

1.) TITLE PAGE

**PHARMACOLOGICAL CHARACTERIZATION OF IW-1973, A NOVEL SOLUBLE
GUANYLATE CYCLASE STIMULATOR WITH EXTENSIVE TISSUE
DISTRIBUTION, ANTI-HYPERTENSIVE, ANTI-INFLAMMATORY, AND ANTI-
FIBROTIC EFFECTS IN PRECLINICAL MODELS OF DISEASE**

Jenny V. Tobin*, Daniel P. Zimmer*, Courtney Shea, Peter Germano, Sylvie G. Bernier,
Guang Liu, Kim Long, Joy Miyashiro, Sheila Ranganath, Sarah Jacobson, Kim Tang, G-
Yoon Jamie Im, James Sheppeck II, Joel D. Moore, Kristine Sykes, James Wakefield,
Renee Sarno, Ali R. Banijamali, Albert T. Profy, G. Todd Milne, Mark G. Currie, and
Jaime L. Masferrer

Ironwood Pharmaceuticals, 301 Binney Street, Cambridge, MA 02142 - JVT, DPZ, CS,
PG, SGB, GL, JM, SJ, G-YJI, JS, KS, JW, RS, ARB, ATP, GTM, MGC, JLM

SUPPLEMENTAL FIGURE LEGENDS

Supplemental figure 1. (A) LC-MS/MS trace of the cGMP measurement with internal standard, ion mass (m/z) for cGMP internal standard were $346 > 152$ and $349 > 155$ respectively with a retention time of 0.62 m for both (B) representative cGMP standard curve representing duplicate measurements from the beginning and end of the LC-MS/MS run.

Supplemental figure 2. IW-1973 induces relaxation of human subcutaneous resistance arteries, each data point represents the concentration dependent % relaxation of one of three tissues from human donors. The dashed line represents the nonlinear curve fit of the average data from which an EC_{50} can be derived.

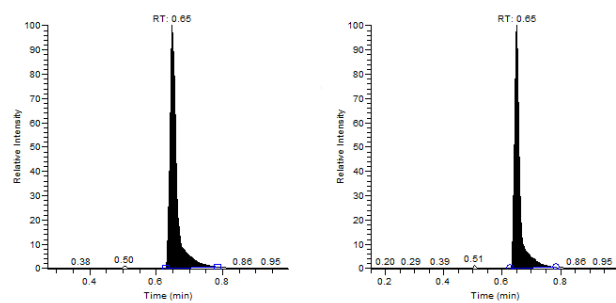
Supplemental figure 3. Body weight in DSS rats throughout 6 weeks of treatment with vehicle, IW-1973 (1, 3, and 10 mg/kg, in chow), or losartan (30 mg/kg, in drinking water), shown as change in weight \pm SEM (g) from start of treatment at week 0, $n = 8$ per group. Rats were provided normal-salt diet or high-salt diet for 2 weeks before initiation of and during treatment with vehicle, IW-1973, or losartan. Significance was determined by two-way ANOVA followed by a Tukey's post-hoc test for multiple comparisons as compared to the high salt group, $*p \leq 0.05$, $**p \leq 0.01$, and $***p \leq 0.001$.

Supplemental figure 4. HR in DSS rats throughout 6 weeks of treatment with vehicle, IW-1973 (1, 3, and 10 mg/kg, in chow), or losartan (30 mg/kg, in drinking water), shown as average HR \pm SEM (BPM), $n = 8$ per group. Rats were provided normal-salt diet or

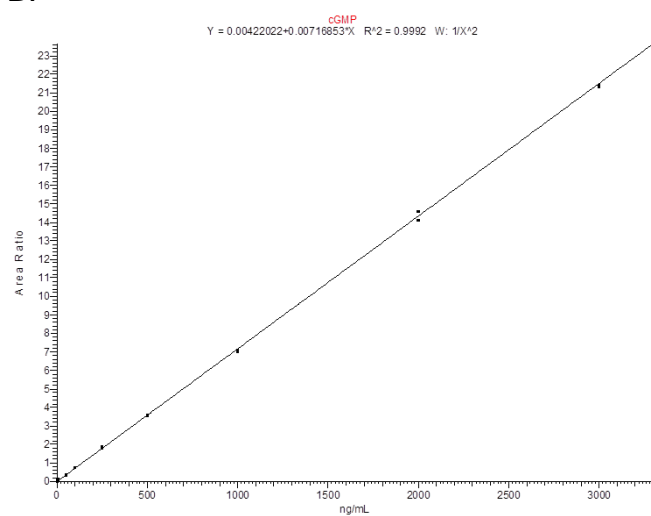
high-salt diet for 2 weeks before initiation of and during treatment with vehicle, IW-1973, or losartan. Baseline measures were determined 1-3 days before initiation of treatment.

Supplemental Figures

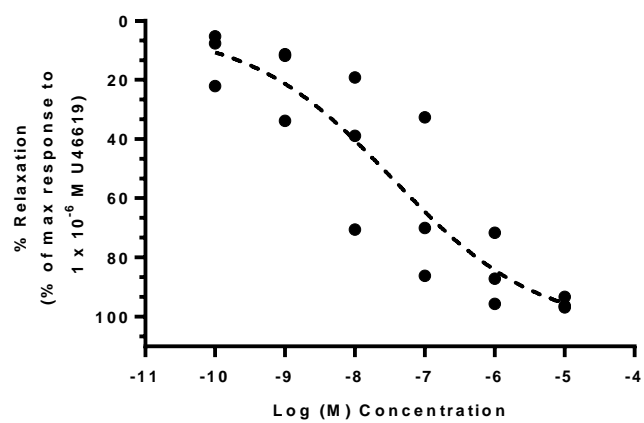
A.



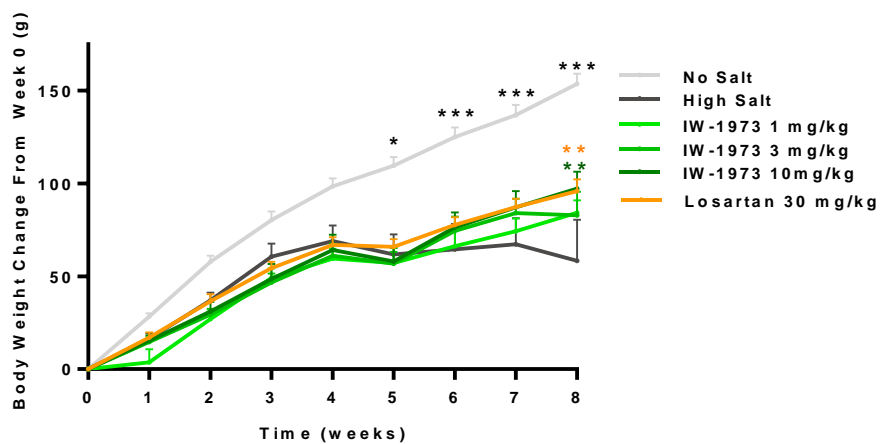
B.



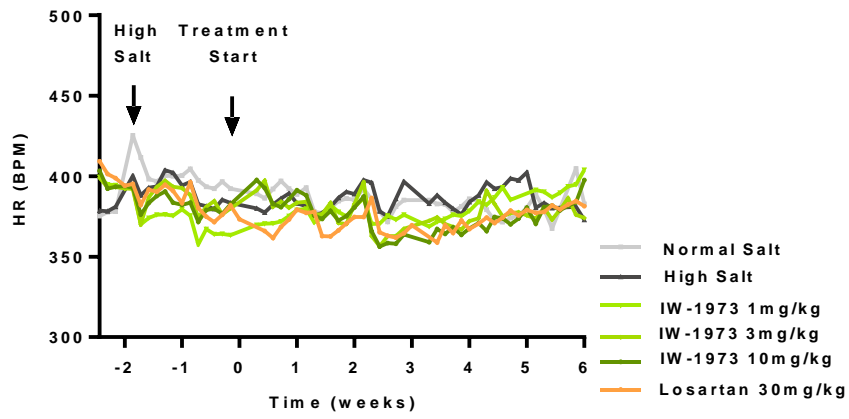
Supplemental Figure 1



Supplemental Figure 2.



Supplemental Figure 3.



Supplemental Figure 4.