Poster Number: P396
Poster Topic: Experimental Models

The novel Bach1 inhibitor HPP971 uniquely activates Nrf2 and reduces disease severity in a mouse model of experimental autoimmune encephalomyelitis

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Abstract

Background: Pharmacological activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is an attractive mechanism for the treatment of Multiple Sclerosis (MS) since Nrf2 promotes both neuroprotection and anti-inflammation. Bach1 is the transcriptional repressor of heme oxygenase-1 (HMOX1), an Nrf2-driven cytoprotective gene. Bach1 deficiency in mice confers cytoprotection against spinal cord injury and experimental autoimmune encephalomyelitis (EAE). We therefore designed potent and specific inhibitors of Bach1 to evaluate as a treatment for MS.

Objectives: Determine the pharmacodynamic and therapeutic effects of Bach1 inhibitor HPP971 after oral administration in naïve and EAE mice.

Methods: Recombinant human MBP-Bach1 protein was overexpressed in E.coli and purified using a protein fusion tag. Direct binding of HPP971 was measured using microscale thermophoresis. HMOX1, NQO1, and GCLC gene expression were measured in human astrocytes *in vitro* and in mouse tissues using the Quantigene Reagent 2.0 system (Affymetrix). Human primary astrocytes were treated with HPP971 for 6hr for reduced glutathione (GSH) measurement and 24 hr for assessment of cytoprotection against H2O2 by propidium iodide staining. In an EAE model, C57Bl/6 female mice were immunized with MOG peptide 35-55/CFA on day 1. HPP971 dosing was on day 2 through day 20. Clinical scores were assessed on days 11-20 (0=no disease, 1=limp tail, 2=affected gait, 3=hind limp paresis, 4=hind limb paralysis).

Results: HPP971 binds human Bach1 protein (IC50=120 nM) and activates HMOX1 expression in an Nrf2-dependent manner. HPP971 acutely elevates GSH levels and protects human astrocytes from H2O2-induced cell death *in vitro*. HPP971 inhibits IFN-g-induced MHC class II expression in bone marrow derived macrophages. Oral HPP971 (PO, 20 mg/kg) induces HMOX1 expression in tissues, and attenuated loss of motor functions in a murine EAE model of MS.

Conclusions: Bach1 inhibitor HPP971 exhibits a unique combination of anti-oxidant, cytoprotective and anti-inflammatory properties. HPP971 attenuates pro-inflammatory cytokine production and MHC class II expression. Oral HPP971 was efficacious in the EAE model with activity comparable to Tecfidera™. Bach1 inhibition offers an innovative approach to Nrf2 activation as this mechanism is electrophile-independent and is not predicated on glutathione depletion.

Identification of Bach1 inhibitors

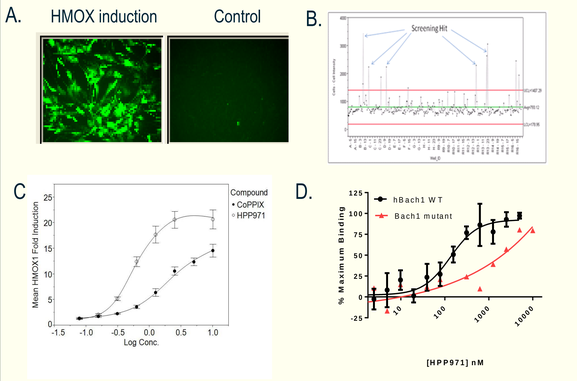


Figure 1. A. and B. Bach1 inhibitors have been identified through high content imaging screening using HMOX1 induction as a surrogate biomarker. Following treatment of normal human lung fibroblasts (NHLF) with compounds, cells were stained with anti-HMOX1 antibody followed by Alexa488-conjugated anti-mouse IgG antibody and fluorescence signal was quantified via InCell imager. C. HPP Bach1 inhibitors induce HMOX in a dose-dependent manner. D. Direct binding between HPP971 and Bach1 was shown through Microscale Thermophoresis with a Kd of 133nM. Mutation of major CP motifs significantly decreased the affinity of HPP971 to this protein (Kd > 1500 nM).

HPP Bach1 inhibitors render astrocytes resistant to H_2O_2-mediated cytotoxicity through augmentation of antioxidant gene expression and cellular GSH

A.

Compound	Conc. (μM)	HMOX1	NQO1	TXNRD1
Tecfidera	60	41.7	2.4	2.8
	30	16.7	2.4	2.9
CoPPIX	10	82.5	4.7	5.5
	3	27.6	2.2	2.3
HPP-971	1	40.7	2.0	4.5
	0.3	10.0	1.4	2.1

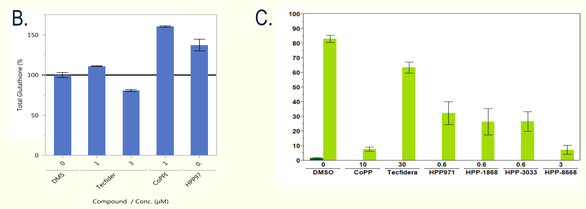


Figure 2. A. Human primary astrocytes were treated with HPP971 for 24 hours; cobalt protoporphyrin (CoPP) and Tecfidera (dimethyl fumarate) were used as controls. *HMOX1, NQO1* and *TXNRD1* mRNA induction was detected from cell lysate using the Quantigene Plex 2.0 Assay Kit (Panomics). B. Rapid Increase in intracellular GSH in astrocytes with HPP-971. Astrocytes were briefly (6hr) treated with indicated compounds. Intracellular glutathione was measured via Promega GSH-Glo Glutathione Assay kit. C. HPP Bach1 inhibitor compounds protect astrocytes from H_2O_2-induced cytotoxicity. Astrocytes were pretreated indicated compounds for 24hr before 0.5 mM of H_2O_2 treatment. Cell viability was assessed using propidium iodide (PI) staining along with Hoescht dye .

HPP Bach1 inhibitors induce HMOX1 expression and suppress IFN-γ-induced MHC class II up regulation in mouse macrophages

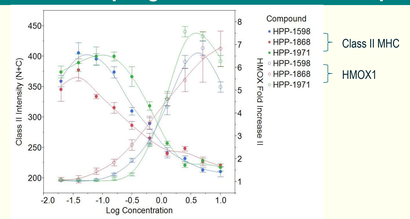


Figure 3. Balb/c mouse bone marrow-derived macrophages (BMDMs) were treated with HPP Bach1 inhibitors for 5hrs before they are treated with 10ng/ml recombinant mouse IFN-γ. Intracellular HMOX1 and surface MHC class II were visualized and quantified via InCell imager.

HPP Bach1 inhibitor, HPP971, robustly induces Bach1-and ARE-regulated anti-oxidant gene expression *in vivo*

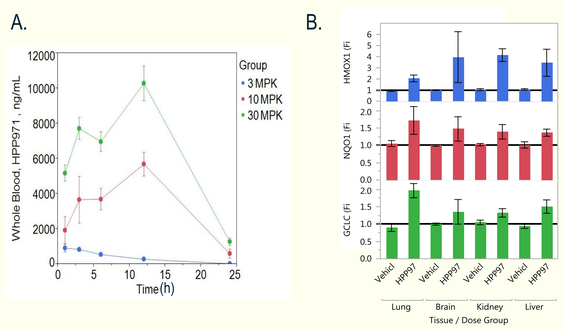


Figure 4. A. HPP971 is orally bioavailable. C57BL/6 mice were dosed orally with indicated dose of HPP-971. Whole blood samples were collected at the indicated time points and compound level was measured via a LC-MS/MS method using Agilent 1100 HPLC. **B.** HPP971 robustly induces anti-oxidant genes in vivo. Tissues from mice given either vehicle or 30mg/Kg of HPP971 were collected at 6 hours. Isolation and quantification of RNA from tissue lysates was carried out according to the Quantigene Reagent System 2.0 protocol (Panomics).

Suppression of pro-inflammatory cytokine gene expression by HPP Bach1 inhibitors *in vitro* and *in vivo*

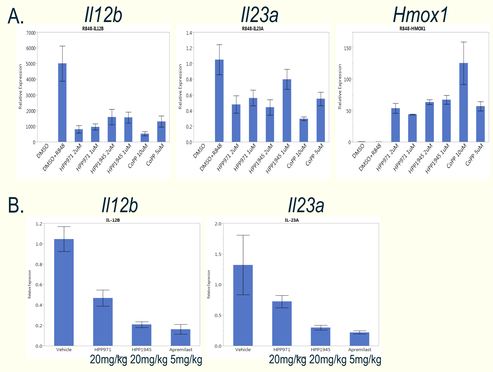


Figure 5. A. Suppression of TLR agonist-induced *IL12b* and *IL23a* gene expression following pre-treatment with Bach1 inhibitors in mouse macrophages. Suppression of cytokine gene expression is correlated with *Hmox1* and other Bach1-regulated gene induction. Mouse macrophages were collected from C57BL/6 mice treated with 4% thioglycollate media. Macrophages were treated with HPP Bach1 inhibitors for 5hours before treatment with TLR7/8 agonist, R848. RNAs were isolated 2hrs after R848 treatment and transcripts were quantified via a Taqman-based RT-PCR method. **B.** Suppression of *IL12b* and *IL23a in vivo* with Bach1 inhibitor treatment in an anti-CD40-induced inflammation model. 10 week-old Balb/c mice were dosed for three days prior to anti-CD40 antibody treatment. Next day, punch biopsy samples were collected from the liver for RNA analysis. Quantification of transcripts were performed via a Taqman-based RT-PCR method.

Attenuation of the disease severity and weight loss with oral administration of HPP Bach1 inhibitors

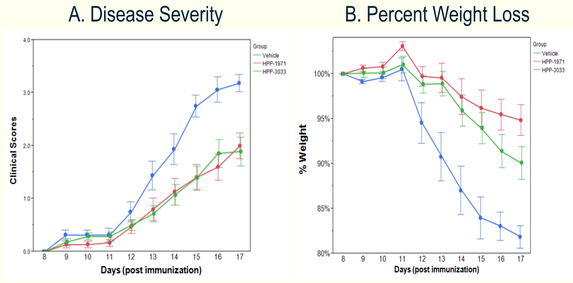
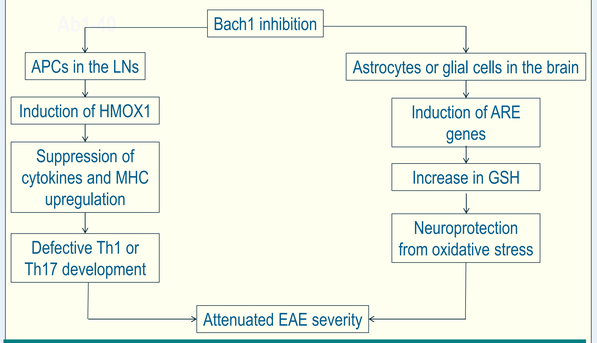


Figure 6. A and B. Attenuation of the EAE disease severity and weight loss with daily treatment with HPP Bach1 inhibitors, HPP971 and HPP3033. 11 weeks old female C57BL/6 mice were subcutaneously injected with MOG35-57/CFA emulsion (Hooke kits). Mice were subsequently injected with pertussis toxin (PTX) on the day of immunization and the next day. Starting one day after immunization, vehicle *(note vehicle composition either here or elsewhere)* (n=10) and 10mg/Kg HPP971 and HPP3033 groups (n=10) were orally administered once a day.

HPP Bach1 inhibitors attenuate the EAE disease severity through both neuroprotection and immune modulation



Conclusions

❖ Bach1 inhibitors have been identified through a high content screening using HMOX1 as a biomarker.

❖ HPP Bach1 inhibitors directly bind to human Bach1 protein.

❖ HPP Bach1 inhibitors efficiently de-repress Bach1-regulated genes in various human and murine cells including *Hmox1*, *Nqo1,Txnrd* and *Gclc.*

❖ HPP Bach1 inhibitor treatment rapidly increase the intracellular GSH level and render human astrocytes resistant to H_2O_2-induced cytotoxicity.

❖ HPP Bach1 inhibitors induce Bach1-regulated genes particularly in murine and human APCs including monocytes, macrophages or dendritic cells.

❖ HPP Bach1 inhibitors suppress IFN-γ-induced MHC class II upregulation in mouse macrophages

❖ HPP Bach1 inhibitor treatment of mouse macrophages lead to suppression of TLR agonist-induced *Il12b* and *Il23a* expression.

❖HPP971, a Bach1 inhibitor, is orally available and pharmacodynamically active as it induces ARE genes in various mouse and rat organs.

❖ HPP Bach1 inhibitor treatment significantly attenuated *IL12b* and *Il23a* expression *in vivo* in anti-CD40-induced inflammation model.

❖Daily HPP Bach1 inhibitor treatment significantly ameliorated the EAE disease severity and weight loss

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