

A new class of compounds for the protection of cells during storage at 2 – 8 °C



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3. Dugbarty, G.J. *et al.* (2014) Dopamine and SUL-121 protect kidney against *in vivo* hypothermia/rewarming-induced injury via CBS/H₂S pathway. FIGON Dutch Medicine Days (page 25).

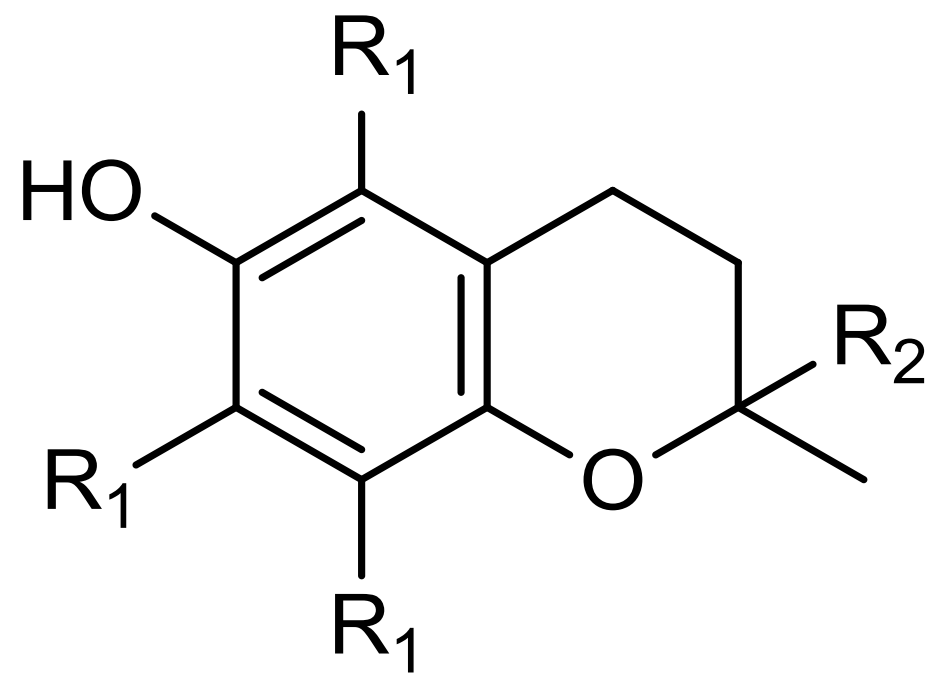
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Introduction

Cell preservation/shipping techniques include live-culture or cryopreservation. Inspired by hibernation¹, nature's solution to low temperature and lack of nutrition, we have designed and synthesized a new class of small molecules (henceforth, SUL-compounds)² complying with Rule-of-5. Efficacy in storage of mammalian cells at ultraprofound hypothermia (2 – 8°C) was tested in a high-throughput screening and compared with compounds with known protective capacities (Trolox, dopamine).



The chemical structure of 60 SUL-compounds². R₁ and R₂ consist of CH₃ or C₃H₇. All compounds were synthesized by Syncom B.V., Groningen.

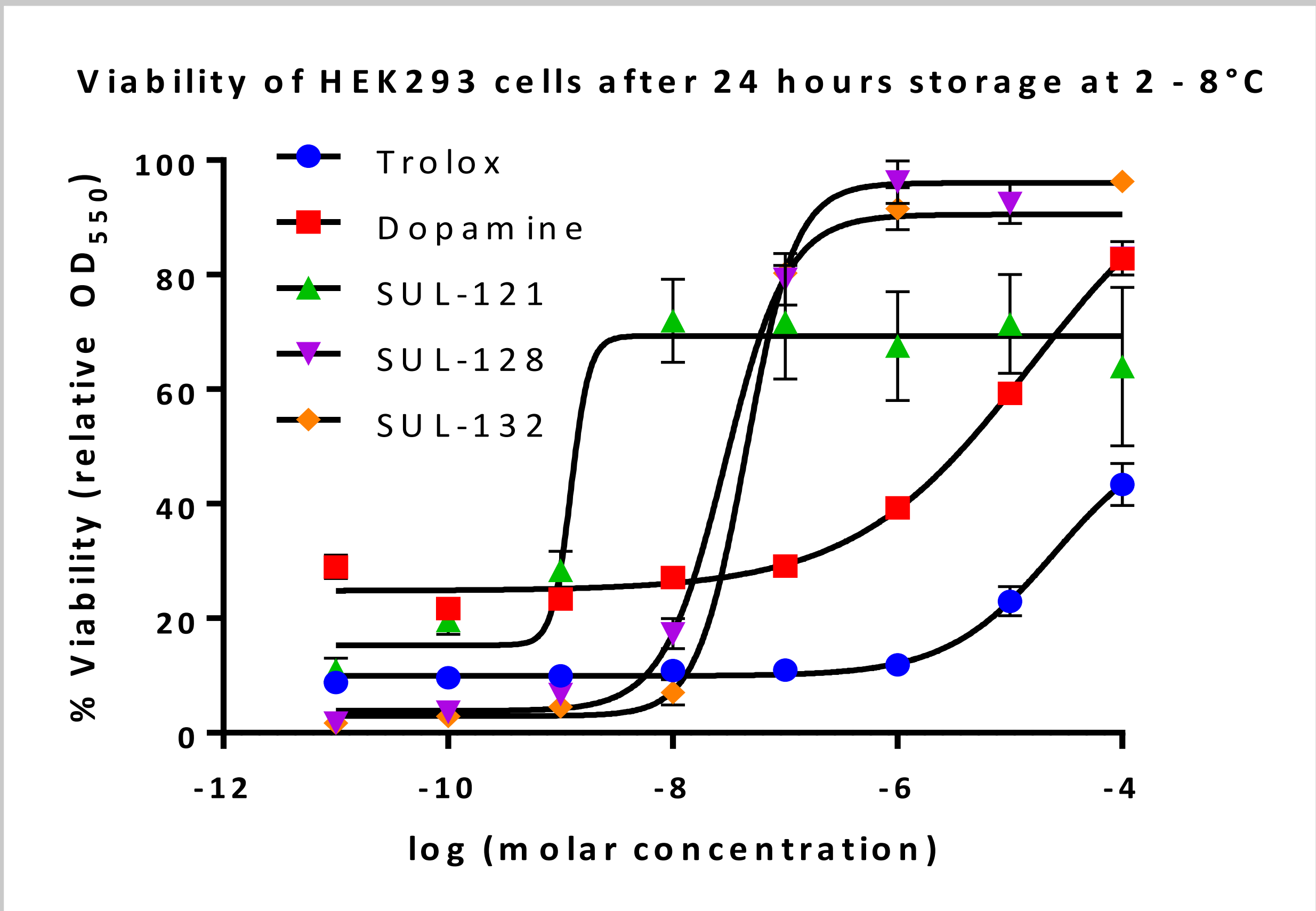


Figure 1. SUL-compounds protect cells from cooling/rewarming at high potency. HEK293 were pre-incubated for 2 hours in standard cell culture before storage at 2–8°C for 24 hours, followed by 4 h rewarming at 37°C. Cell viability was assessed by Neutral Red uptake. High doses do not show toxicity. Data are mean ±SEM (n=6).

Table 1. Chemicophysical properties of SUL-compounds.

MW, molecular weight; TEAC, Trolox Equivalent Antioxidant Capacity.

Compound	MW (kDa)	ClogP	TEAC	EC ₅₀ (M)
SUL-121	< 400	2,60	0,84	5,66E-09
SUL-128	< 400	4,06	1,05	1,08E-08
SUL-138	< 400	1,84	0,82	2,47E-08
SUL-132	< 400	1,84	0,90	3,00E-08
SUL-134	< 400	3,05	0,90	5,05E-08
SUL-122	< 450	1,56	0,52	5,48E-08
SUL-125	< 400	2,91	1,01	1,23E-07
SUL-135	< 400	3,05	0,54	2,08E-07
SUL-137	< 450	3,57	0,22	3,30E-07
SUL-95	< 400	2,84	0,41	5,45E-07
SUL-142	< 400	0,04	0,97	6,06E-07
SUL-131	< 400	3,88	n.d.	6,23E-07
SUL-141	< 400	0,04	0,98	6,57E-07
SUL-139	< 450	1,01	0,91	3,37E-06
Trolox	250,29	n.d.	1,00	1,17E-05
Dopamine	189,4	n.d.	n.d.	1,54E-05

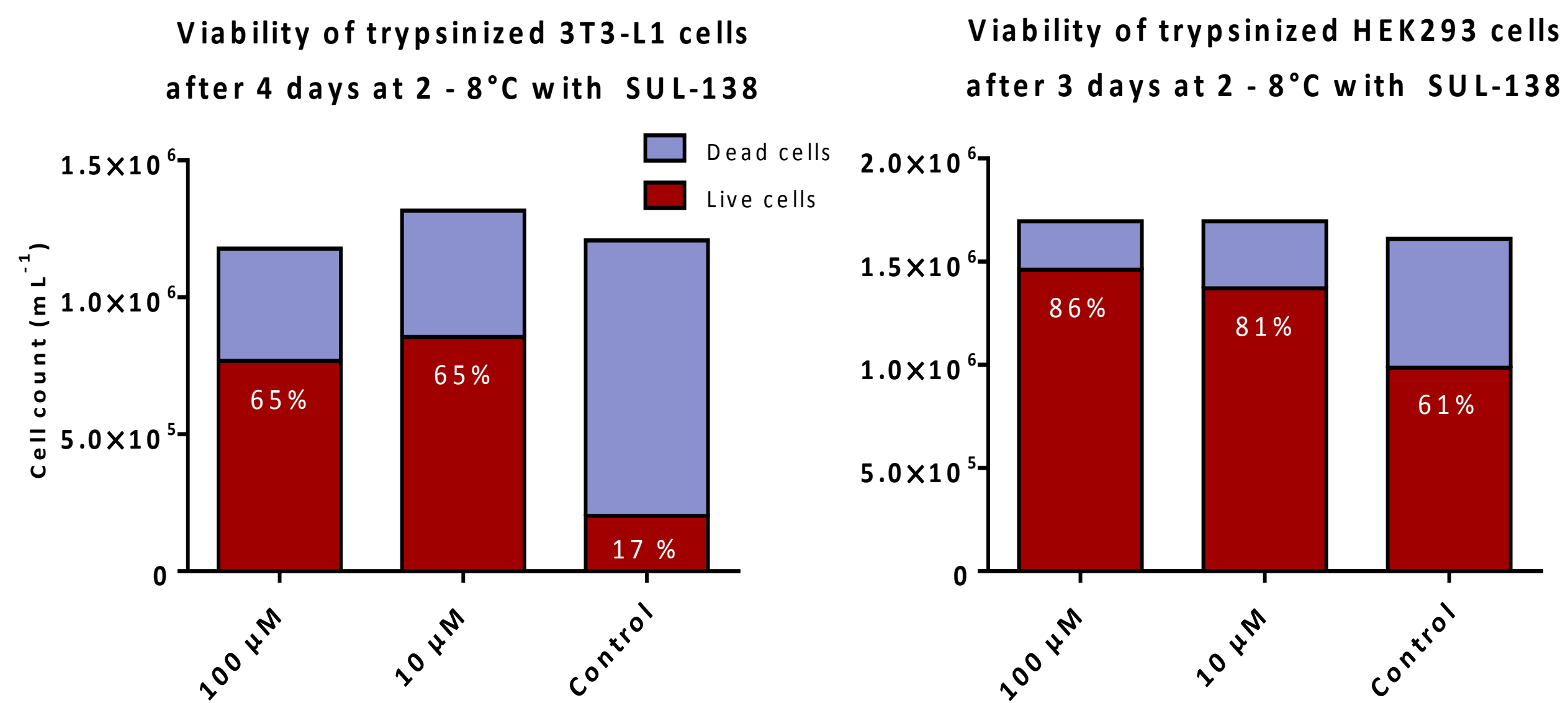
Results

SUL-compounds protect cells stored at 2–8°C at 1.000 and 10.000 fold potency compared to dopamine and Trolox (fig 1).

Cell growth rate and morphology is maintained after storage at 2–8°C in cells protected by SUL-compounds (fig 2).

SUL-compounds were successfully used to store 7 human and rodent cells at 2 – 8° for up to 21 days.

EC₅₀ of SUL compounds did not correlate with Trolox Equivalent Antioxidant Capacity (table 1).



Cell line	Stored days	Application
HUVEC	13 days	Model for endothelial damage
HEK293	7 days	Host for production of recombinant proteins
3T3-L1	7 days	<i>In vitro</i> cytotoxicity cell assays
SH-Sy5y	7 days	Model for neuronal function and differentiation
MS5	5 days	Feeder layer for stem cells and iPS cells
CaCo-2	7 days	Model for human intestinal drug absorption
NRK-52E	21 days	Model for kidney epithelial damage

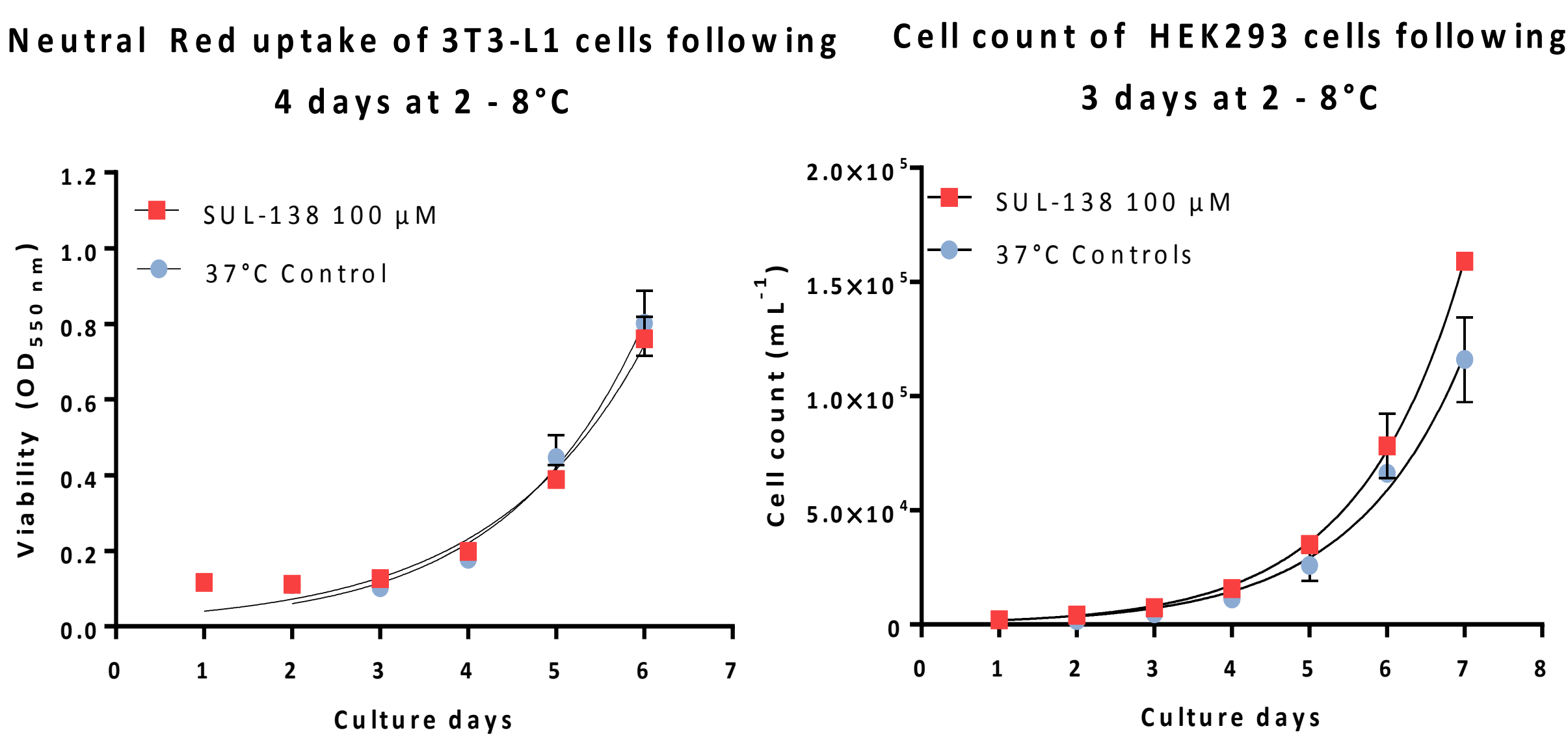


Figure 2. (Top Left) Cells were immediately stored at 2 – 8°C after trypsinization. At this temperature, attachment of cells to the culture vessel is absent. SUL-compounds protect freshly trypsinized cells during storage at 2 – 8 °C. **(Top Right)** Cell growth of cells treated with 100 μM SUL-138 showed unaltered growth characteristics as compared to 37°C cells. **(Lower left)** Morphology of NRK-52E and HUVEC cells after storage at 2 – 8°C.

Conclusions

SUL-compounds represent a new class that protect cells during storage at 2–8°C, independent of antioxidant characteristics in the micro- to nanomolar range.

SUL-compounds are now tested to protect kidney against hypothermia/rewarming injury³ and to hypothermic DNA damage⁴.

SUL-compound 121 protects from ROS associated damage in a model for chronic obstructive pulmonary disease⁵.

SUL-109 is launched as a Research Only product to store mammalian cells and tissues at 2 – 8°C (ROKEPIE®-S01)⁶.



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