Supplement to:

Pharmacologic IRE1/XBP1s Activation Confers Targeted ER Proteostasis Reprogramming

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Running Title: Establishment of IRE1/XBP1s Activator Compounds

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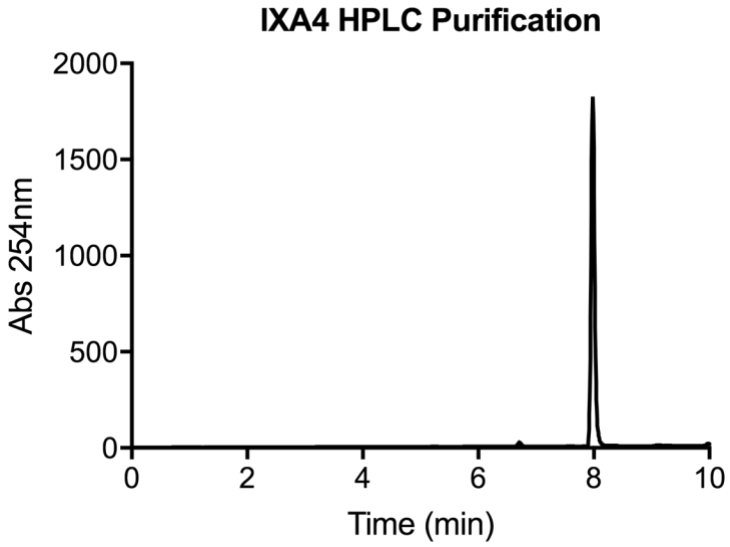
FIGURE LEGENDS

Figure 1. (Chemical characterization of compound IXA4)

HPLC trace from **IXA4** purification confirms >95% purity. $_{1}$H NMR and $_{13}$C NMR confirm identity of compound **IXA4**.

Figure 1.



$_{1}$H NMR (400 MHz, Acetone-d_6) δ 9.32 (s, 1H), 7.99 (s, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.28 (t, *J* = 7.8 Hz, 2H), 7.10 (t, *J* = 8.5 Hz, 2H), 6.93 (dd, *J* = 12.5, 8.1 Hz, 3H), 6.84 (d, *J* = 8.0 Hz, 1H), 5.15 (d, *J* = 56.7 Hz, 2H), 4.34 (t, *J* = 6.2 Hz, 2H), 4.21 (t, *J* = 5.2 Hz, 1H), 4.10 (t, *J* = 5.7 Hz, 1H), 3.77 – 3.58 (m, 1H), 3.25 (s, 2H), 3.00 (s, 1H), 2.80 (t, *J* = 6.2 Hz, 2H), 2.26 (d, *J* = 2.9 Hz, 3H).

$_{13}$C NMR (126 MHz, Acetone-d_6) δ 205.31, 129.85, 129.80, 129.37, 120.57, 114.48, 114.32, 114.30, 63.94, 47.66, 35.96, 19.57.

Table 1. Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	Cell-based, luminescence screen
	Target	ER proteostasis pathways (IRE1/XBP1s activation)
	Primary measurement	Detection of luminescence for XBP1s-Rluc reporter
	Key reagents	Renilla-Glo Luciferase detection reagent (Promega Part E2750)
	Assay protocol	5ul of cells at 2500 cells per well were added to white 1536 well TC treated plates (Greiner, 789173-F) and incubated overnight at 37C, 5%CO2, 95% RH. Compounds were added using a 30nL pintool and plates were incubated for an additional 18hrs under the same conditions. 5uL of Renilla luciferase substrate was added and plates were incubated for 10 minutes at RT prior to reading for luminescence using the PerkinElmer Viewlux.
	Additional comments	
Library	Library size	645,000+ compounds
	Library composition	The Scripps Drug Discovery Library (SDDL) currently consists of over 650,000 unique compounds, representing a diversity of drug-like compound scaffolds targeted to traditional and non-traditional drug-discovery biology. The SDDL contains more than 20,000 compounds unique to Scripps and a number of focused sub-libraries for screening popular target classes (e.g. kinases, transferases, GPCRs, ion channels, nuclear receptors, hydrolases, transporters), also diversity discovery sets (e.g. click-chemistry, PAINS-free, FSp3 enriched, covalent inhibitors and natural product collections) as well as clinically relevant compounds and a +3,500 FDA-approved set for drug repurposing studies.
	Source	Scripps Drug Discovery Library (SDDL)
	Additional comments	
Screen	Format	1536 well
	Concentration(s) tested	5.17µM
	Plate controls	Vehicle (DMSO) and Tg (500nM)
	Reagent/ compound dispensing system	Kalypsys/GNF PinTool compound transfer unit
	Detection instrument and software	PerkinElmer ViewLux

	Assay validation/QC	EC50 of positive control within 3-fold, Z'>0.5, %CV of DMSO wells <10%
	Correction factors	None applied
	Normalization	Based on DMSO reference wells (0% activation) and 500 nM Tg (100% activation)
	Additional comments	
Post-HTS analysis	Hit criteria	> 13.83% of Tg positive control
	Hit rate	1.56%
	Additional assay(s)	Confirmation screening in triplicate, dose response, cytotoxicity
	Confirmation of hit purity and structure	All selected hits were validated through LCMS and/or NMR for purity and structural identification to provide adequate QC for future studies. Note that purity acceptance is set at ≥80%.
	Additional comments	

Table 2. Representative 128 compounds from structural analysis of small molecule IRE1/XBP1s activators (see Excel spreadsheet)

Table 3. Internal Library Numbers for Selected IRE1/XBP1s Activators (see Excel spreadsheet)

Table 4. GO analysis of prioritized IRE1/XBP1s activator transcriptional profiles (see three tabs in the included Excel spreadsheet)