Supplemental Data

Small-Molecule Inhibitors of Pendrin (Slc26a4) Potentiate the Diuretic Action of Furosemide

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Legends to Supplemental Figures

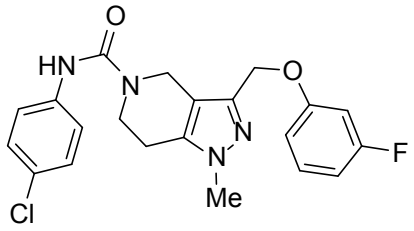
Supplemental Figure 1. Structure-activity analysis of a second class of small molecule pendrin inhibitors. **A.** Chemical structure of class A inhibitor PDS_{inh}-A01. **B**. Concentration-dependent inhibition of pendrin-facilitated Cl^-/anion exchange by PDS_{inh}-A01 in FRT cells coexpressing murine pendrin and a YFP halide sensing protein (S.E., $n = 4$). **C.** Structural determinants of pendrin inhibition activity of class A pendrin inhibitors.

Supplemental Figure 2. Characterization of pendrin inhibitors. **A.** Washout (*left*) and kinetics of action (*right*) of PDS_{inh}-A01 and PDS_{inh} -C01. Assays were done with 25 μM inhibitor and 70 mM NaI gradients, as described in the Concise Methods section of the manuscript. For washout studies cells were washed in PBS, 15 min per wash. For kinetic studies cells were incubated with inhibitor for indicated times prior to assay. **B.** Competition studies, showing inhibitor concentration-dependence with NaI gradients of 70, 35 and 17.5 mM. IC_{50} values for all gradients were ~10 μM. **C.** pH sensitivity of PDS_{inh}-C01 in which cells were incubated with PBS containing different concentrations of inhibitor for 20 min, exchanged with weakly buffered solution containing 140 mM NaCl and inhibitor (5 mM Hepes, 140 mM NaCl, pH 7.4), and then exposed to a NaI-containing solution at pH 5 or pH 8 (20 mM Hepes, 20 mM Na citrate, 140 mM NaI, pH 5 or pH 8) to drive Cl^-/I^- exchange.
D. Effect of extracellular NaCl in cells equilibrated in a buffered solution containing 100 mM NaCl and 37 mM NaI, in which pendrin-mediated exchange elevated intracellular I^- and reduced YFP-HIF fluorescence. Cells were then exposed to solutions containing indicated NaCl (with 5 Hepes, pH 7.4) to drive I^-/Cl^- exchange seen as increased fluorescence.

Supplemental Figure 3. Effects of PDS_{inh}-A01 on urine volume and osmolality. 3-h urine volume and osmolality after intraperitoneal administration of 10 mg/kg PDS_{inh}-A01 at zero time alone or together with furosemide (20 mg/kg) or hydrochlorothiazide (HCTZ, 20 mg/kg) (mean ± S.E., 4-6 mice per group). ** $p < 0.01$, *** $p < 0.001$, ns: not significant, one-way analysis of variance with post-hoc Newman-Keuls test.

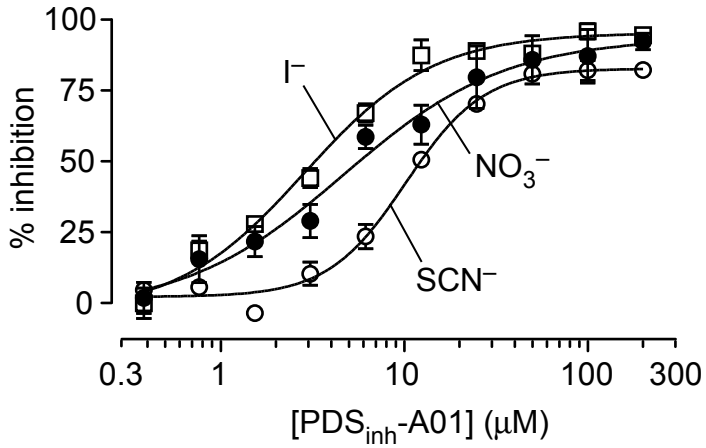
Supplemental Figure 4. PDS_{inh}-C01 and HCTZ do not interact functionally. **A.** PDS_{inh}-C01 inhibition of pendrin in the absence (*black*) and presence (*grey*) of 25 μM HCTZ. PDS_{inh}-C01 inhibition of pendrin I^-/Cl^- exchange was not reduced by HCTZ. **B.** HCTZ inhibition of NCC in the absence (*grey*) and presence (*black*) of 25 μM PDS_{inh}-C01. NCC activity was measured in transiently transfected COS-7 fibroblasts as described in the Concise Methods section of the manuscript. Control data (without inhibitors) shown for reference. HCTZ inhibition of NCC inhibition was not reduced by PDS_{inh}-C01.

A

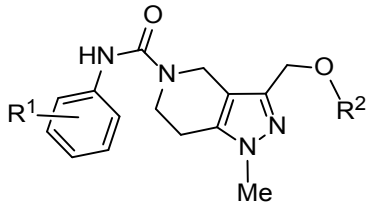


PDS_{inh}-A01

B

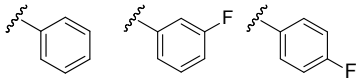


C



Best R^1= 4-OMe, 4-Et, 4-Cl, 4-Me, 4-SMe, 3-F, 4-OMe

Best R^2 =



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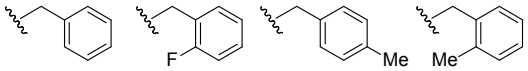
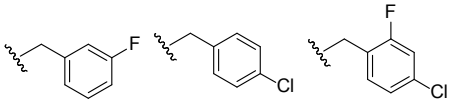
Moderate R^2 =

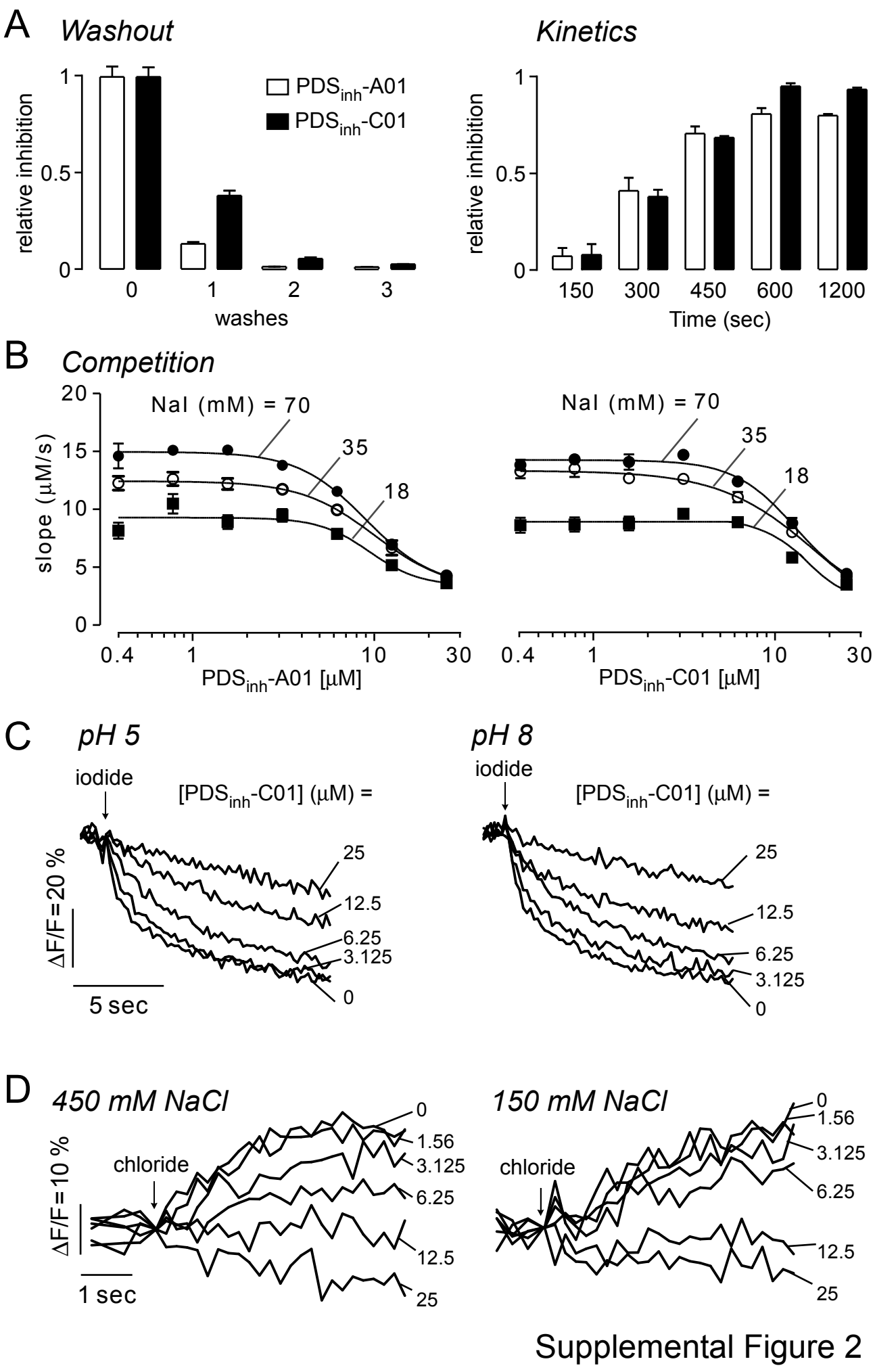


Inactive R^1 = 2-OMe 2-Cl, 2-F, 2-CF_3, 2-Me, 2,5-di-Cl, 2,5-di-Me, 2-Me,5-OMe

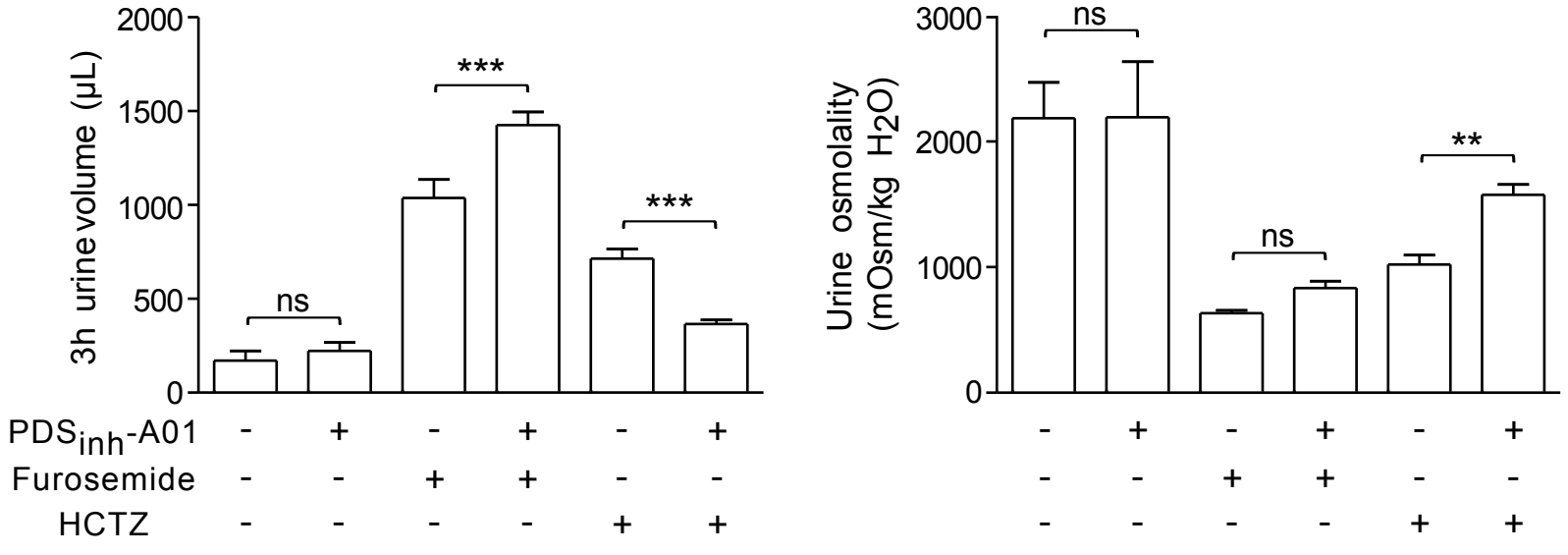
Inactive R^2 =



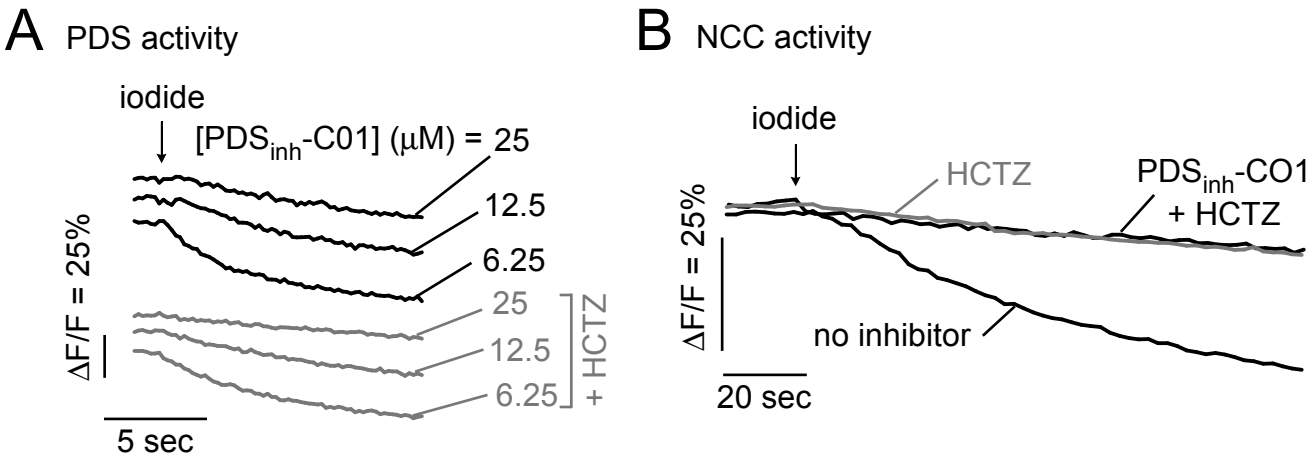
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4