

## Supplementary Material

# Screening of 5- and 6-substituted amiloride libraries identifies dual-uPA/NHE1 active and single target-selective inhibitors

Benjamin J. Buckley<sup>1,2,3,4</sup>, Ashna Kumar<sup>1,2,3</sup>, Ashraf Aboelela<sup>1,2</sup>, Richard S. Bujaroski<sup>1,2,3</sup>, Xiuju Li<sup>5</sup>, Hiwa Majed<sup>1,2</sup>, Larry Fliegel<sup>5</sup>, Marie Ranson<sup>1,2,3,4</sup> and Michael J. Kelso<sup>1,2,3\*</sup>

<sup>1</sup> Illawarra Health and Medical Research Institute, Wollongong, NSW 2522, Australia.

<sup>2</sup> School of Chemistry and Molecular Bioscience, University of Wollongong, NSW 2522, Australia.

<sup>3</sup> Molecular Horizons, University of Wollongong, NSW 2522, Australia.

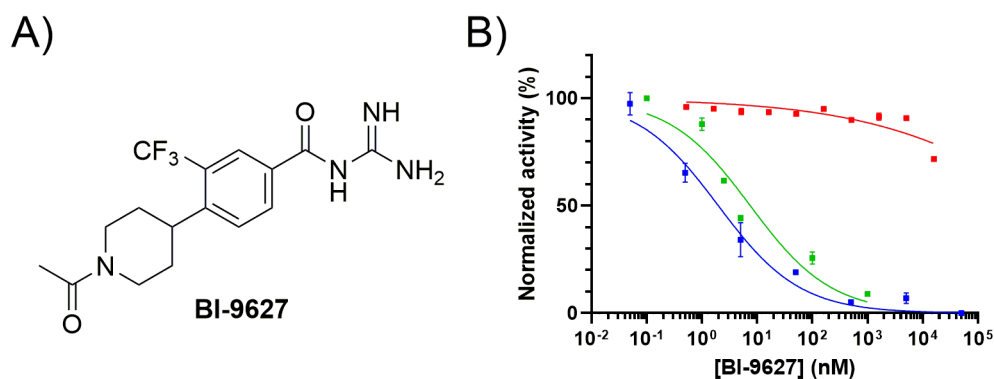
<sup>4</sup> CONCERT-Translational Cancer Research Centre, NSW 2750, Australia.

<sup>5</sup> Department of Biochemistry, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada.

\*Correspondence authors: bbuckley@uow.edu.au, mkelso@uow.edu.au; Tel.: +61-2-4221-5085

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**Figure S1.** Structure of BI-9627 and **B)** NHE1 and uPA inhibition curves for BI-9627. Blue = NHE1 inhibition determined using the plate reader assay. Data points = mean  $\pm$  SEM (n = 3 technical replicates, IC<sub>50</sub> of curve shown = 1.4 nM). Green = NHE1 inhibition determined using the cuvette assay. Data points = mean  $\pm$  SEM (n = 6 technical replicates/concentration, IC<sub>50</sub> = 7.5 nM). Red = inhibition of human uPA activity determined using the reported fluorometric method [25]. Data points = mean  $\pm$  SEM (n = 3 technical replicates). IC<sub>50</sub> was not reached at the highest concentration tested (50  $\mu$ M).

**Table S1.** POLARstar OMEGA settings used for plate reader uPA and NHE1 inhibition assays. <sup>a</sup>biochemical assay using low molecular weight active human uPA. <sup>b</sup>cell-surface assay using exogenous HMW active human uPA.

Parameter	Value		
uPA inhibition with Z-Gly-Gly-Arg-AMC			
Measurement type	fluorescence intensity, top optic		
Read mode	single excitation, single emission		
No. of cycles	60		
Cycle time	60 seconds		
Scan mode	well average		
Scan diameter/ No. of flashes	<sup>a</sup> whole well averaging, 20 flashes per well		
Plate shake mode	<sup>a</sup> double orbital, 3 s prior to each read (600 rpm), <sup>b</sup> off		
Assay temperature	37 °C		
Filter settings	Excitation	Emission	Gain
	355 $\pm$ 10 nm	450 nm	900
NHE1 inhibition assay with BCECF-AM			
Measurement type	fluorescence intensity, top optic		
Read mode	plate multichromatic		
No. of cycles	5		
Cycle time	61 seconds		
Scan mode	orbital averaging		
Scan diameter/ No. of flashes	1 mm/ 8 flashes per well		
Plate shake mode	off		
Assay temperature	37 °C		
Filter settings	Excitation	Emission	Gain
1. pH-sensitive couple	485 $\pm$ 12 nm	520 nm	1100
2. Isosbestic couple	440 $\pm$ 10 nm	520 nm	1100