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Selective inhibitors of death in mutant huntingtin cells

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Huntington disease (HD) is an inherited neurodegenerative disorder with unclear pathophysiology. We developed a high-throughput assay in a neuronal cell culture model of HD, screened 43,685 compounds and identified 29 novel selective inhibitors of cell death in mutant huntingtin–expressing cells. Four compounds were active in diverse HD models, which suggests a role for cell death in HD; these compounds are mechanistic probes and potential drug leads for HD.

Huntington disease (HD) is a fatal autosomal dominant neurodegenerative disease caused by polyglutamine expansion in a stretch of glutamine repeats in the huntingtin (htt) protein[1]. Mutant htt (>35 glutamine repeats) has been proposed to cause toxicity by multiple mechanisms[2] (**Fig. 1a**). However, a clear understanding of how these mechanisms contribute to HD remains elusive. Striatal neuronal cell loss is ubiquitous in HD-afflicted individuals[1]. Though the role for cell death in HD pathophysiology is debated[1], prevention of neuronal death can ameliorate disease phenotype in mouse models[3,4]. However, the mechanisms by which mutant htt perturbs the cell death machinery are unclear. To address the role of mutant htt in cell death, we devised a screen to identify small molecules that selectively reverse cell death in a mutant htt–expressing neuronal cell line. This approach could potentially identify (i) chemical probes for elucidating the mechanisms underlying mutant htt toxicity and (ii) potential drug leads.

We chose a cell culture HD model that uses immortalized striatal neurons (ST14A). ST14A cells were stably transfected with the N-terminal 548-amino-acid fragment of mutant (120 glutamine repeats) human htt to generate the N548 mutant cell line[5] (**Supplementary Methods** online). In serum-deficient medium at 39 °C, N548 mutant cells undergo cell death at a rate greater than that of the parental ST14A[5]. We developed a high-throughput screening assay for detecting N548 mutant cell death in a 384-well plate format (**Supplementary Methods**). We screened 43,685 compounds and

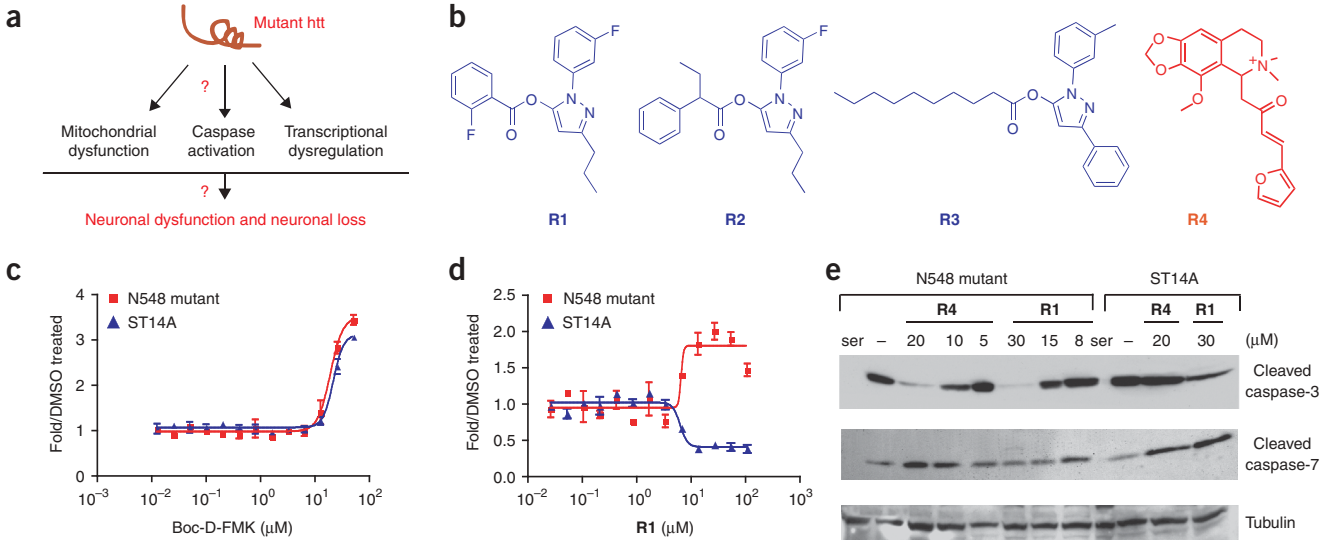

Figure 1 Novel compounds selectively suppress cell death and caspase-3 activation in N548 mutant cells. (**a**) Diagram of multiple mechanisms implicated in HD. (**b**) Structures of compounds **R1**, **R2**, **R3** and **R4**. **R1**, **R2** and **R3** (colored blue) are structurally related, whereas **R4** (red) is structurally distinct. (**c**) Dose-response curves of nonselective rescue by the pan-caspase inhibitor Boc-D-FMK (**30**) (**Supplementary Methods**). Viability is shown as fold increase above DMSO-treated cells. Error bars represent one s.d. (**d**) Selective rescue of N548 mutant cells by **R1** (see **Supplementary Fig. 3** for dose responses for **R2**, **R3** and **R4**). (**e**) Effect of **R1** and **R4** on cleavage of caspase-3 and caspase-7 induced by serum-deficient medium for 24 h; DMSO vehicle (–) and 10% serum (ser) were controls (see **Supplementary Fig. 4** for data on **R2** and **R3**).

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Table 1 Activity of novel compounds R1 to R4 in diverse HD models

	Model				
Cmpd	N548 mut (μM)	PC12 (μM)	Yeast	*C. elegans* (mM)	HD brain slice (μM)
R1	12	145	i.a.	3	15
R2	11	i.a.	i.a.	i.a.	15
R3	10	100	i.a.	i.a.	i.a.
R4	9	22	i.a.	2.2	i.a.

i.a., inactive; Cmpd, compound. All concentrations are effective concentrations for half-maximum response (EC_{50}) values, except for the *C. elegans* assay, in which the highest effective concentrations are shown.

identified 29 novel compounds, designated **R1** through **R29** (**Fig. 1b** and **Supplementary Table 1** online), that selectively rescue N548 mutant cell viability, without rescuing cell viability in ST14A cells. We confirmed the enhanced viability by two additional viability criteria (**Supplementary Methods**).

To prioritize hits for further study, we subjected these 29 compounds to additional filters (**Supplementary Fig. 1** online). We excluded a decrease in mutant htt protein expression upon treatment with selective hits as a mechanism for selective suppression of cell death in N548 mutant cells (**Supplementary Fig. 2** online). Most compounds rescued cell death in at least one additional striatal cell line expressing a distinct mutant htt construct (N63 or full-length htt)[5], thereby assuring us of selectivity for mutant htt–expressing cells relative to parental ST14A cells. We tested all compounds for rescue of mutant htt–induced phenotype in rat pheochromocytoma (PC12)[6], yeast[7] and *Caenorhabditis elegans*[8] HD models (results in **Supplementary Table 2** online). Four new compounds (**Fig. 1b**) were active in multiple HD models (**Table 1** and **Supplementary Fig. 3** online); of these four, **R1**, **R2** and **R3** are structurally related. We next tested these four compounds in an *in vivo*–like brain slice HD model (**Supplementary Methods**). **R1** and **R2** rescued degeneration in this HD model, whereas **R3** and **R4** were inactive (**Table 1** and **Supplementary Fig. 3**).

Because these compounds were selective for cells expressing mutant htt, we used them to gain insight into the mechanisms of mutant htt toxicity. Caspases have been implicated in htt toxicity, both as targets activated by mutant htt[9] and as regulators of mutant toxicity that act by cleaving mutant htt to generate toxic htt fragments[10]. We found that caspase activation contributes to cell death in both ST14A and N548 mutant cells (**Fig. 1c** and **Supplementary Fig. 4** online). We tested the possibility that mechanisms leading to caspase activation differ between N548 mutant and ST14A cells. Both **R1** and **R4** selectively inhibited cleavage of caspase-3, but not caspase-7, in a dose-dependent manner; inhibition of caspase-3 cleavage correlated with selective rescue of N548 mutant cells by these compounds (**Fig. 1d**,**e**). These results, together with reports of enhanced caspase-3 processing in N548 mutant cells[5] and increased caspase-3 expression in the R6/2 HD mouse model[9], suggest a role for aberrant caspase-3 activation in HD.

As these compounds were active in multiple HD models, we also assessed their potential as drug leads. **R1**, **R2** and **R4** passed *in silico* medicinal chemistry filtering, which suggests adequate oral bioavailability (**Supplementary Table 3** online). We analyzed the structure-activity relationship by testing 57 analogs of **R1** and 23 analogs of **R4**; we identified several active analogs for both scaffolds (**Supplementary Table 3**). This information will be useful for synthesizing more potent analogs. These compounds are potential drug leads and should be tested in other HD models and in models of other polyglutamine diseases[11].

Given that these compounds were active in different assays, they may target mechanisms of mutant htt toxicity that are conserved across the models tested. Considering the undefined mechanisms involved in neuronal loss in HD, these compounds should be valuable tools for investigating the mechanisms by which mutant htt affects caspases and induces neuronal loss. Furthermore, the assays and hit prioritization strategies described should be useful for identifying other HD drug leads.

Note: Supplementary information and chemical compound information is available on the Nature Chemical Biology website.

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AUTHOR CONTRIBUTIONS

H.V. and B.R.S. designed the screen and wrote the manuscript. E.C. provided essential reagents, cell lines and advice. H.V. performed the high-throughput screen and tested compounds in PC12 and yeast HD models. H.V., C.V. and A.C.H. designed the *C. elegans* assay; H.V. and C.V. performed the *C. elegans* assay. C.T.D. and D.C.L. designed the brain slice assay, C.T.D. tested compounds in the assay and D.C.L. performed analysis of the data.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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