Reducing mutant Huntingtin protein expression in living cells by a newly identified RNA CAG binder

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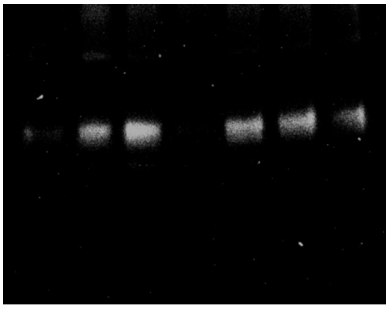
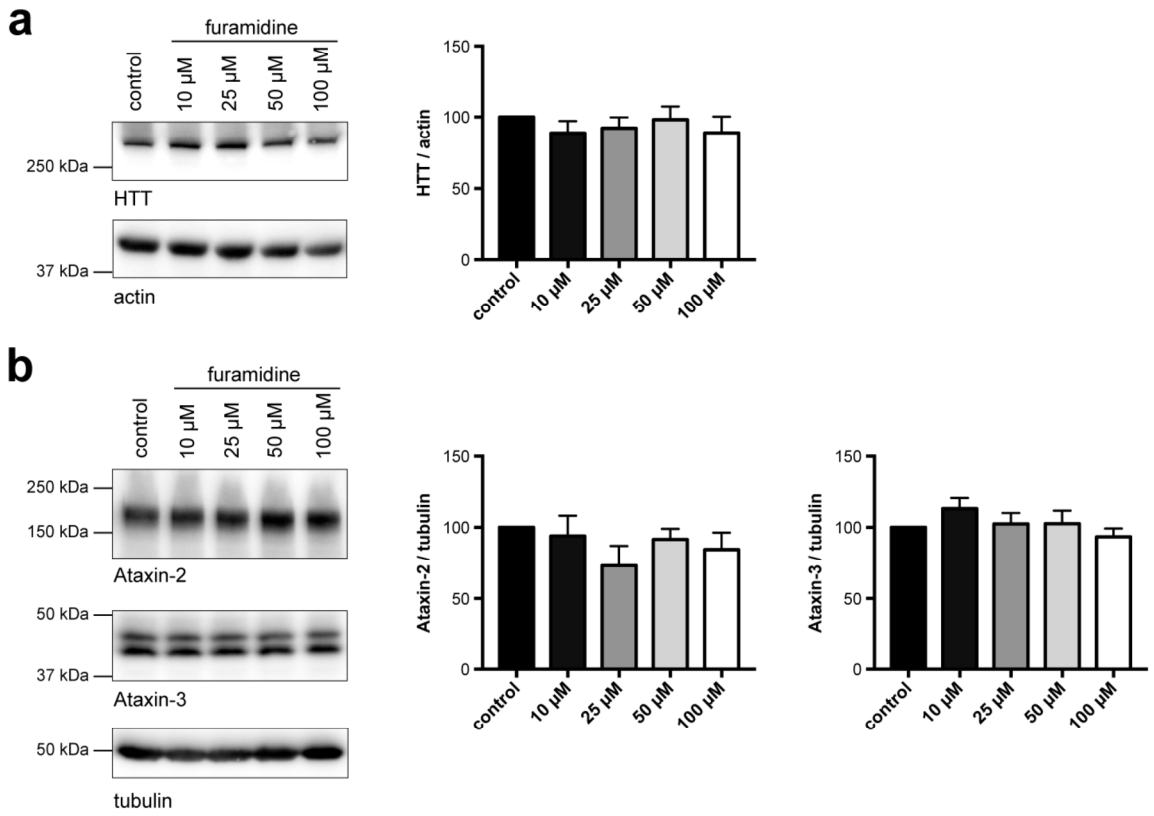
SUPPORTING INFORMATION

SUPPLEMENTARY FIGURES



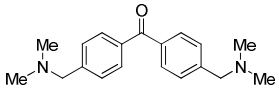
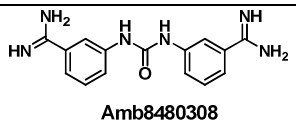
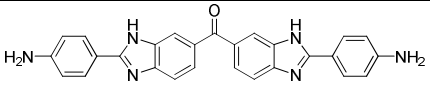
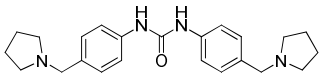
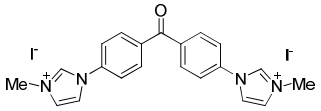
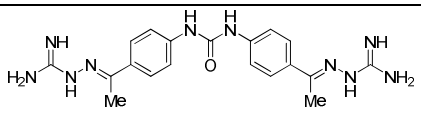
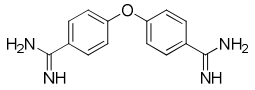
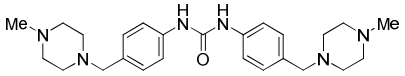
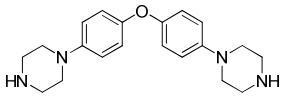
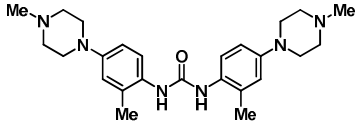
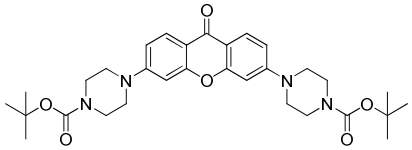
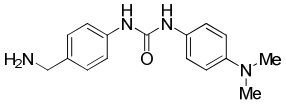
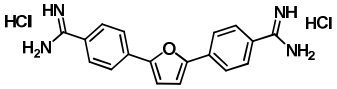
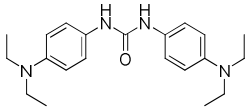
Supplementary Figure S1: Full-length blot of Figure 2a.

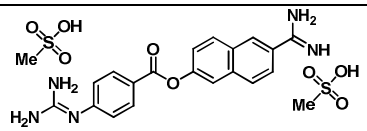
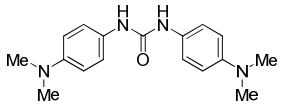
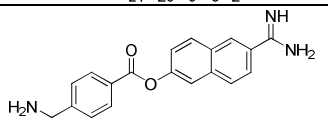
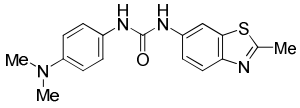
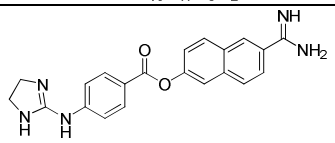
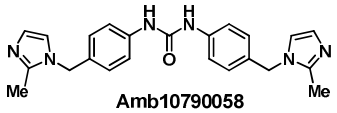
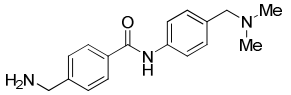
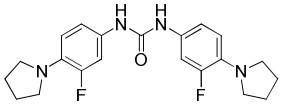
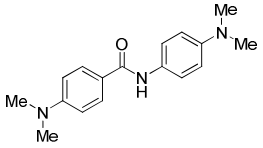
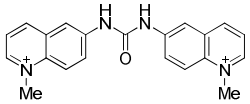
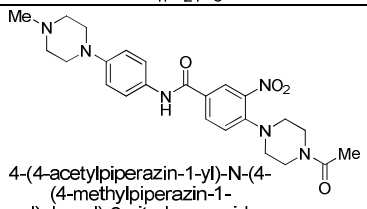


Supplementary Figure S2: Furamidine does not affect the protein level of endogenous non-mutant polyglutamine proteins. HEK293T-cells were treated with or without different doses of furamidine for 24 hours. (a) HTT protein level was analyzed on a western blot using anti-HTT antibodies for detection. Actin was detected as loading control. (b) Ataxin-2 and Ataxin-3 protein level was analyzed on a western blot using anti-Ataxin-2 or anti-Ataxin-3 antibodies for detection. Tubulin was detected as loading control. A representative western blot of an n=3 experiments together with quantification of relative protein levels is shown. Mean values +/- SEM are shown.

Supplementary Table

Table S1. Chemical structure of the 25 molecules selected in this study.

 bis(4-((dimethylamino)methyl)phenyl)methanone $C_{19}H_{24}N_2O$		 **Amb8480308** 3,3'-carbonylbis(azanediyl)dibenzimidamide $C_{15}H_{16}N_6O$
 bis(2-(4-aminophenyl)-1H-benzo[d]imidazol-6-yl)methanone $C_{27}H_{20}N_6O$		 1,3-bis(4-(pyrrolidin-1-ylmethyl)phenyl)urea $C_{23}H_{30}N_4O$
 1,1'-(4,4'-carbonylbis(4,1-phenylene))bis(3-methyl-1H-imidazol-3-ium) iodide $C_{21}H_{20}I_2N_4O$		 2,2'-(1,1'-(4,4'-carbonylbis(azanediyl)bis(4,1-phenylene))bis(ethan-1-yl-1-ylidene))bis(hydrazinecarboximidamide) $C_{19}H_{24}N_{10}O$
 4,4'-oxydibenzimidamide $C_{14}H_{14}N_4O$		 1,3-bis(4-((4-methylpiperazin-1-yl)methyl)phenyl)urea $C_{25}H_{36}N_6O$
 1,1'-(4,4'-oxybis(4,1-phenylene))dipiperazine $C_{20}H_{26}N_4O$		 **Amb8201157** 1,3-bis(2-methyl-4-(4-methylpiperazin-1-yl)phenyl)urea $C_{25}H_{36}N_6O$
 *tert*-butyl 4,4'-(9-oxo-9*H*-xanthene-3,6-diyl)dipiperazine-1-carboxylate $C_{31}H_{40}N_4O_6$		 1-(4-(aminomethyl)phenyl)-3-(4-(dimethylamino)phenyl)urea $C_{16}H_{20}N_4O$
 **furamidine dihydrochloride** 4,4'-(furan-2,5-diyl)dibenzimidamide dihydrochloride $C_{18}H_{18}Cl_2N_4O$		 1,3-bis(4-(diethylamino)phenyl)urea $C_{21}H_{30}N_4O$

 **nafamostat dimesylate** 6-carbamimidoylnaphthalen-2-yl 4-(diaminomethyleneamino)benzoate dimethanesulfonate $C_{21}H_{25}N_5O_8S_2$		 1,3-bis(4-(dimethylamino)phenyl)urea $C_{17}H_{22}N_4O$
 6-carbamimidoylnaphthalen-2-yl 4-(aminomethyl)benzoate $C_{19}H_{17}N_3O_2$		 1-(4-(dimethylamino)phenyl)-3-(2-methylbenzo[d]thiazol-6-yl)urea $C_{17}H_{18}N_4OS$
 6-carbamimidoylnaphthalen-2-yl 4-(4,5-dihydro-1H-imidazol-2-ylamino)benzoate $C_{21}H_{19}N_5O_2$		 **Amb10790058** 1,3-bis(4-((2-methyl-1H-imidazol-1-yl)methyl)phenyl)urea $C_{23}H_{24}N_6O$
 4-(aminomethyl)-N-(4-((dimethylamino)methyl)phenyl)benzamide $C_{17}H_{21}N_3O$		 1,3-bis(3-fluoro-4-(pyrrolidin-1-yl)phenyl)urea $C_{21}H_{24}F_2N_4O$
 4-(dimethylamino)-N-(4-(dimethylamino)phenyl)benzamide $C_{17}H_{21}N_3O$		 6,6'-carbonylbis(azanediyl)bis(1-methylquinolinium) $C_{21}H_{20}N_4O^{2+}$
 4-(4-acetylpiperazin-1-yl)-N-(4-(4-methylpiperazin-1-yl)phenyl)-3-nitrobenzamide $C_{24}H_{30}N_6O_4$		
Furamidine, nafamostat mesylate, and Amb compounds were purchased and tested.		

SUPPORTING INFORMATION

Description of furamidine binding poses in minima M1-M4 and U.

The representative structures for each cluster are reported in Figure 6 of the main text. The lowest free energy pose (B) is located in the FES at d_{CM} ≈ 4.8 Å and n_{HB} ≈14. The M1 basin is observed at d_{CM}≈10 Å, n_{HB}≈2 and is ≈ 2.5 kcal/mol higher in free energy then B. The cluster analysis of the poses corresponding to this basin shows, in the most populated family, that the A_4 base partially flips outward to stack with one of the compound's phenyl rings. The furan and the other phenyl ring are fully solvent exposed. Two salt bridges between the ligand amidine tails and the phosphate groups of the nucleotides G_5 and G_2 are also observed. The minima M2-M3 are ≈ 4 kcal/mol higher in free energy than B. In M2 (d_{CM}≈5 Å, n_{HB}≈8) the amidine groups establish, on one end, a salt bridges with the A_{17} phosphate group and on the other end and H-bond with N7 atom of the A_4 base. In the basin M3 (d_{CM}≈5 Å, n_{HB}≈3) Furamidine, almost fully solvated, is bound to the RNA major groove through two H-bonds, established by its amidine tails with the A_4 phosphate group and the O_6 atom of the C_5 base. In the shallow free-energy minimum M4 (d_{CM} ≈ 18 Å, n_{HB} ≈ 1), ≈ 6 kcal/mol higher in free energy then B, Furamidine is bound to the 5'-end of the RNA sequence, loosing the interactions with both the minor and the major groove floors. One of the two compound guanidine tails forms a salt-bridge with C_{20} phosphate. In the minimum U (d_{CM} ≈ 30 Å, n_{HB} ≈ 0), about ≈7.5 kcal/mol higher in free energy than B, direct intermolecular furamidine/CAG RNA interactions are absent.