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Degradation of huntingtin mediated by a hybrid molecule composed of IAP antagonist linked to phenyldiazenyl benzothiazole derivative

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ABSTRACT

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by aggregation of mutant huntingtin (mHtt), and removal of mHtt is expected as a potential therapeutic option. We previously reported protein knockdown of Htt by using hybrid small molecules (Htt degraders) consisting of BE04, a ligand of ubiquitin ligase (E3), linked to probes for protein aggregates. Here, in order to examine the effect of changing the ligand, we synthesized a similar Htt degrader utilizing MV1, an antagonist of the inhibitor of apoptosis protein (IAP) family (a subgroup of ubiquitin E3 ligases), which is expected to have a higher affinity and specificity for IAP, as compared with BE04. The MV1-based hybrid successfully induced interaction between Htt aggregates and IAP, and reduced mHtt levels in living cells. Its mode of action was confirmed to be the same as that of the BE04-based hybrid. However, although the affinity of MV1 for IAP is greater than that of BE04, the efficacy of Htt degradation by the MV1-based molecule was lower, suggesting that linker length between the ligand and probe might be an important determinant of efficacy.

Huntington's disease (HD), one of a group of neurodegenerative disorders including Alzheimer's disease and Parkinson's disease, has autosomal dominant inheritance, causes various symptoms such as motor dysfunction and cognitive slowing with neuronal cell death, and leads to death within 15–20 years. The disease is caused by abnormal expansion of the CAG repeat sequence in *HTT* exon 1 gene to more than 35 repeats, which results in the expression of aggregation-prone mutant huntingtin (mHtt) with an extended *N*-terminal polyglutamine tract (polyQ).[1] Currently, there is no effective therapy. Based on reports showing that mHtt aggregates, as well as soluble oligomer intermediates, are cytotoxic, several groups have proposed that removal of mHtt aggregates might be an effective therapeutic approach.[2–4] Based on this idea, we previously developed hybrid small molecules that induce degradation of Htt,[5] based on protein knockdown technology. Specifically, we synthesized hybrid molecules consisting of a ligand for the protein of interest (POI; Htt in this case) and a ligand for ubiquitin ligase (E3). These hybrid molecules work as a tether bringing the POI and E3 into close proximity, thereby inducing ubiquitination and subsequent proteasomal degradation of the POI (Fig. 1a).[6] For this purpose, we utilized probes for protein aggregates as the mHtt-recognizing moiety, because no specific ligand for Htt has yet been discovered (Fig. 1b). The developed hybrid molecules successfully reduced Htt levels in fibroblasts derived from HD patients.

In recent years, various small-molecular E3 ligands have been reported, including bestatin derivatives,[7–13] inhibitor of apoptosis protein (IAP) antagonists,[14–17] thalidomide analogs[18–21] and ligands for von-Hippel Lindau,[22–24] and all of them are potentially available for protein knockdown. An appropriate choice is important, because the use of different E3 ligands to prepare hybrid molecules for protein knockdown targeting the same protein can result in different activity.[25] The Htt degraders **1** and **2** (Fig. 2) in our previous report consist of a bestatin derivative, BE04 (**3**), which is a specific ligand of cellular inhibitor of apoptosis protein 1 (cIAP1), conjugated to a probe for protein aggregates, benzothiazole derivative BTA (**4**),[26] or phenyldiazenyl benzothiazole derivative PDB (**5**)[27], respectively (Fig. 2). In this study, we focused on MV1 (**6**), an IAPs antagonist, as an alternative E3 ligand because, piecing together the binding tests from two literatures, MV1 is likely to have a higher affinity for cIAP1 than BE04[7,28] and besides, MV1 is also able to interact with other IAPs.[28] Additionally, MV1 is a specific antagonist to IAPs while BE04 shows weak aminopeptidases-inhibitory activity. We synthesized the Htt degrader **7** composed of MV1 conjugated to PDB by coupling the PDB derivative **8**[5] with Boc-protected MV1 (**9**),[15] followed by removal of *N*-Boc group, as shown in Scheme 1.

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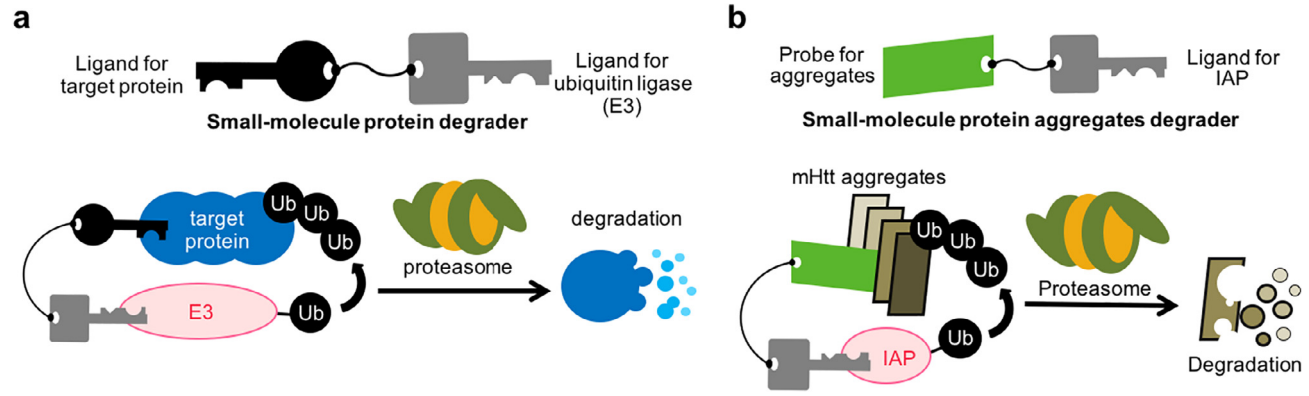


Fig. 1. (a) Schematic illustration of the principle of protein knockdown technology. (b) Schematic illustration of the present system for degrading mHtt aggregates. A small-molecular protein aggregates degrader induces complex formation between the target protein and inhibitor of apoptosis protein (IAP), a kind of E3, followed by ubiquitination (Ub) and proteasome-mediated degradation of mHtt aggregates.

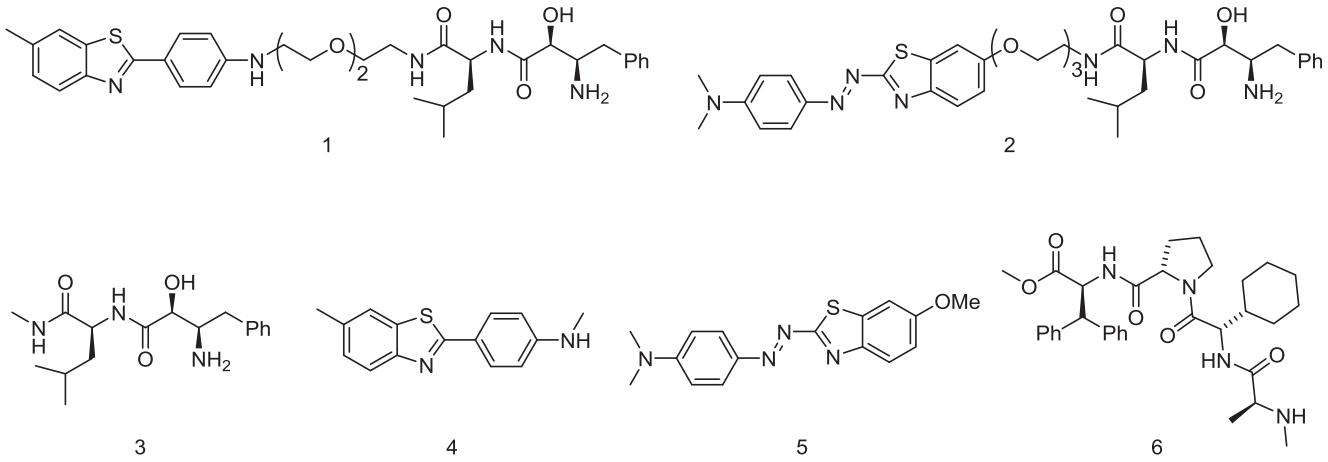
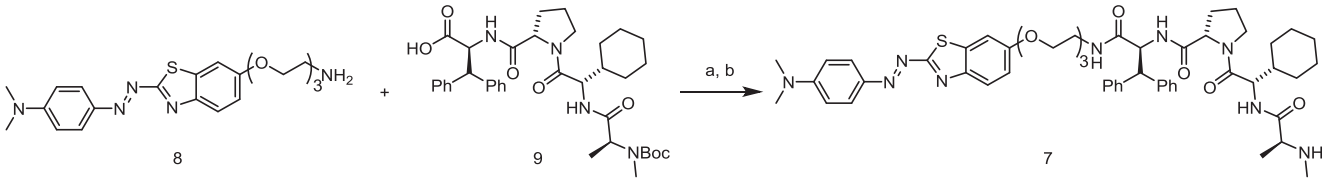


Fig. 2. Structures of Htt degraders **1**, **2**, BE04 (**3**), BTA (**4**), PDB (**5**) and MV1 (**6**).



Scheme 1. Synthesis of the new Htt degrader **7**. Reagents and conditions: (a) EDC, HOBt·H_2O, DIEA, DMF, rt, 24 h, 82%; (b) $ZnBr_2$, DCM, r.t., overnight, 44%.

First, we examined the mHtt degradation-inducing activity of **7** by means of western blot analysis. Compound **7** dose-dependently decreased the mHtt level in fibroblasts derived from an HD patient (HD fb) after treatment for 24 h. Its efficacy did not increase further upon more prolonged incubation (Fig. 3). However, even though MV1 should interact with IAPs with higher affinity than BE04, as mentioned above, the efficacy of compound **7** was less than that of **1** and **2**, which significantly decrease mHtt levels and make the signal on western blotting almost invisible. Since the linker length of hybrid molecules is critical for protein knockdown,[8,15] we speculated that the linker length of **7** might not provide an optimal distance between mHtt and IAPs for ubiquitination. As another possibility, a research group of Naito recently reported that BE04-type degrader for TACC3 protein recruits anaphase-promoting complex/cyclosome in complex with CDH1 (APC/C^{CDH1}), a physiological E3 for TACC3, as well as IAP.[29] Although they did not evaluate whether BE04-type degraders for other target proteins universally recruits their physiological E3s, BE04-type Htt degrader might also recruit physiological E3s for Htt such as C-terminus of Hsp70-interacting protein (CHIP)[30] and enhance its ubiquitination to Htt.

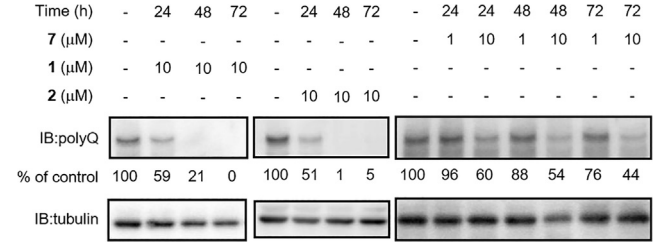


Fig. 3. Compound **7** reduced the mHtt level in HD fb. Western blot analysis with anti-polyQ antibody was performed after treatment with Htt degraders **1**, **2** and **7** at the indicated concentrations for the indicated times.