Supplemental data for manuscript # MOLPHARM-AR-2020-000184

Cysteine modification by ebselen reduces the stability and cellular levels of 14-3-3 proteins

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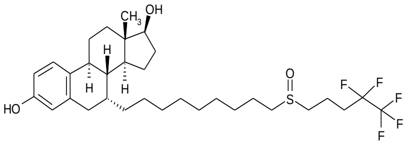
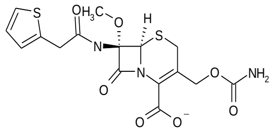
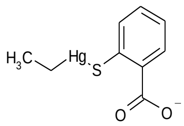
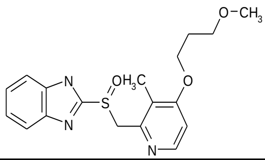
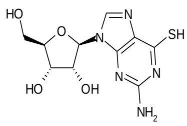
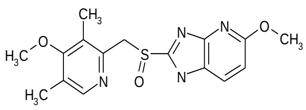
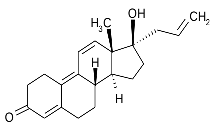
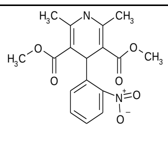
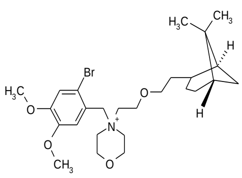
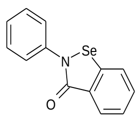
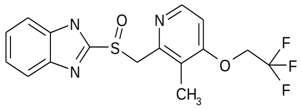
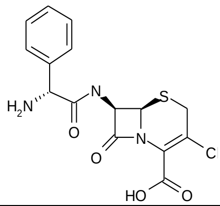
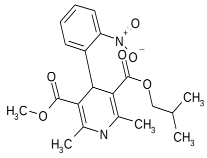
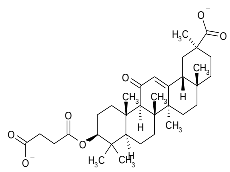
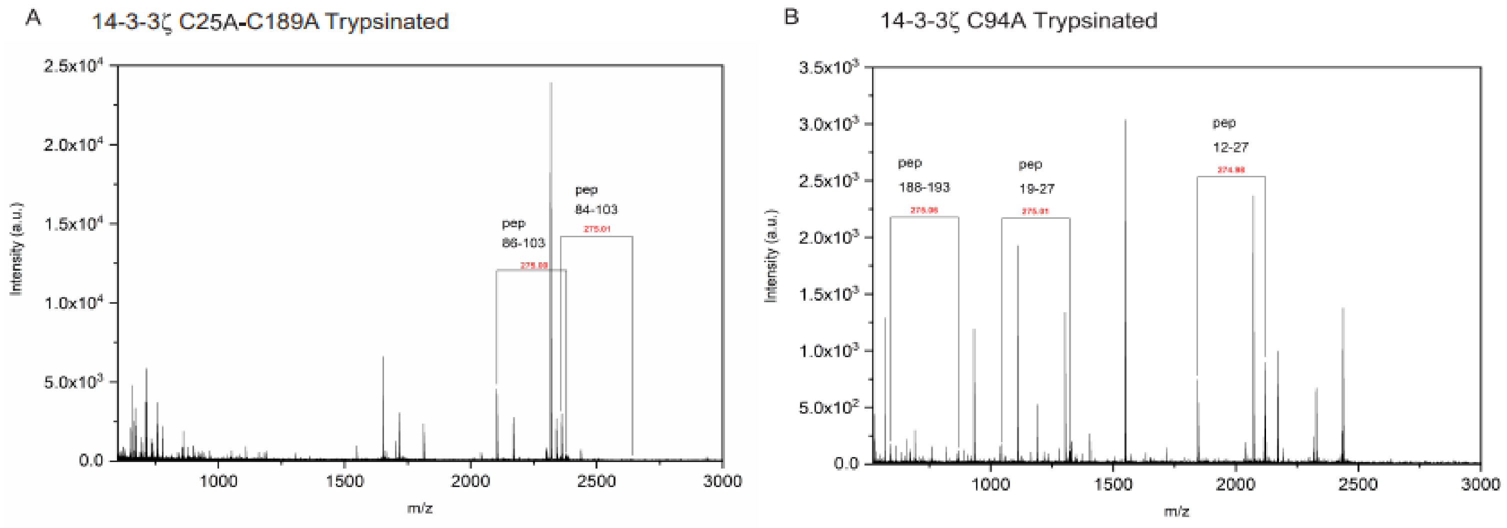
ID	Drug name	Formula	Therapeutic class/ therapeutic effect	ΔT_m at 400 μM (◦C)
1	Fulvestrant*		Endocrinology/ Antineoplastic	-23.6
2	Cefoxitin		Metabolism/ Antibacterial	-17.3
3	Thimerosal		Infectiology/ Antiseptic	-11.7
4	Rabeprazole[1]		Metabolism/ Antiulcer	-11.4
5	Mercaptopurine		Immunology/ Immunosuppressant	-10.8
6	Thioguanosine		Metabolism/ Antineoplastic	-9.2
7	Tenatoprazole[1]		Metabolism/ Antiulcer	-8.6
8	Altrenogest*		Endocrinology/ Progestogen	-8.4
9	Nifedipine[2]		Cardiovascular/ Antianginal	-7.2
10	Pinaverium[2]		Neuromuscular/ Antispastic	-6.7
11	Ebselen		Metabolism/ Anti-inflammatory	-7.0

12	Lansoprazole[1]		Metabolism/ Antiulcer	-6.0
13	Cefaclor		Infectiology/ Antibacterial	-6.0
14	Nisoldipine[2]		Cardiovascular/ Antianginal	-5.8
15	Carbenoxolone*,[3]		Metabolism/ Antiulcer	-5.1

Supplemental Table 1. Small-molecule drug hits obtained from the primary DSF screen. The midpoint melting temperature (T_m) of 14-3-3ζ with 4% DMSO was 61.1 ± 0.5°C, and 15 hits that caused IΔT_mI ≥ 10 x SD (5.1ºC), used as a cut-off value, were selected for concentration dependent studies. Based on its consistent concentration-dependent effect and reduced toxicity, ebselen was selected for further studies. *Steroids. [1]Proton pump inhibitor. [2]Calcium channel blocker [3]Probable calcium channel blocker

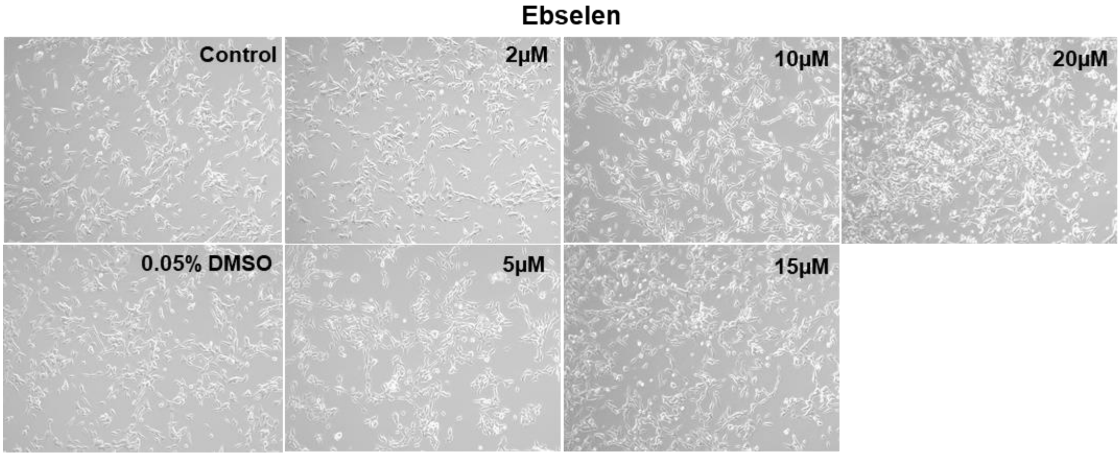
Supplemental Figure S1. Amino acid sequences of the seven isoforms of 14-3-3 are presented. The cysteine residues are highlighted in yellow.



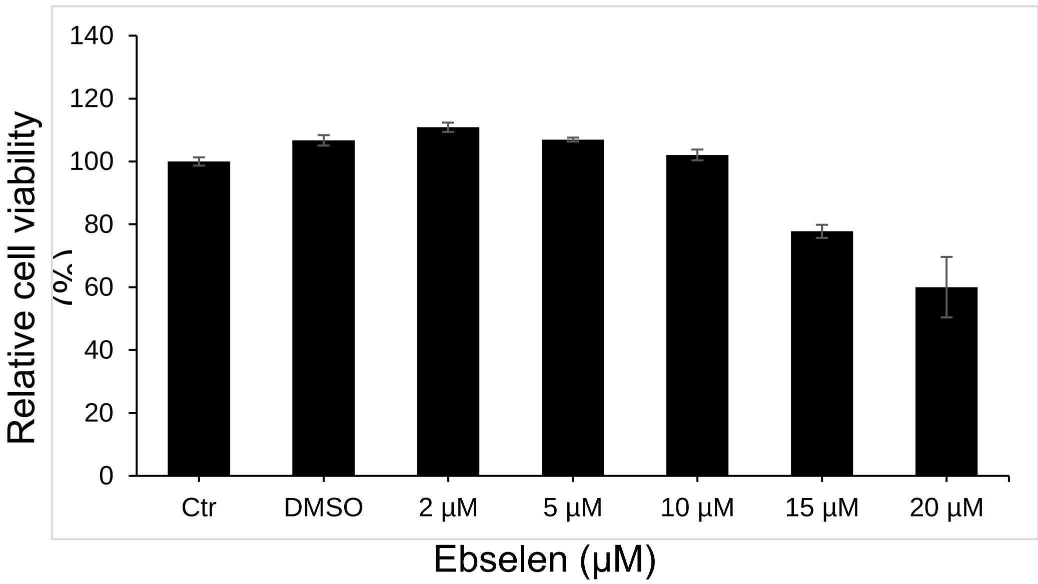
Supplemental Figure S2. MALDI-TOF MS/MS peptide analysis of trypsin digested 1433ζ mutant constructs. a) 1433ζ-C25A-C189A and b) 1433ζ-C94A, after ebselen treatment. Peptides containing cysteines in tagged and untagged form (peptides with a mass shift of 275 Da) are denoted. In a) the figure shows two peptides involving ebselen tagging of C94 in very small amounts. In b) the figure shows a peptide involving C189 and two peptides involving C25.

Supplementary Figure S3. Viability of SHSY5Y cells treated with increasing concentration of ebselen and ebselen oxide. A Cell Titer-Blue assay was performed to examine cell viability of SHSY5Y cells treated with increasing concentration of ebselen and ebselen oxide. The cells were plated in 96-well plates at the density of 25000 cells per well and 5 h later were treated with different concentration of ebselen, ebselen oxide and DMSO (0.05%). Cells were then incubated for 16 h and then 20 µL of Cell Titre Blue reagent was added to each well. The plates were incubated for 2hrs before the fluorescence (λ=590) was measured with Victor 3 1420 Multilabel counter plate reader. A, Cell images using ebselen, B, relative cell viability using ebselen, C Cell images using ebselen oxide D, relative cell viability with ebselen oxide. Statistical analyses by 2-tailed Student's t test. (*) p< 0.05, (**) p < 0.005, (***) p < 0.001, (****) p < 0.0001. All data are presented as mean ± SEM.

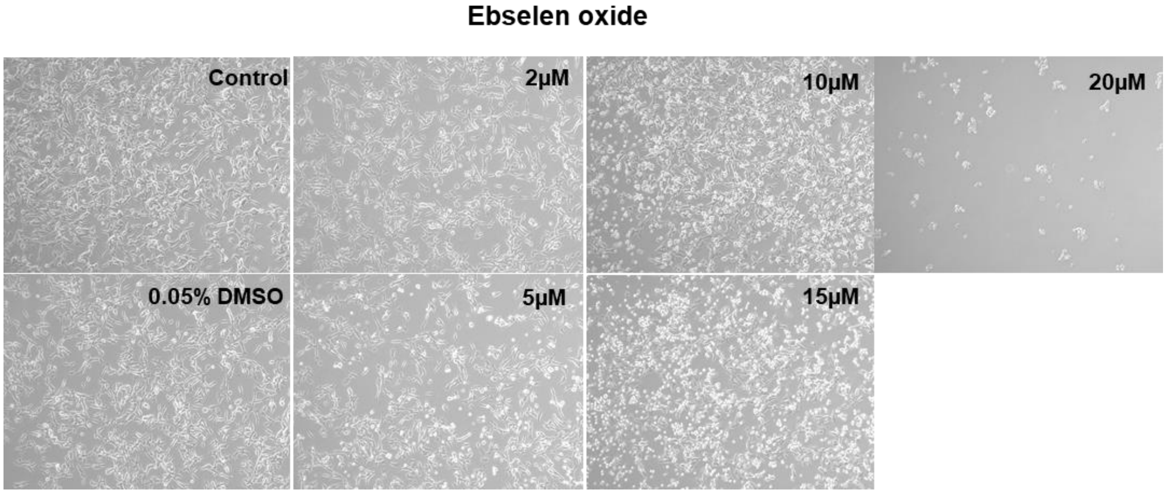
A



B

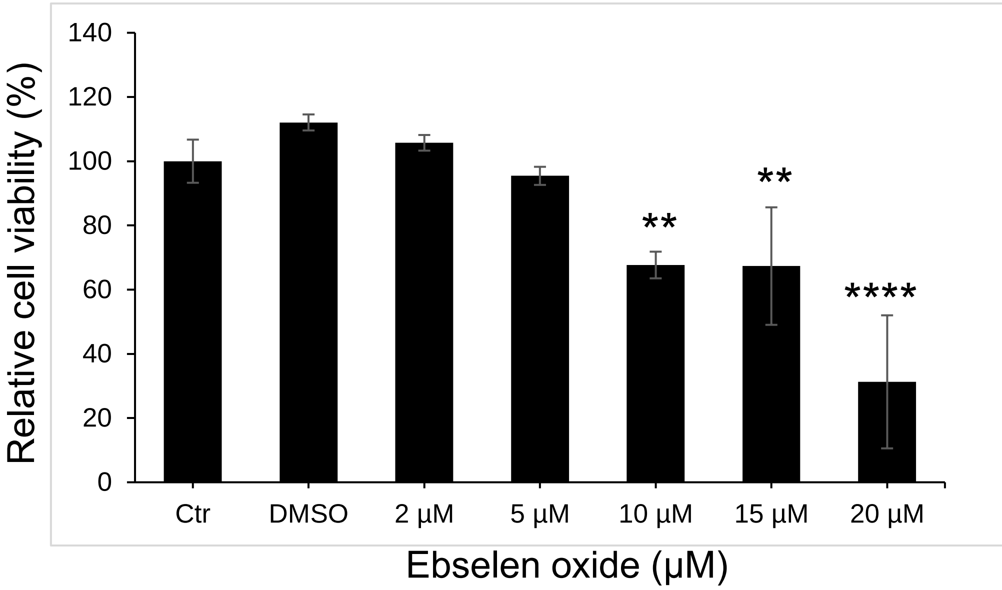


C



Ebselen oxide
Control
2μM
10μM
20μM
0.05% DMSO
5μM
15μM

D



Relative cell viability (%)
140
120
100
80
60
40
20
0
**
**
****
Ctr
DMSO
2 μM
5 μM
10 μM
15 μM
20 μM
Ebselen oxide (μM)