Discovery of a potent small molecule inhibiting Huntington's disease pathogenesis *via* targeting CAG repeat RNA and Poly Q protein

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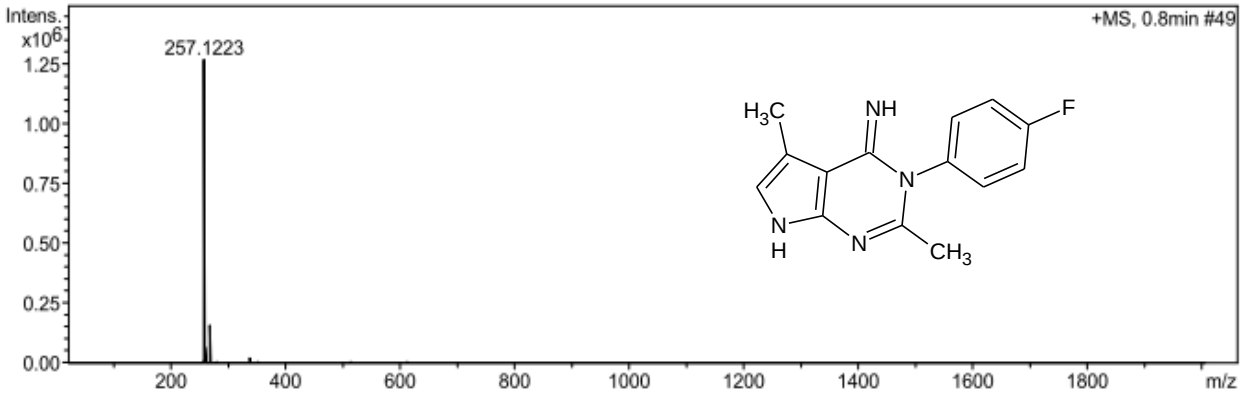
CP1=NSC351550 M.W.= 256.28



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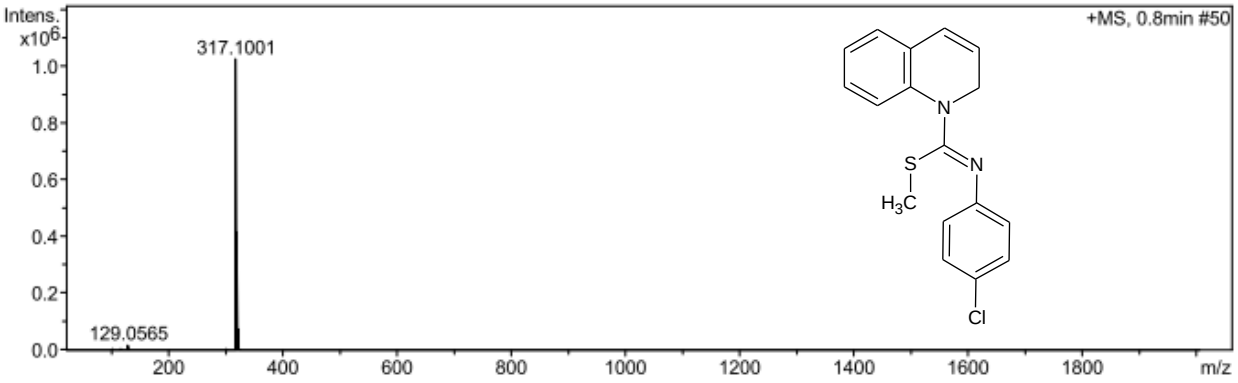

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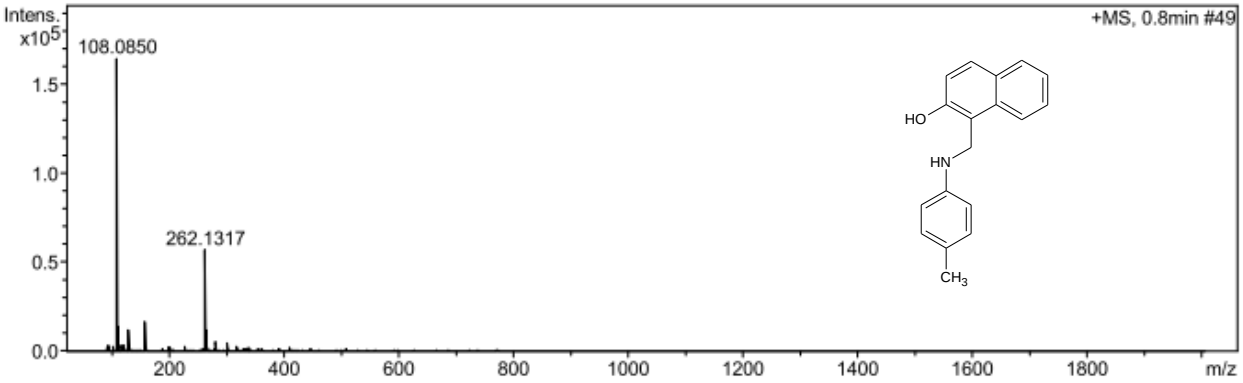

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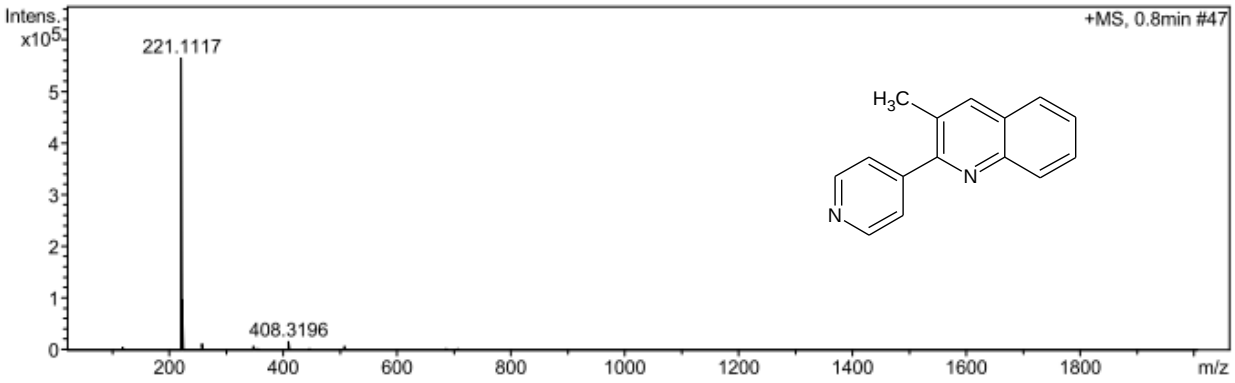

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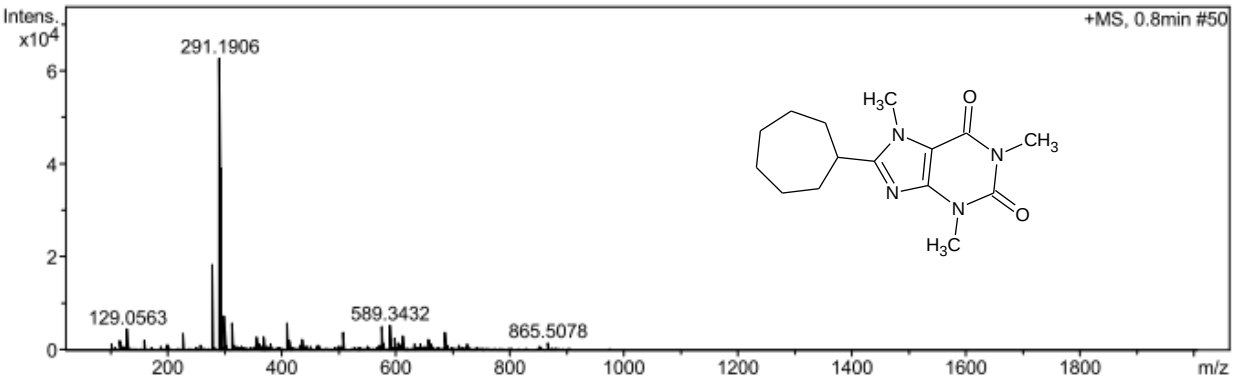

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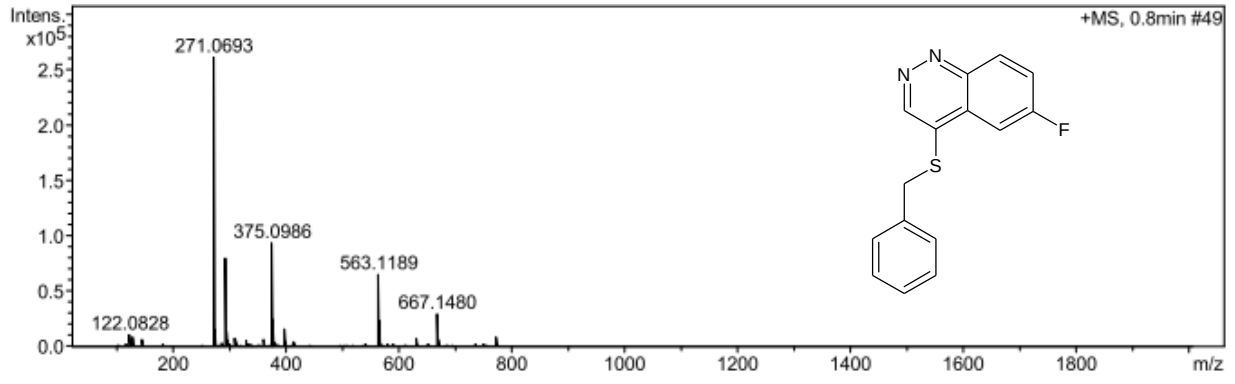

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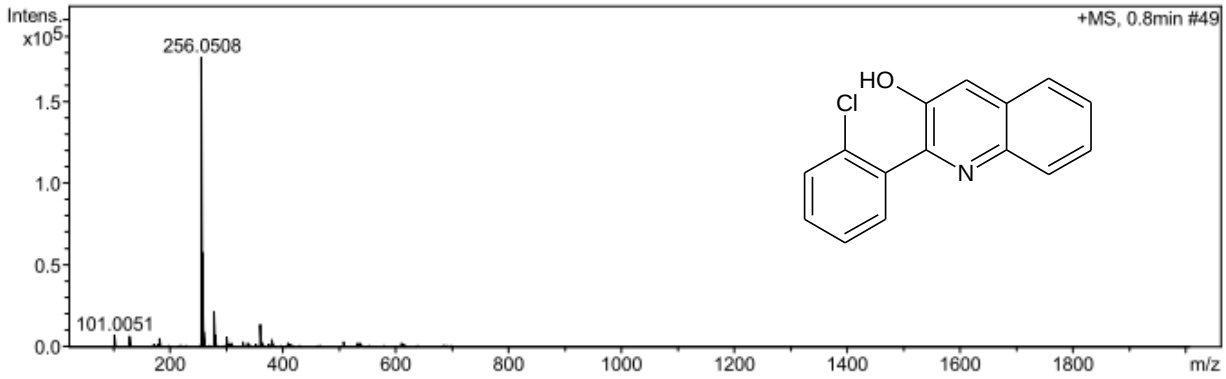

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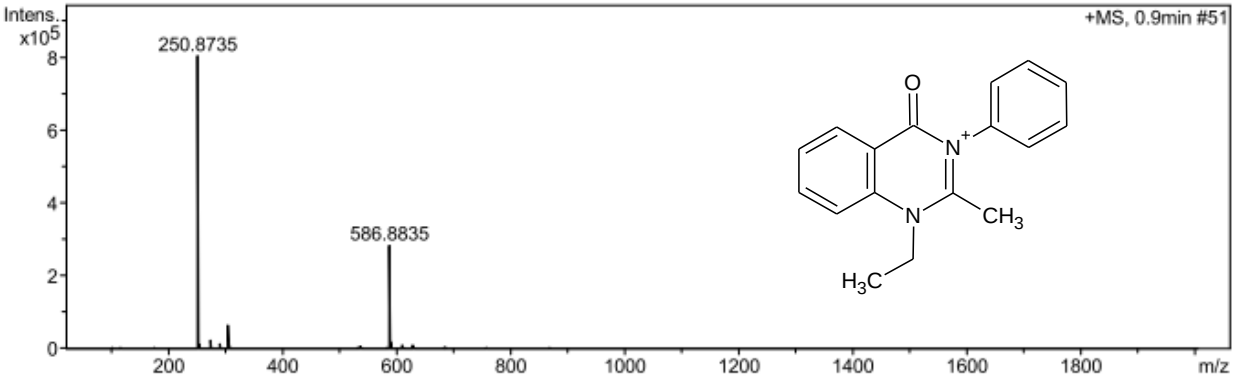

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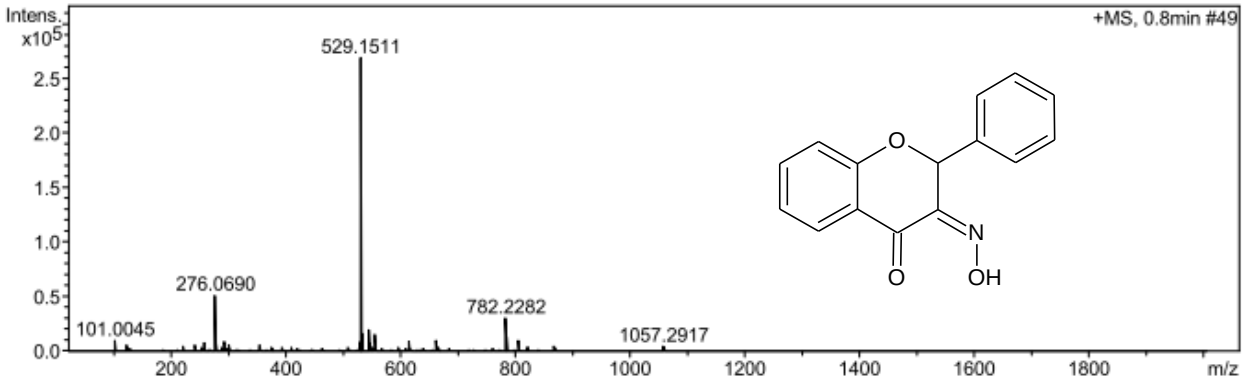

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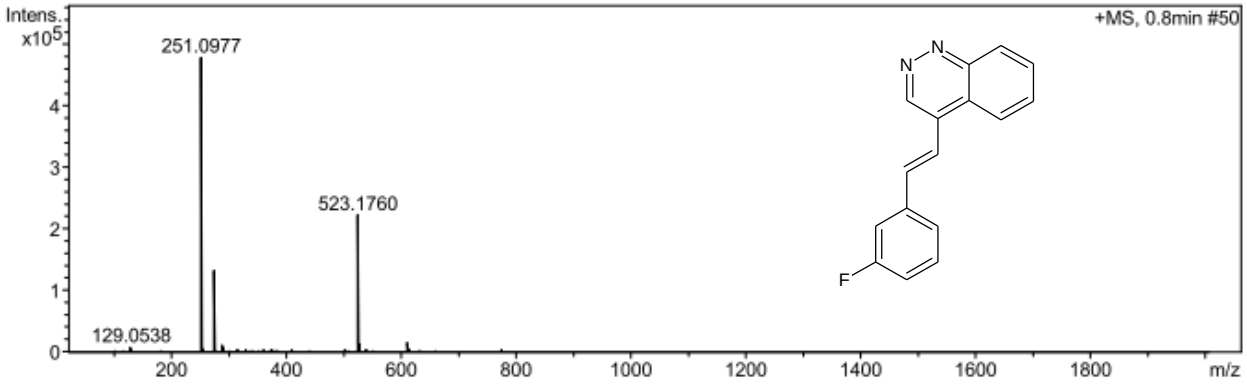

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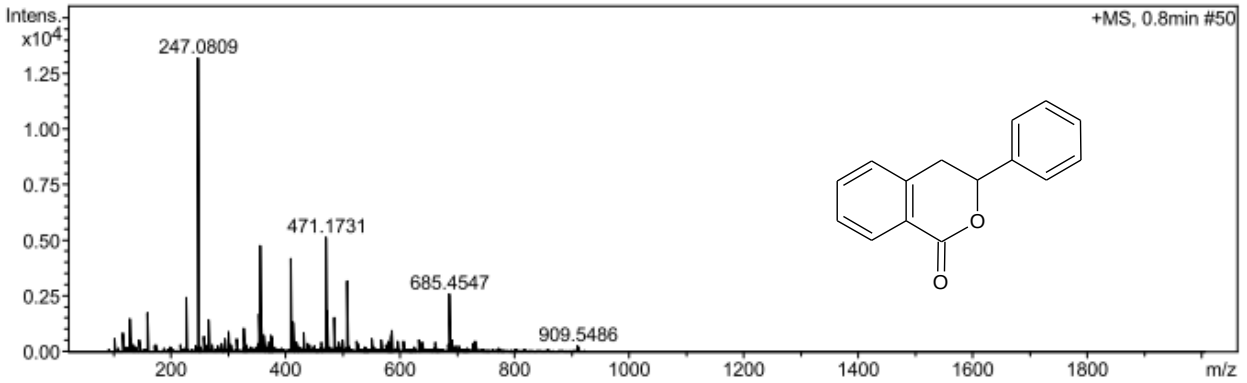

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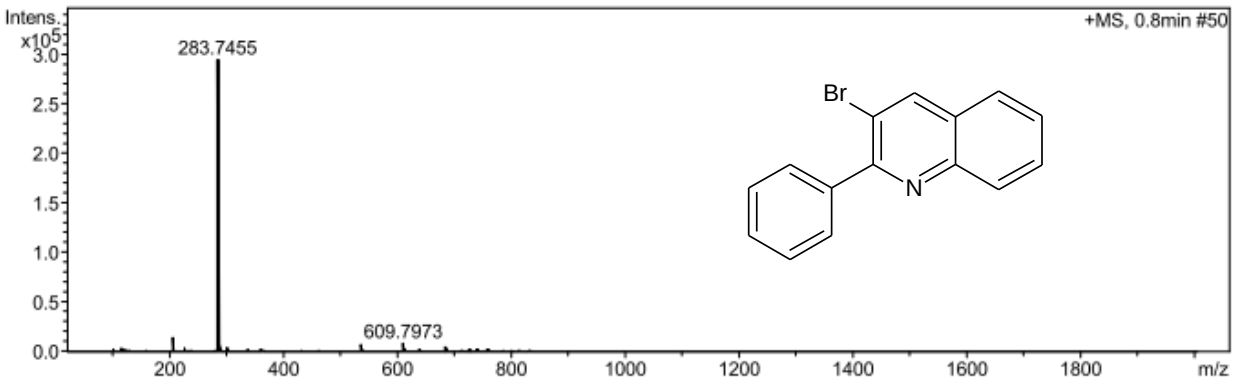

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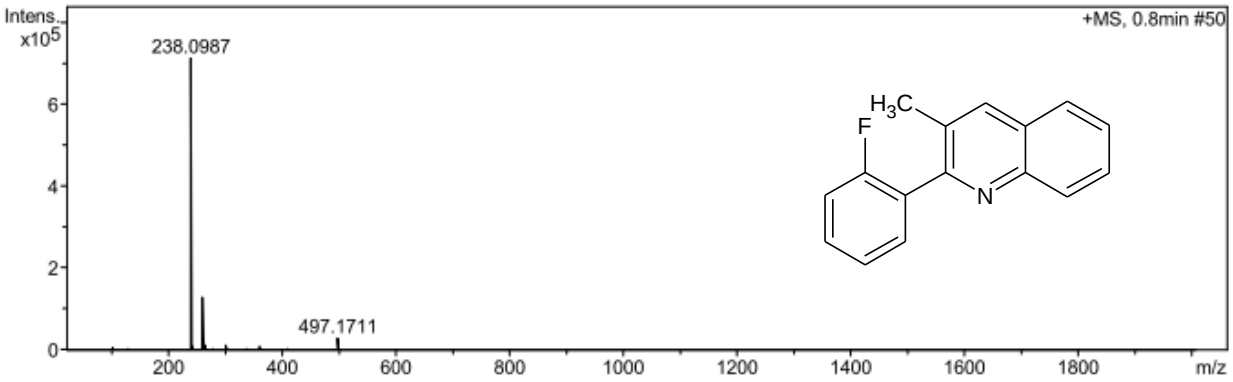

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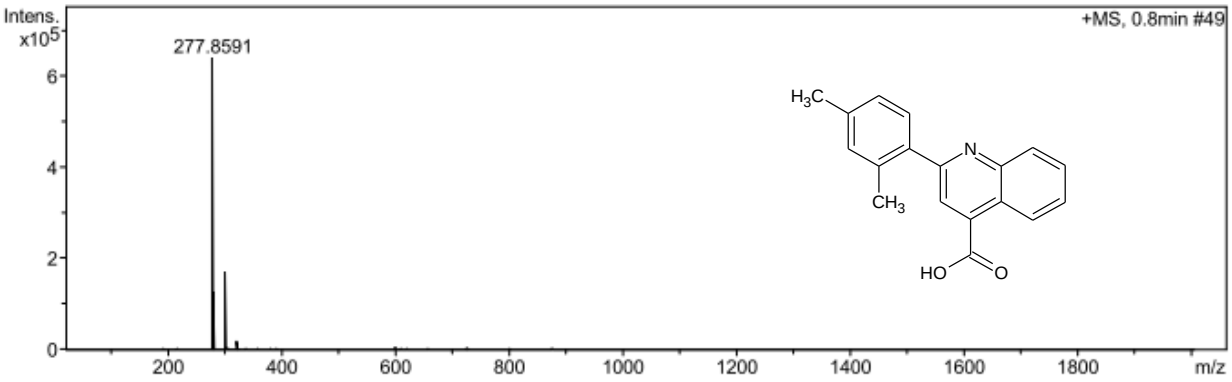

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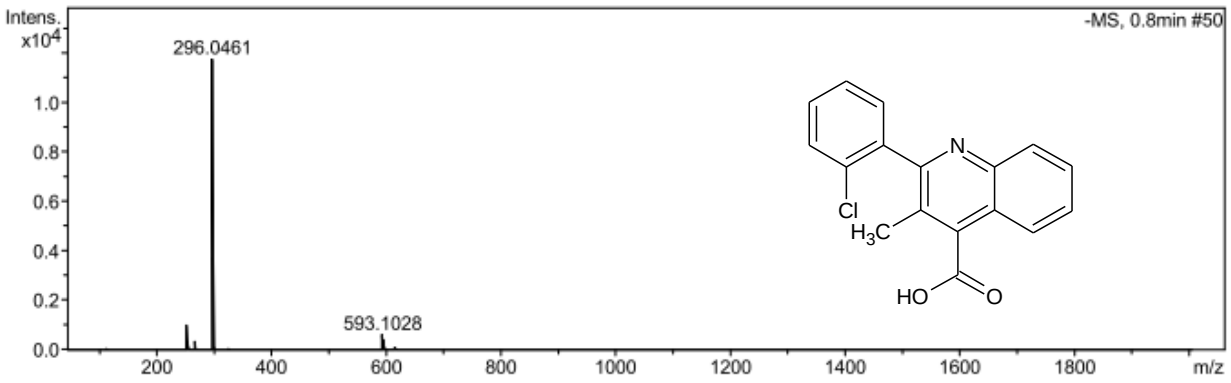

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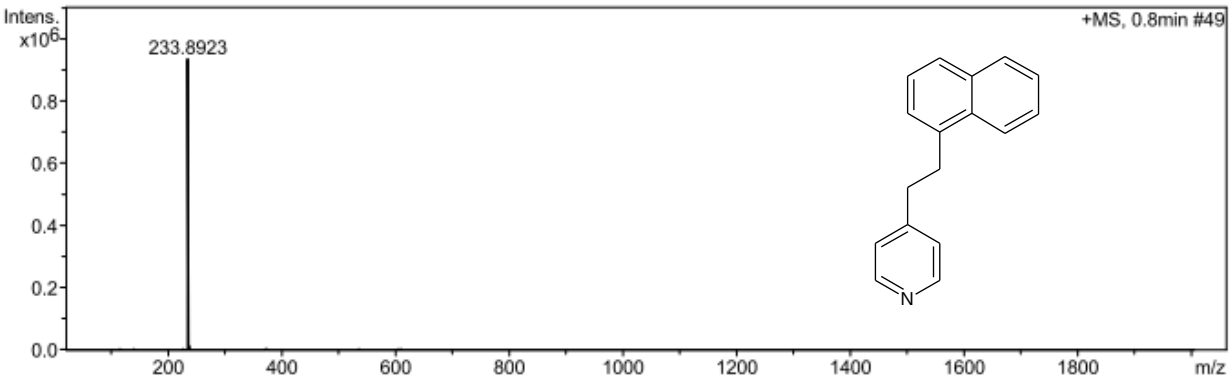

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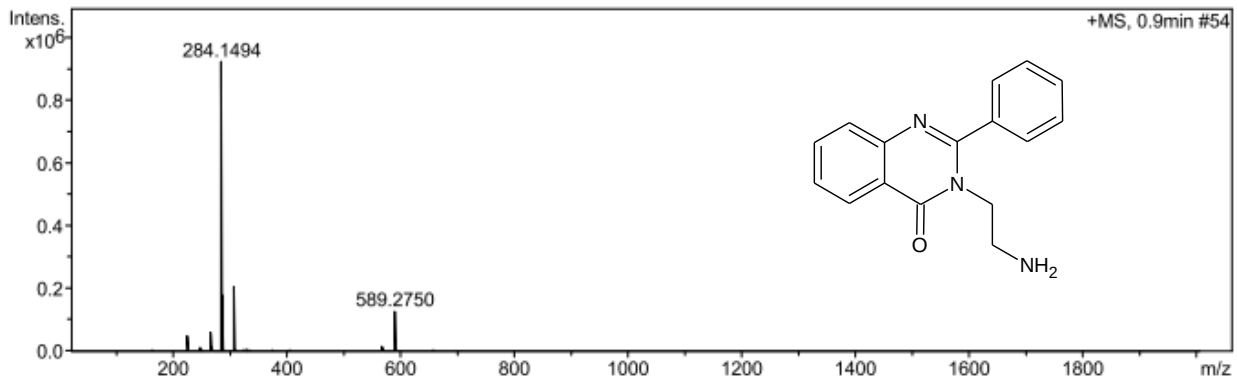

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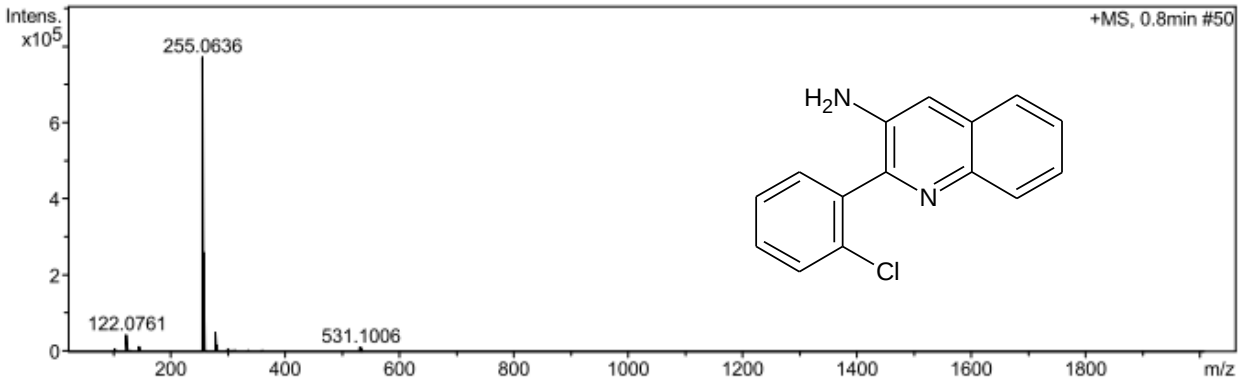

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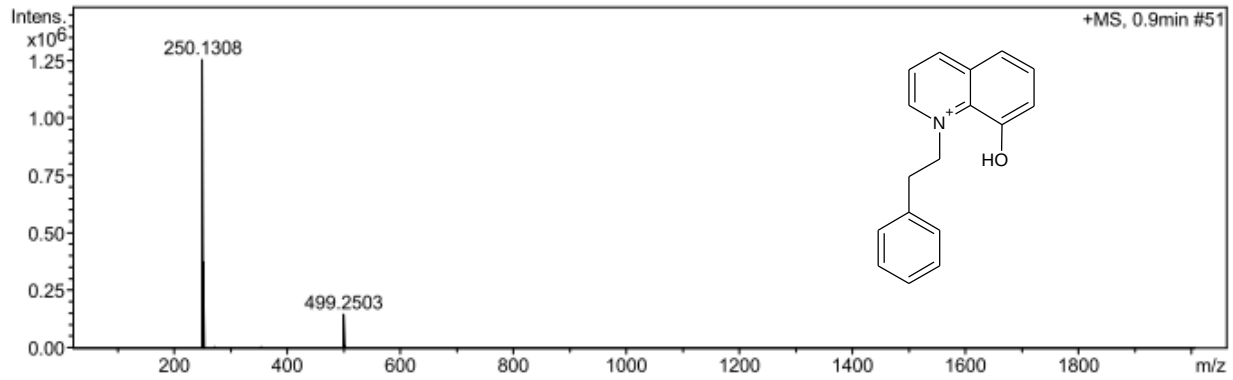
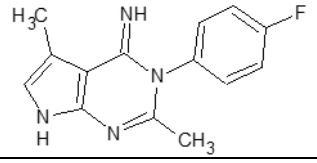
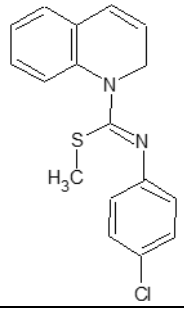
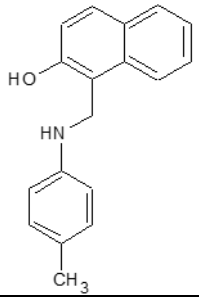
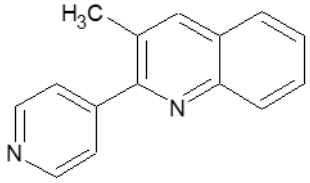
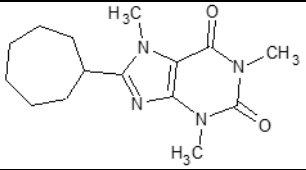
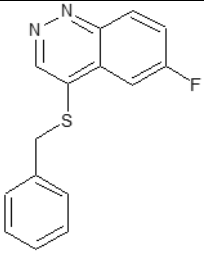
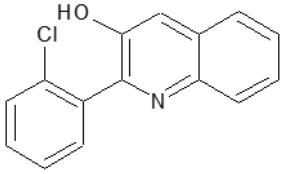
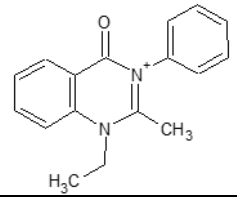
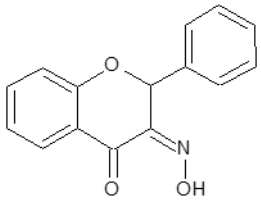
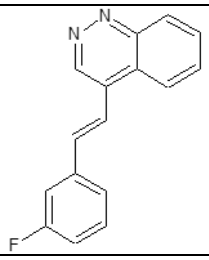
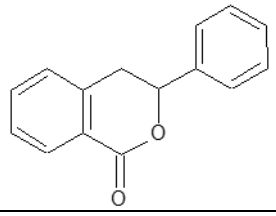
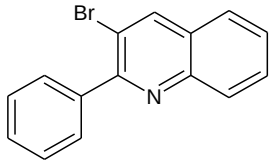
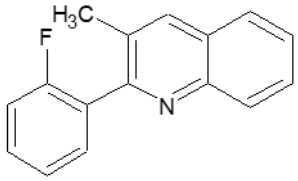
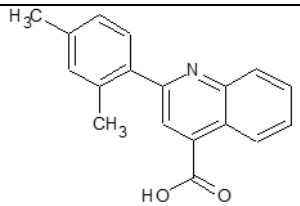
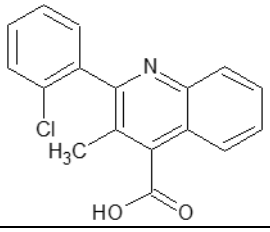


Table S1 Chemical structures of the small-molecules used in screening for repeats containing RNA

Compound Code	Structure
CP1	
CP2	
CP3	
CP4	
CP5	
CP6	
CP7	

CP8	
CP9	
CP10	
CP11	
CP12	
CP13	
CP14	
CP15	

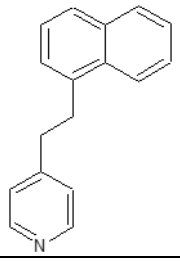
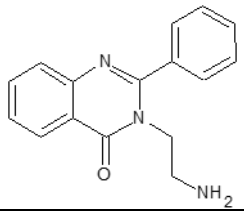
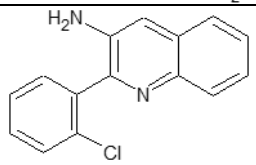
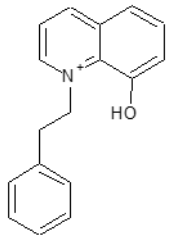
CP16	
CP17	
CP18	
CP19	

Table S2: The binding constant (K_d (μM)) values of compounds with RNA 5' CAG/3'GAC and 5' CAG/3'GUC

Compound	Binding constant values (μM)			
	RNA 5' CAG/3'GAC		RNA 5' CAG/3'GUC	
	K_d1	K_d2	K_d1	K_d2
CP1	0.1672	11.4500	0.0280	0.4521
CP2	0.0540	2.1215	0.5662	0.5662
CP3	0.0893	1.5579	0.2031	0.7697
CP4	0.0009	0.7255	0.0092	0.7178
CP5	1.3840	1.3840	0.6657	0.6657
CP6	0.0476	0.9693	0.7339	0.7339
CP7	0.0178	2.4435	0.0005	1.1453
CP8	0.0103	4.7000	0.6154	0.6154
CP9	0.6778	$1.91x10^7$	0.0124	0.9227
CP10	0.9087	$1.81x10^7$	0.4957	0.4957
CP11	1.4307	1.4307	1.2764	1.2764
CP12	2.1650	2.1650	0.6115	0.6115
CP13	0.3455	1.0217	1.2717	1.2717
CP14	0.0675	4.1180	0.5012	1.9855
CP15	0.0135	0.1948	0.0172	0.6402
CP16	1.5816	1.5816	0.4933	0.4933
CP17	N.D.	N.D.	N.D.	N.D.
CP18	1.0361	1.0361	0.6931	0.6931
CP19	0.3463	4048.3000	N.D.	N.D.

Table S3: The binding constant (K_d, (μM)) values of compounds with multiple loop RNAs

Compounds	Binding constant values (μM)			
	RNA (5'CAG/3'GAC)x6		RNA (5'CAG/3'GUC)x6	
	K_d1	K_d2	K_d1	K_d2
CP2	0.0003	0.1749	0.6203	0.6203
CP3	0.0495	0.1588	0.2452	0.2452
CP4	0.2188	0.49	0.4407	0.7459
CP6	0.0005	0.1344	0.2021	0.2021
CP8	0.2061	0.1768	0.1150	0.1758
CP13	0.0015	0.387	0.3038	0.3038
CP14	0.1823	0.1823	0.2823	0.2823
Myricetin	0.0730	0.4699	N.D.	2.4757

Table S4. The binding constant (K_d) values of compounds with multiple loop RNAs calculated using ITC experiment.

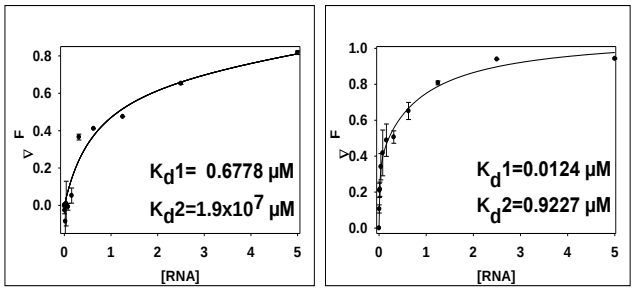
Compounds	Binding constant values			
	RNA (5'CAG/3'GAC)x6		RNA (5'CAG/3'GUC)x6	
	K_d^1 (μM)	K_d^2 (μM)	K_d^1 (μM)	K_d^2 (μM)
CP2	0.028	12.3	9.52	N.D.
CP3	27.700	2.13	-	-
CP4	62.500	3.18	-	-
CP6	0.709	10.86	30.3	0.42
CP8	38.010	2.35	-	-
CP13	0.011	1.54	3.89	174.2
CP14	60.200	6.89	-	-
Myricetin	0.253	26.24	-	-

Table S6. The IC_{50} values of compounds with HEK293 cells calculated by MTT assay

Compounds	IC_{50} Values (μM)		
	HEK293 Cells	Normal fibroblast cells	HD cells
CP2	19.06	56.33	46.63
CP3	42.44	-	-
CP4	76.99	-	-
CP6	40.24	55.30	44.44
CP8	17.38	-	-
CP13	62.86	53.17	52.70
CP14	17.25	-	-
Myricetin	22.45	-	-

Table S5. The melting temperature values of different 5'CAG/3'GAC & 5'CAG/3'GUC RNA loop in absence and presence of compounds

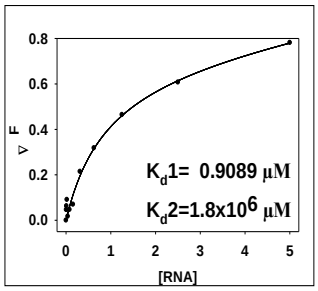
Compounds	Drug/Nucleic acid ratio	Melting temperature (°C) of different loop containing RNAs		
		AAx20	AAx6	AUX6
CP2	DN0.0	67.6	57.6	61.8
	DN1.0	70.4	61.8	61.9
	DN2.0	72.1	63.0	62.5
	DN3.0	75.2	-	-
CP6	DN0.0	67.8	57.0	60.4
	DN1.0	70.2	59.8	60.8
	DN2.0	71.9	62.8	61.4
	DN3.0	74.9	-	-
CP13	DN0.0	66.8	51.8	62.0
	DN1.0	69.1	55.4	61.8
	DN2.0	72.2	57.9	62.2
	DN3.0	75.2	-	-



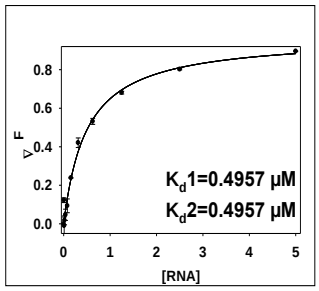
(5'CAG/3'GAC)×1

(5'CAG/3'GUC)×1

CP9

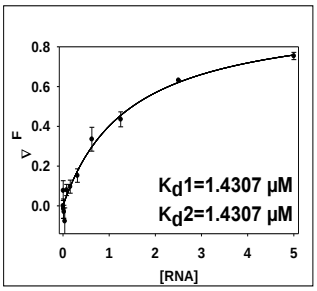


(5'CAG/3'GAC)×1

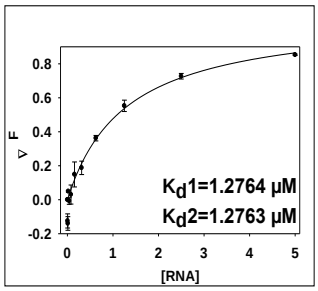


(5'CAG/3'GUC)×1

CP10

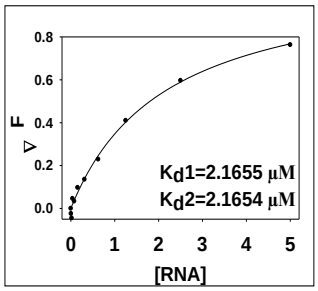


(5'CAG/3'GAC)×1

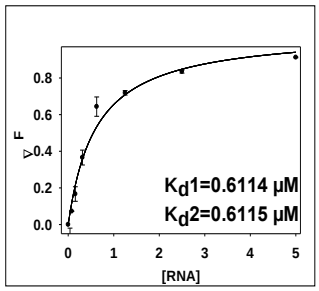


(5'CAG/3'GUC)×1

CP11

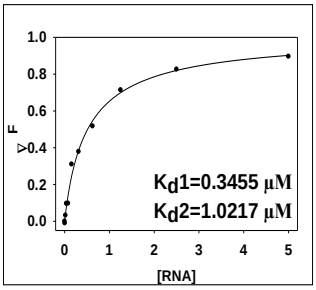


(5'CAG/3'GAC)×1

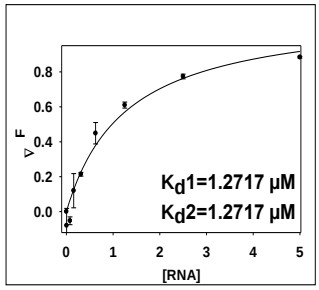


(5'CAG/3'GUC)×1

CP12

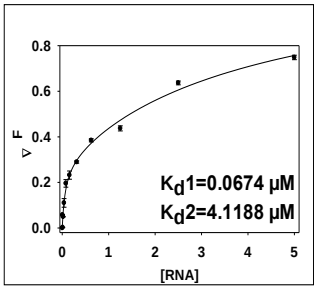


(5'CAG/3'GAC)×1

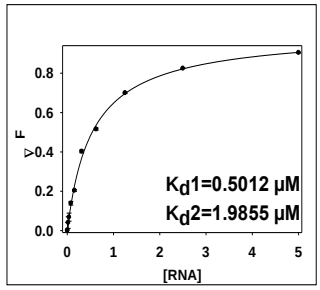


(5'CAG/3'GUC)×1

CP13

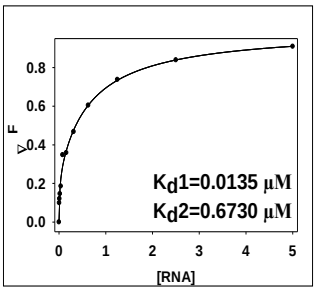


(5'CAG/3'GAC)×1

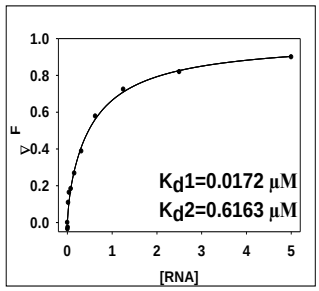


(5'CAG/3'GUC)×1

CP14

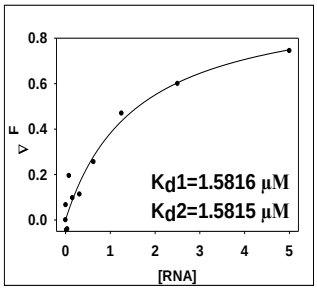


(5'CAG/3'GAC)×1

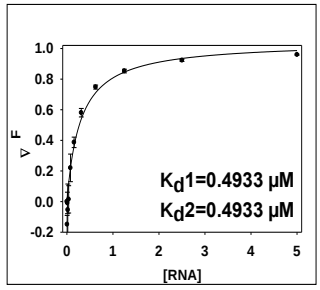


(5'CAG/3'GUC)×1

CP15



(5'CAG/3'GAC)×1



(5'CAG/3'GUC)×1

CP16

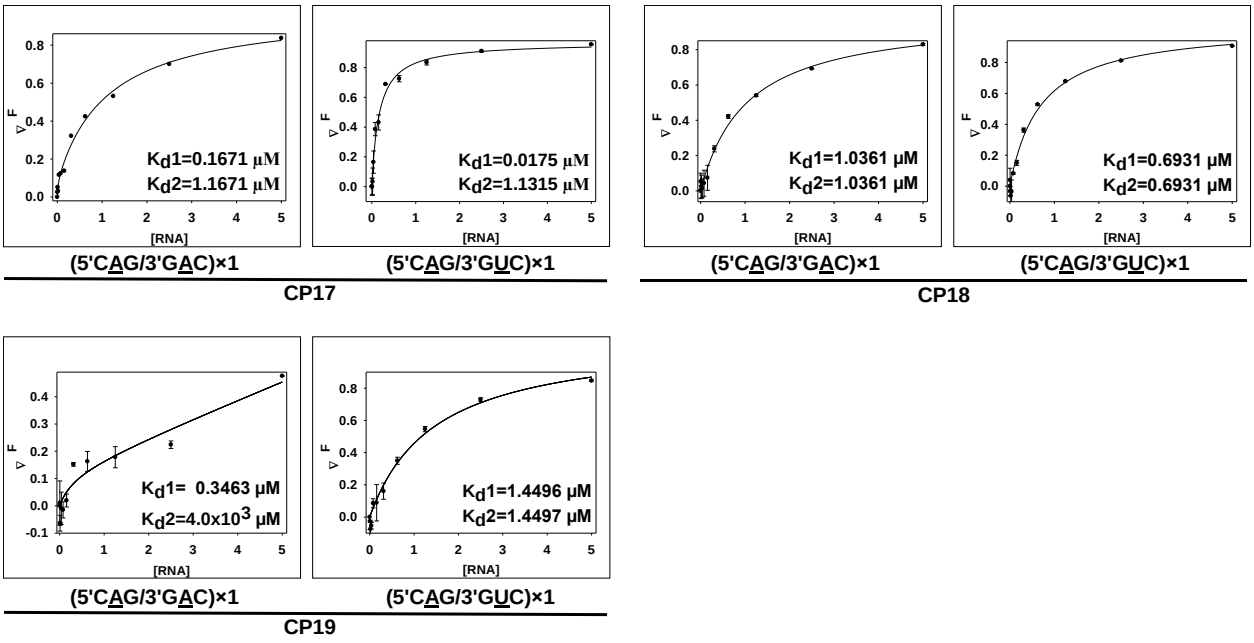


Figure S1. Fluorescence titration curves of RNAs (5'CAG/3'GAC)×1 & (5'CAG/3'GUC)×1 RNA respectively with different compounds. Solid lines represent the data fitting by two sites saturation ligand binding mode. Values of binding constant (s) (K_d) are mentioned in right bottom side of the plot.

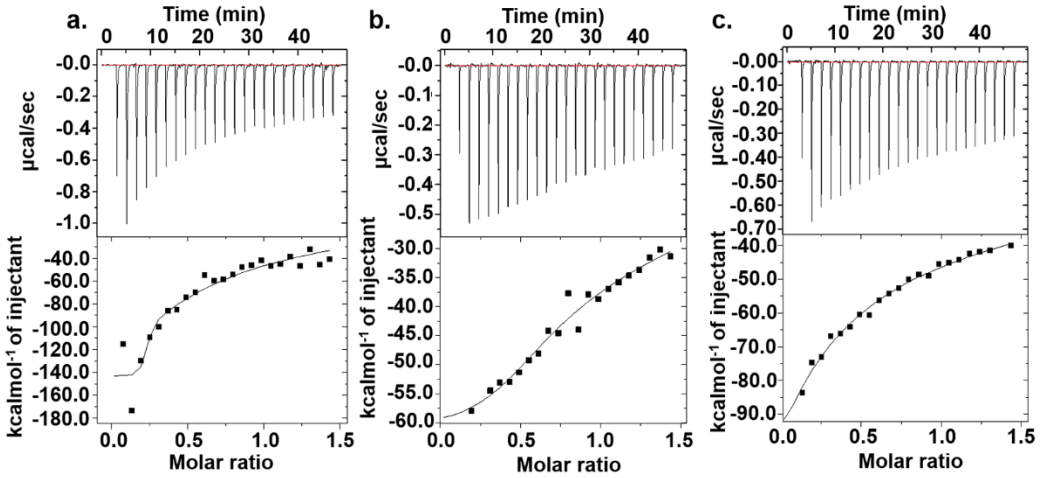


Figure S2. Isothermal titration calorimetry assay of (5′C**A**G/3′G**U**C)x6 RNA with compounds **(a)** CP2 **(b)** CP6 **(c)** CP13

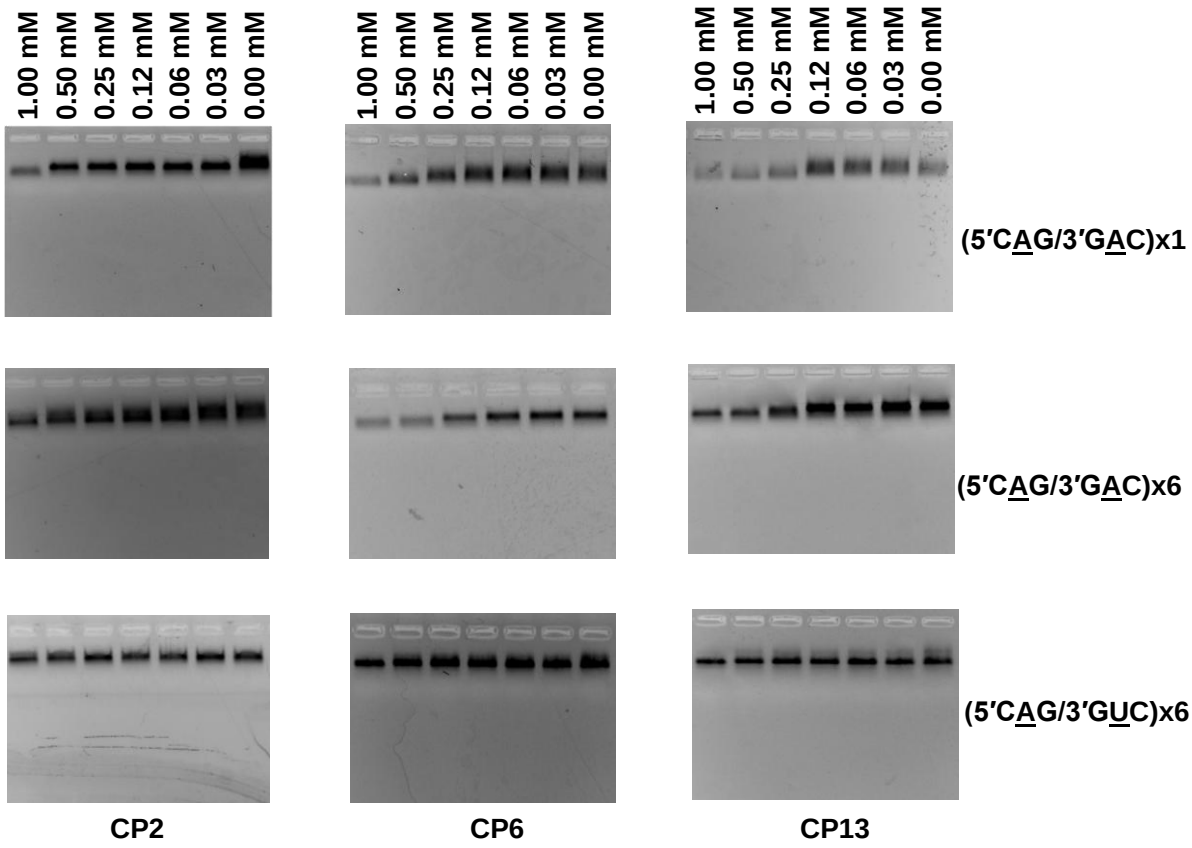


Figure S3a. PCR stop assay of compounds CP2, CP6 & CP13 **(Left to right)** with different motif DNA templates (5'C**A**G/3'G**A**C)x1, (5'C**A**G/3'G**A**C)x6 & (5'C**A**G/3'G**U**C)x6 respectively **(top to bottom).**

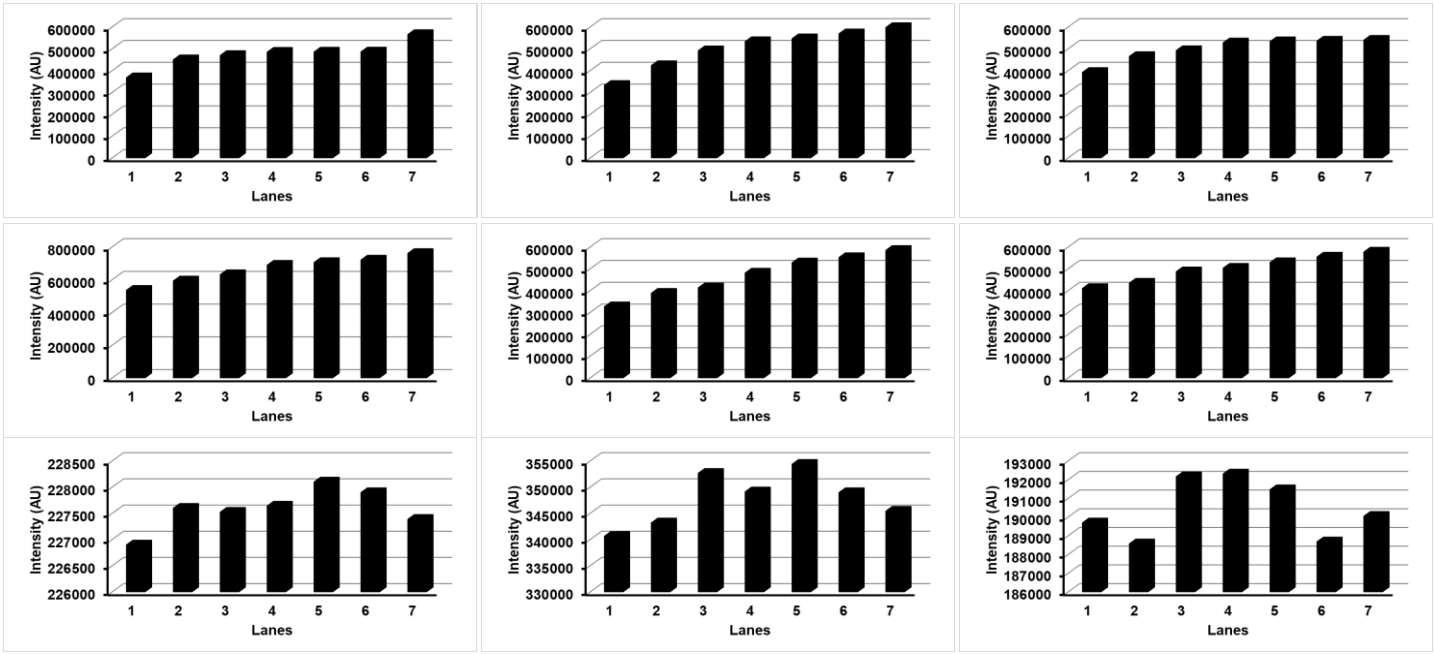


Figure S3b. Gel intensity calculated using ImageJ for PCR stop assay. Plots depicts the results for CP2, CP6 & CP13 **(Left to right)** with different motif DNA templates (5'C**A**G/3'G**A**C)x1, (5'C**A**G/3'G**A**C)x6 & (5'C**A**G/3'G**U**C)x6 respectively **(top to bottom).**

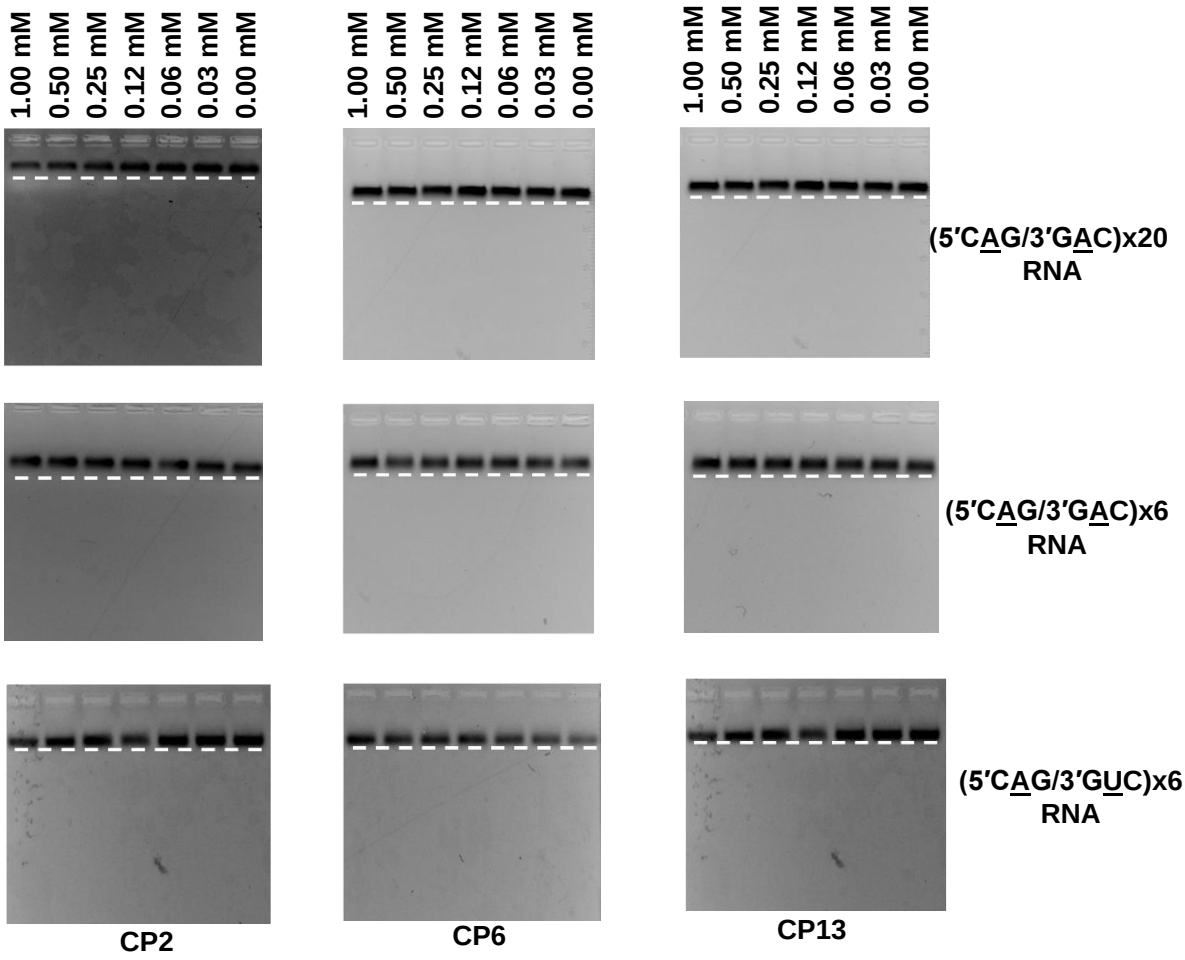


Figure S4a. Gel retardation assay of compounds CP2, CP6 & CP13 **(Left to right)** with different motif RNA templates (5'C**A**G/3'G**A**C)x20, (5'C**A**G/3'G**A**C)x6 & (5'C**A**G/3'G**U**C)x6 respectively **(top to bottom).**

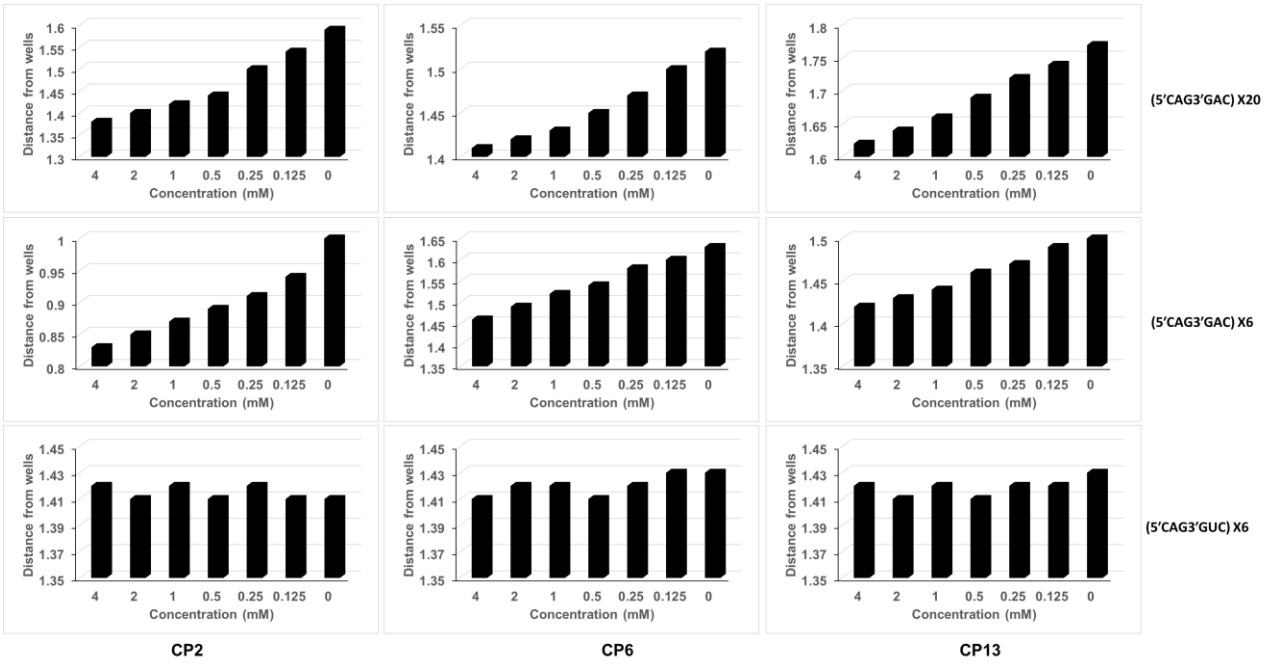


Figure S4b. Plots for distance travelled from wells in gel retardation assay of compounds CP2, CP6 & CP13 (Left to right) with different motif RNA templates (5'C**A**G/3'G**A**C)x20, (5'C**A**G/3'G**A**C)x6 & (5'C**A**G/3'G**U**C)x6 respectively (top to bottom).

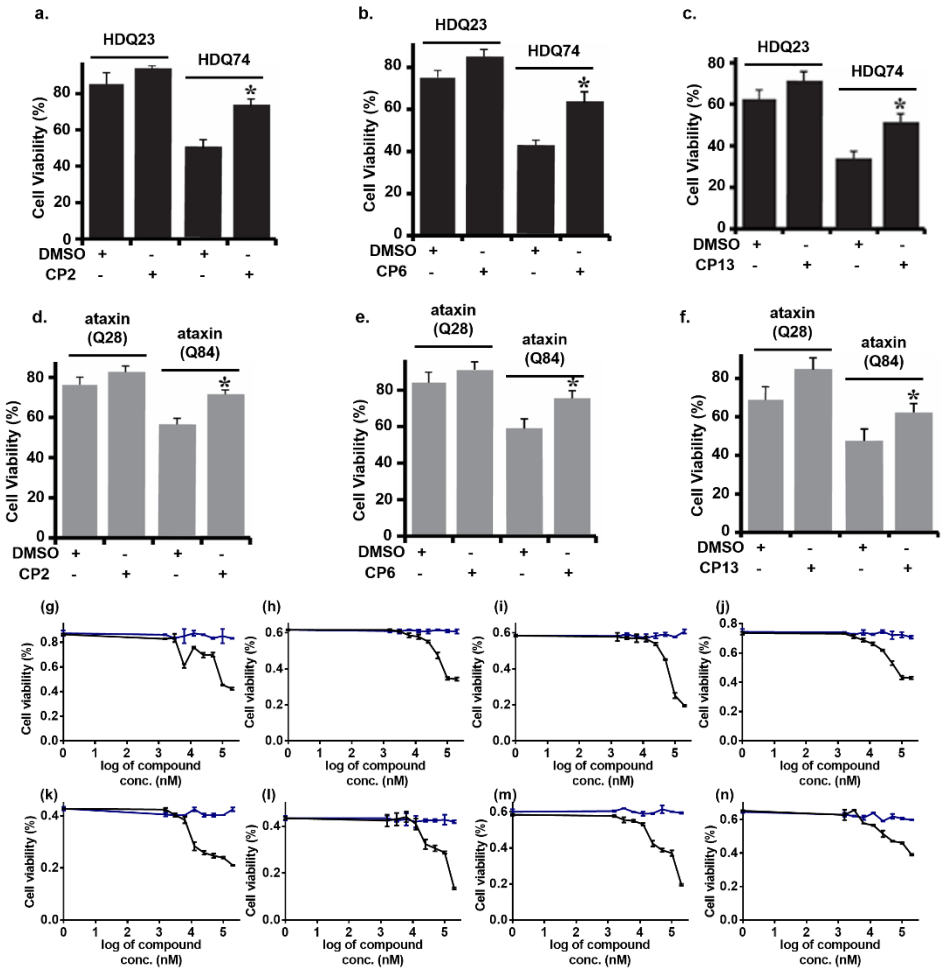


Figure S5. Cell viability assay in HD cellular models. **(a-f)** Cos-7 cells were transfected with polyglutamine encoding plasmids (HDQ23, HDQ74, ATX28 and ATX84) and treated with compounds to assess the cyto-protection effects of compounds. **(g-n)** Viability of cells treated with compounds on normal HEK293 cells with **(g)** CP2 **(h)** CP3 **(i)** CP4 **(j)** CP6 **(k)** CP8 **(l)** CP13 **(m)** CP14 **(n)** Myricetin. Blue line showing the curve for vehicle (control) while black lines represents viability of cells treated with compounds. Values shown are the mean ± SD from three independent sets of experiments. *p<0.05 compared to control

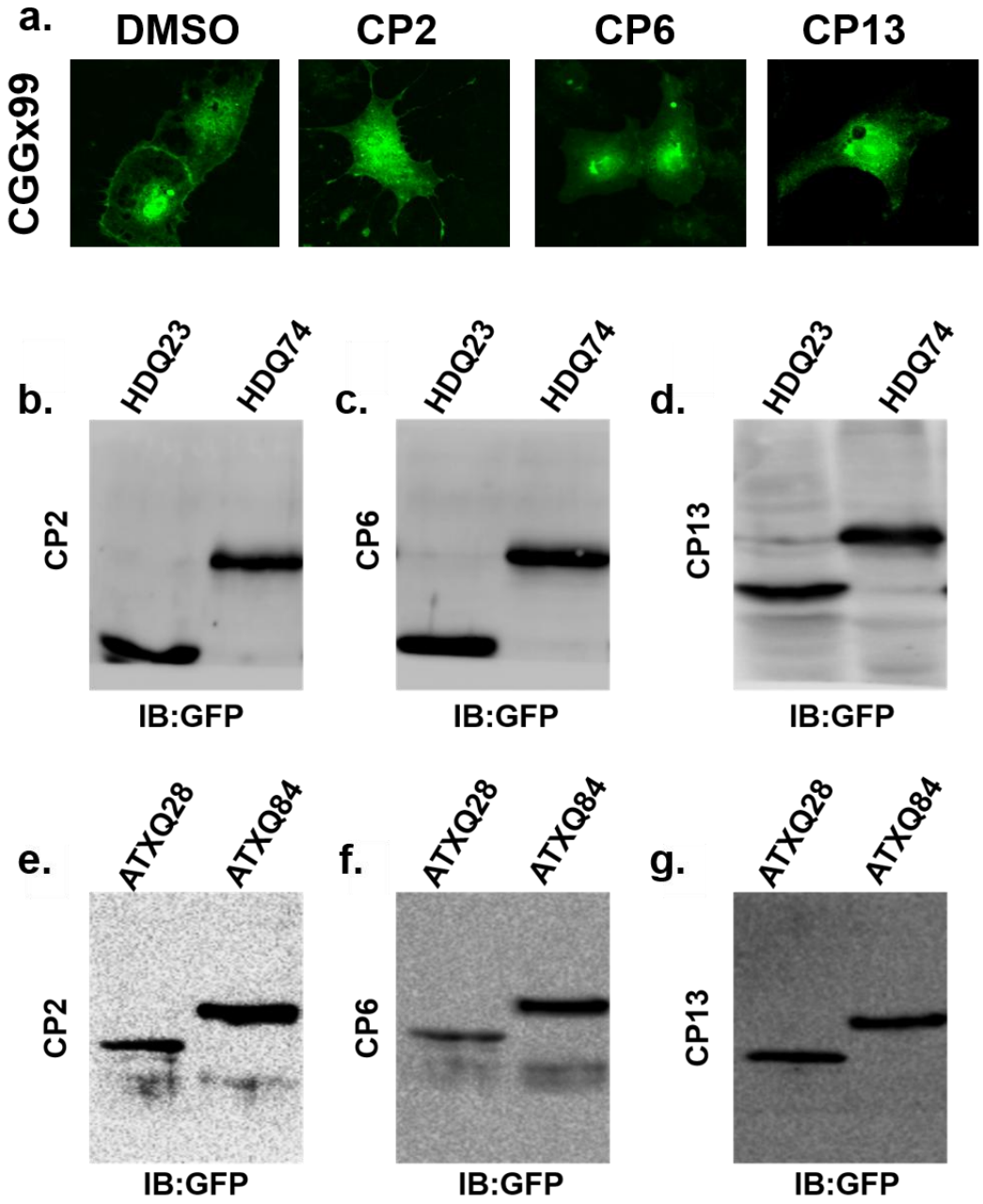


Figure S6 **(a)** Micrographs of EGFP-CGG99, transiently transfected COS-7 cells, treated with control (DMSO) as well as compounds (100.0 µM) for 12 hours **(b-g) Full images of western blots.** Plasmid expression analysis of the cells was confirmed by immunoblot, using anti-GFP antibodies **(top lane)** HDQ plasmid treated with **(a)** CP2 **(b)** CP6 **(c)** CP13 **(bottom lane)** ATX plasmid treated with **(d)** CP2 **(e)** CP6 **(f)** CP13