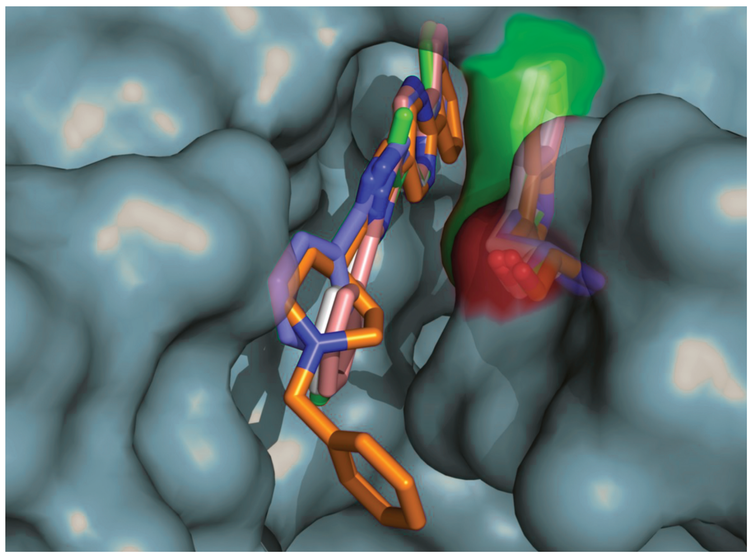


Figure 1: Overlay of a subset of Cayman's H-PGDS co-crystallization structures. Overall, Cayman's molecules show how the core pyrimidine ring π-π stacks with Trp104 (sticks, green surface). H-PGDS is shown as a Surface representation (light purple), while Cayman's molecules KMN010019 (orange), KMN-698-261 (pink), KMN-872-29 (purple), KMN010002 (grey), and KMN-872-42 (green) are shown as a stick representation. ***Crystallography performed by Cayman Protein Structure Services Core.***

METHODS

Cellular Assay

After an overnight incubation with compound or DMSO control, PGD_2 production was stimulated by the addition of 0.5 μg/ml. After stimulation with LPS for 4 hours, the supernatants were harvested and PGD_2 concentrations were quantified with the Cayman Chemical PGD_2 EIA assay kit (Cayman Chemical #512031).

Fluorescence Polarization (FP) Binding Assay

One microliter of compound diluted in DMSO or DMSO control was added to every well of black, non-stick 384-well plates (Corning 3654) containing 40 μl of assay buffer. Five microliters of H-PGDS (Cayman Chemical #10006593) was added to a final assay concentration of 30 nM in all wells except positive control wells, which received 5 μl of assay buffer. After a 15 minute pre-incubation, 5 μl of H-PGDS FP fluorescent probe (Cayman Chemical #600025) was added to a final assay concentration of 5 nM. The assays were allowed to incubate for 60 minutes at ambient temperature before fluorescence polarization analysis (excitation: 485 nm, emission: 528 nm) on a BioTek Synergy H4.

CONCLUSIONS

Prostaglandin D_2 synthesized by HPGDS in damaged DMD muscle fibers contributes to the necrosis of muscle. Plasma levels of the primary metabolite of PGD_2 (Tetranor PGDM) are elevated in Duchene MD patient's plasma at rest, and in Becker-MD patients after exercise. Inhibition of the production of PGD_2 in the mdx mouse model reduces the degree of muscle necrosis and inflammation after 5 days of treatment. We have used crystallography guided SAR in order to discover a series of novel and potent PGDS inhibitors that could be possible new treatments for ameliorating the condition of muscular dystrophy in human patients.

References
1. Mohri, I. *et al*., *Am. J. Pathol.*, **2009**, 174(5), 1735-1744.
2. Aldous, Suzanne C. *et al*., United States Patent 8,202,863
3. Blake, Tanisha D. *et al*., United States Patent 7,582,643
4. Endres, Gregory W. *et al*., PCT Application Publication WO 2010/033977