Identification of novel 5-HT$_7$ antagonists with efficacy in animal models of IBD

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Abstract: Over 3 million people in the US and Canada suffer from inflammatory bowel disease (IBD), a serious, chronic inflammatory conditions of the human bowel that is rarely fatal, but often severely debilitating. The two major forms of this condition, Crohn's disease (CD) and ulcerative colitis (UC), are both associated with pain, persistent diarrhea, rectal bleeding, fistula formation, intestinal strictures, abscess, and perforated bowels, all of which contribute to reduced quality of life, decreased capacity for work, and increased disability. There is no cure for IBD and the current IBD standard of care focuses on disease management and symptom mitigation. Thus, development of improved therapies is warranted to halt the progression of IBD. It has been demonstrated that the mucosal inflammation observed in IBD patients and IBD animal models is associated with increased serotonin (5-hydroxytryptamine; 5-HT) signaling. We have previously demonstrated that 5-HT signaling via the 5-HT receptor type 7 (5-HT$_7$) receptor in the gastrointestinal (GI) tract is a key component of colitis progression. These studies were conducted in the dextran sodium sulfate (DSS) mouse models of colitis using the selective 5-HT$_7$ receptor antagonist SB-269970 or genetic ablation of 5-HT$_7$ in mice. We also demonstrated that our selective 5-HT$_7$ antagonist, 170073, showed efficacy in the acute and chronic DSS models. We now report the separation of 170073 into its R (230079) and S (230078) enantiomers, their *in vivo* PK properties, and the impact of 230078 on the acute DSS induced mouse models of colitis and the CD4+CD45RBhigh T-cell transfer model of colitis. Amelioration of intestinal inflammation along with decreased histopathological damage, and lower levels of pro-inflammatory cytokines were observed in mice treated with 170073 or 230078 in both DSS and T-cell transfer models of colitis. This data suggests that antagonizing GI 5-HT$_7$ signaling may be a viable treatment option for treating intestinal inflammatory disorders including IBD patients.

Inflammatory Bowel Disease (IBD)

Severe and Chronic Inflammation of the Gastrointestinal Tract

Two Major Classes

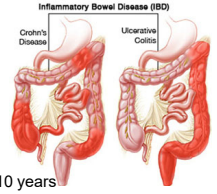
- **Crohn's Disease** (6.5 in 100,000)
  - Can affect any part of G.I tract
  - Inflammation in patches
- **Ulcerative colitis** (11 in 100,000)
  - Continuous inflammation of colon

Symptoms

- Pain
- Persistent diarrhea
- Rectal Bleeding
- Formation of fistulas
- Intestinal strictures
- Abscess
- Perforated bowels
- Malnutrition

No Cure

- Medication focused on symptom mitigation
- Surgery when medication fails



Ulcerative colitis
- 33% require serious surgery
- Ileal pouch-anal anastomosis
  - Removal of colon and rectum
- Attach small intestines to anus

Crohn's Disease
- 70% require surgery
- Removes affected area
  - Not permanent fix
- Require additional surgery
  - 30% @ 2 years , 60% @10 years

New Treatments Required to Halt Disease Progression

IBD, Serotonin (5-HT), and the 5-HT$_7$ Receptor

Serotonin (5-HT) and the GI Tract
- ~95% of the body's 5-HT is in the GI tract
- GI Enterochromaffin (EC) cells produce 5-HT
- 5-HT is critical to enteric mucosal signaling
- *5-HT signaling altered in IBD*
  - *Increased number of EC cells*
  - *Increased 5-HT concentration in GI tract*

GI DCs and ECs are co-located in GI tract
- 5-HT from ECs activates DCs 5-HT$_7$ receptor
- DC release inflammatory cytokines
  - TNF-α, IL-1β, IL-6
- Immune cells activated
- Intestinal inflammation occurs

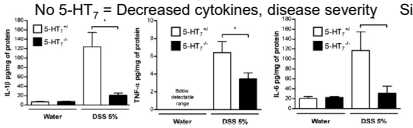
5-HT$_7$ receptor and the GI Tract
- Cell surface GPCR
- Activated by the 5-HT
- High expression in GI dendritic cells (DCs)
- *Key role in:*
  - *GI immune response*
  - *intestinal inflammation*
  - ***IBD severity tied to:***
    - *5-HT production*
    - *5-HT$_7$ activation*

Hypothesis: 5-HT$_7$ antagonist will modulate the immune response, reduce intestinal inflammation, and slow/stop IBD progression

5-HT$_7$ Receptor, IBD Progression, and DSS Mouse Model

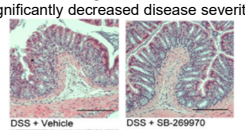
5-HT$_7$ (-/-) mice

No 5-HT$_7$ = Decreased cytokines, disease severity



5-HT$_7$ antagonist SB-269970

Significantly decreased disease severity



Strongly supports 5-HT$_7$ antagonists as potential IBD therapy

Kim, J., Bridle, B. W., Ghia, J., Wang, H., Syed, S. N., Manocha, M. M., Rengasamy, P., Shajib, M. S., Wan, Y., Hedlund, P. B., Khan, W. I. *J of Immun.*, **2013**, 190, 4795-4804

SB-269970 not viable IBD clinical candidate
- Penetrates CNS (17%-24% brain/plasma, rat)
- 5-HT$_7$ plays role in memory, learning
- CNS penetration is unacceptable risk

Hypothesis: *5-HT$_7$ antagonist without CNS penetration will slow/stop IBD progression without CNS side effect risk.*

170073: Proof of Concept Lead Compound

- Potent 5-HT$_7$ binder
- Functional antagonist
- Highly selective:
  - IC_{50} = 89 nM
  - EC_{50} = 18.3 nM
  - 100% efficacy
  - >10 μM @ 5-HT$_{1-6}$

MW	TPSA	cLogP	5-HT$_7$ K_i (nM)	5-HT$_7$ K_b (nM)	5-HT$_{1A}$ 5-HT$_{1B}$ 5-HT$_{1D}$ 5-HT$_{1E}$ 5-HT$_{2A}$ 5-HT$_{2B}$ 5-HT$_{2C}$ 5-HT$_3$ 5-HT$_4$ 5-HT$_{5A}$ 5-HT$_6$ Ki (nM)
346	53	3.1	89	22	>10000

MW	TPSA	cLogP	CYP3A4 IC_{50} (nM)	CYP2C9 IC_{50} (nM)	CYP2D6 IC_{50} (nM)	Sol (μM)	MLM $T_{1/2}$ min.	HLM $T_{1/2}$ min.
346	53	3.1	10000	10000	10000	168	17	60

Route	Dose (mg/kg)	Cmax (ug/ml)	Tmax (h)	AUC (ug*h/ml)	$T_{1/2}$ (h)	Cl (L/h)	%F
IV	1	0.081	0.25	0.1415	4.4	0.125	
PO	5	0.049	0.25	0.1237	3.4	0.73	17%

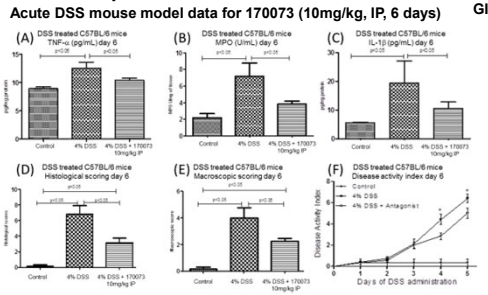
Mouse PK

Orally bioavailable (F% = 17%)

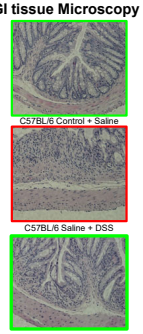
Moderate $T_{1/2}$ (4.4 h IV, 3.4 h PO)

170073 Improves Outcome in Acute DSS Mouse IBD Model

Acute DSS mouse model data for 170073 (10mg/kg, IP, 6 days)



GI tissue Microscopy

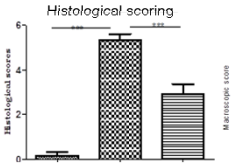


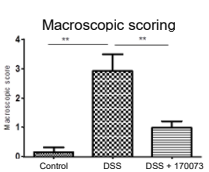
Inflammatory markers lowered
Intestinal damage mitigated

170073 Improves Outcome in Chronic DSS Mouse IBD Model

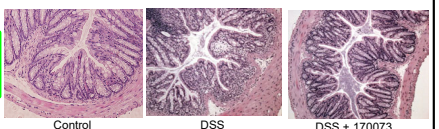
Chronic Mouse DSS* Colitis Model

- DSS administered
  - 5 days 4% DSS (cycle 1)
  - 7 Days water
  - 5 days 2% DSS (cycle 2)
  - 7 days water
  - 5 days 2% DSS (cycle 3)
- 10 mg/kg 170073 IP
  - Initiate 1 day before cycle 3
  - Once daily for 6 days

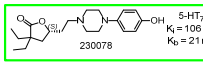




Intestinal damage mitigated
Chronic damage reversed
Proof of concept established



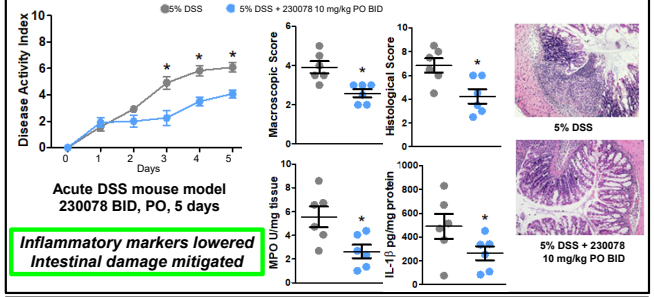
In vivo mouse PK of enantiomer 230078 and 230079



Compound	Route	Dose (mg/kg)	Cmax (ug/ml)	Tmax (h)	AUC (ug/ml*h)	$T_{1/2}$ (h)	Cl (L/h)	%F
S-enantiomer	IV	1	0.527	0.083	0.277	3.97	0.084	
(230078)	Oral	5	0.123	0.5	0.19	3.78	0.511	13.7
R-enantiomer	IV	1	0.148	0.167	0.097	0.335	0.252	
(230079)	Oral	5	0.057	0.5	0.0525	0.582	2.07	10.8

230078 $T_{1/2}$ %F suitable for *in vivo* efficacy studies

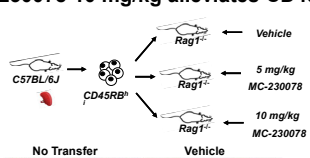
230078 attenuates the severity of DSS-induced colitis

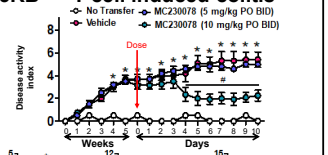


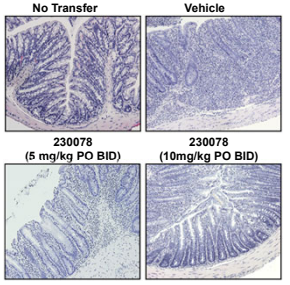
Acute DSS mouse model
230078 BID, PO, 5 days

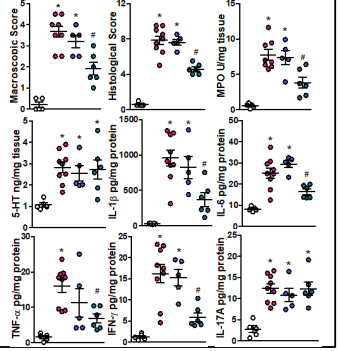
Inflammatory markers lowered
Intestinal damage mitigated

230078 10 mg/kg alleviates CD45RB^{high} T-cell induced colitis









Inflammatory markers lowered
Intestinal damage mitigated

Conclusions: Proof of concept established

- 170073 - Efficacious in acute and chronic DSS mouse model
- 230078 - Efficacious in acute DSS and T-cell induced colitis mouse model
- *5-HT$_7$ is a valid IBD therapeutic target*

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