Electronic Supplementary Material (ESI) for Analyst.
This journal is © The Royal Society of Chemistry 2021

Electronic Supplementary Material

A DNA radar-like monitor for RNase H-targeted natural compounds screening and RNase H activity *in situ* detecting

Yalei Hu[1#], Qian Xie[2#], Li Chang[3#], Xueqing Tao[1], Chunyi Tong[1*], Bin Liu[1], Wei Wang [2*]

[1]*College of Biology, Hunan Province Key Laboratory of Plant Functional Genomics and Developmental Regulation, Hunan University, Changsha, 410082, China.*

[2]*TCM and Ethnomedicine Innovation & Development International Laboratory, Innovative Material Medical Research Institute, School of Pharmacy, Hunan University of Chinese Medicine, Changsha, 410208, China.*

[3] *Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha, 410008, PR China*

# These authors contributed equal and should be signed as co-fist author.

∗ Corresponding author.

E-mail addresses: sw_tcy@hnu.edu.cn (C. Tong), wangwei402@hotmail.com (W. Wang).

Experimental section

Table S1. Sequences of Oligonucleotides used in this work

Oligo	Sequences (5' to 3')
L1	ATTTATCACCCGCCATAGTAGACGTATCACCAGGCAGTTGAGACGAACATTCCTAAGTCTGAA
(Dabcyl)-L2	**(Dabcyl)-** CTTCTCTACGTTCCGGACCTTTTACATGCGAGGGTCCAATACCGACGATTACAGCTTGCTACAC G A TTCAGACTTAGGAATGTTCG
L3	ACTACTATGGCGGGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATC C
L4	ACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCG
L5	ACATGCGAGGGTCCAATACCGACGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCG
L6- (FAM)	GGTCCGGAAC r（GUAGAGAAG) - **(FAM)**
L7- (FAM)	GGTCCGGAACGTAGAGAAG - **(FAM)**

Results and discussion

Optimization of the experimental conditions

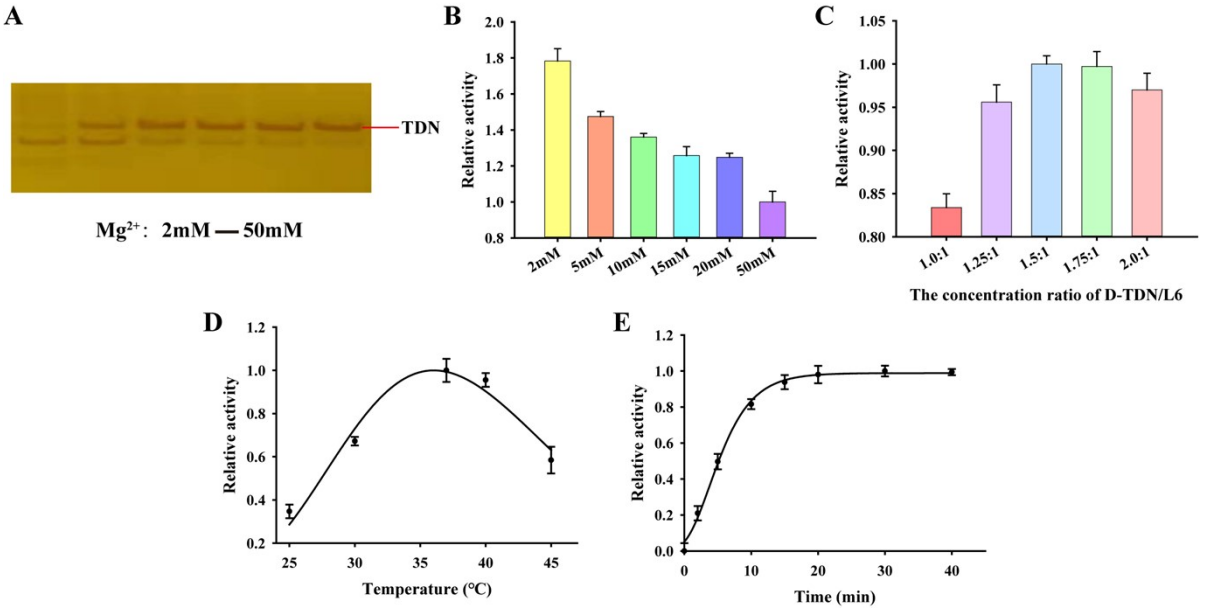


Fig. S1. The optimization of assay conditions. (A) Synthesis results of D-TDN under different concentrations of Mg^{2+} (2mM; 5mM; 10mM; 15mM; 20mM; 50mM). (B) Fluorescence results optimized for Mg2+ concentration in the sensing system. (C) The optimization of the ratio of L6 to D-TDN. [L6]=100 nM. (D) The fluorescence intensity changes with different

temperature. (E) The fluorescence intensity changes at different time point. [L1]=[L2]=[L3]=[L4]=150 nM, [RNase H]=10 U/mL. [L6] =100 nM.

Sensitivity analysis

Table S2. Comparison of the limit of detection of RNase H

Detection methods	Detection limit (U/mL)	Year	Reference
Molecular beacon	5	2008	[1]
Iridium (III)	0.125	2016	[2]
Tb^{3+}	2	2017	[3]
Graphene oxide	0.005	2017	[4]
DNAzyme	0.01	2017	[5]
Gold nanoparticles / DNAzyme	0.023	2019	[6]
DNA Tetrahedron / G-quadraplex	3.41	2019	[7]
TDN	0.01	-	This work

Specificity analysis of RNase H

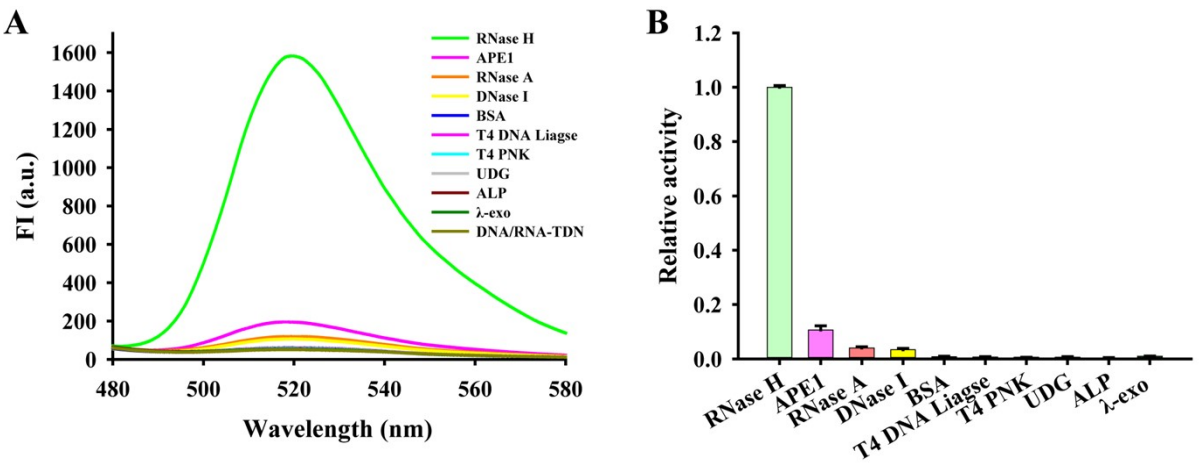


Fig. S2. Influence of other enzymes on the specificity of the RNase H activity assay. [L1]=[L2]=[L3]=[L4]=150 nM, [L6]=100 nM, [RNase A]=[BSA]=1 μg/mL, [ALP] = [T4 PNK] = [RNase H] = [APE1] = [UDG] = [DNase I] = [T4 DNA Ligase] = 20 U/mL. Ex/Em = 450/521 nm. Error bars SD, n = 3

Time optimization in the cell

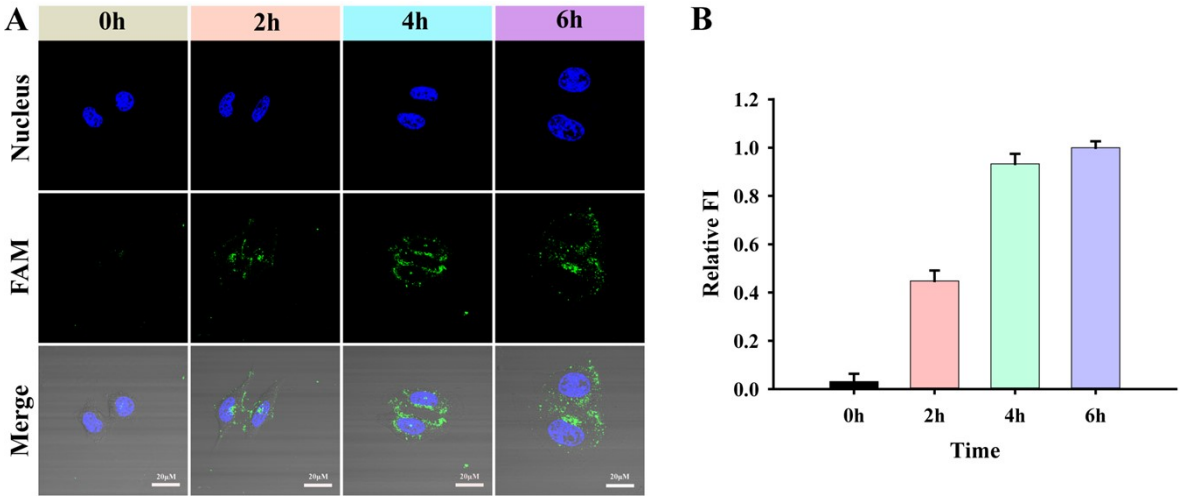


Fig. S3. (A) Optimization of incubation time for RNA/DNA-TDN with living cells. (B) Perform relative quantitative analysis on the image data in Fig. A.Scale bars are 20 μm.

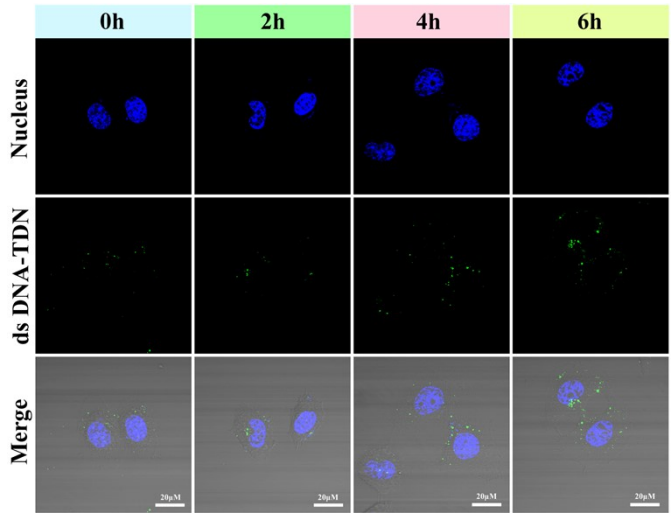


Fig. S4. Imaging by dsDNA-TDN in HepG2.

Cytotoxicity analysis of natural compounds

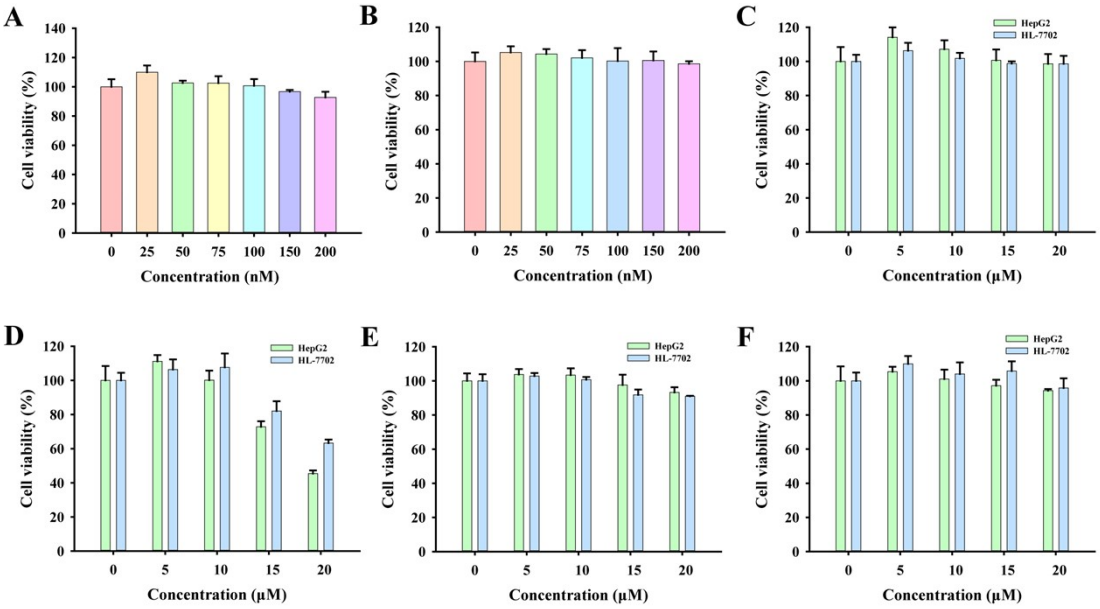
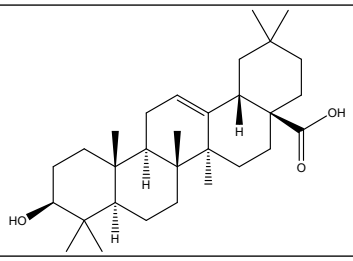
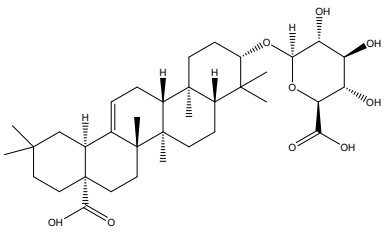
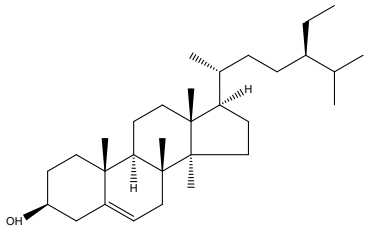
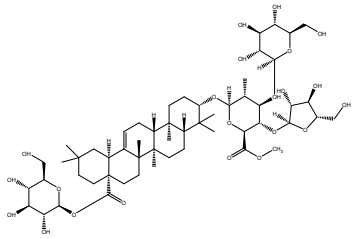
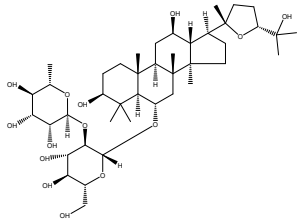
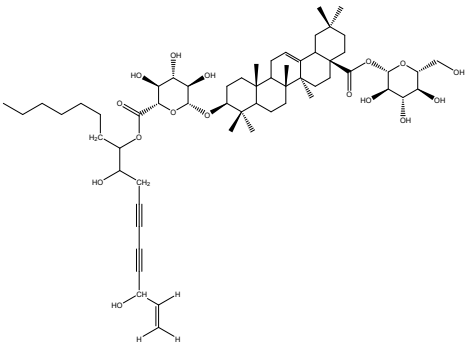
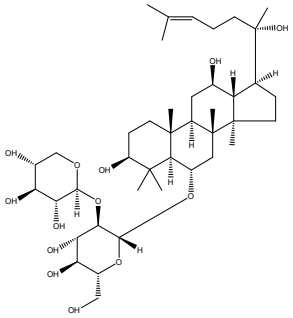


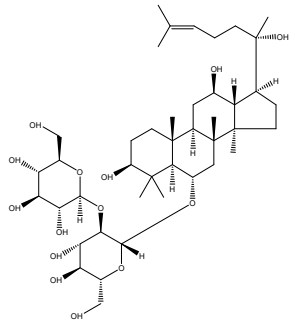
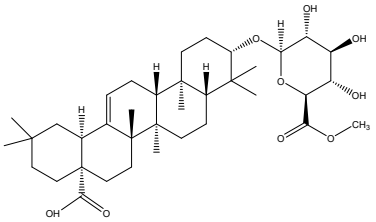
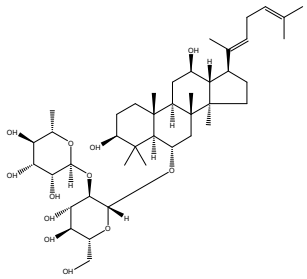
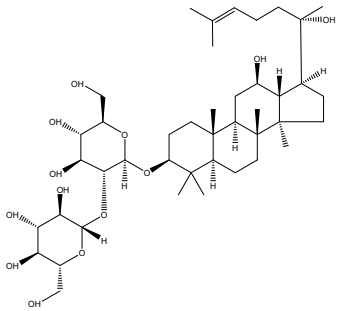
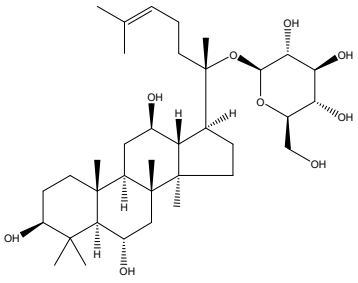
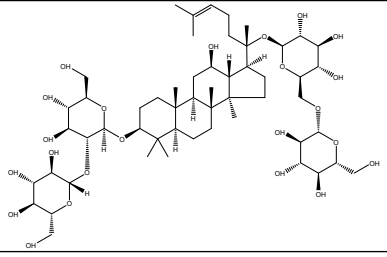
Fig. S5. (A) Cell viability of HepG2 cells treated with various concentrations of nanoprobes for 24 h. (B) Cell viability of HL-7702 cells treated with various concentrations of nanoprobes for 24 h. (C) Cell viability of two kinds of cells treated with various concentrations of natural compound 35 for 24 h. (D) Cell viability of two kinds of cells treated with various concentrations of natural compound 2 for 24 h. (E) Cell viability of two kinds of cells treated with various concentrations of natural compound 1 for 24 h. (F) Cell viability of two kinds of cells treated with various concentrations of natural compound 3 for 24 h.

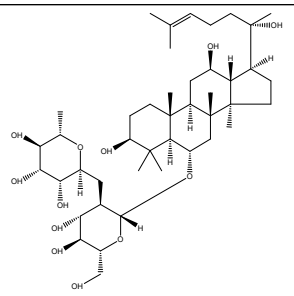
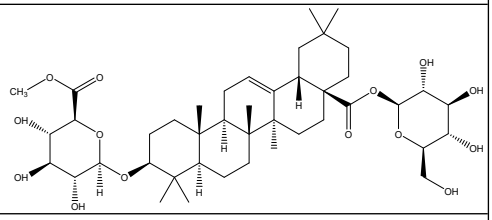
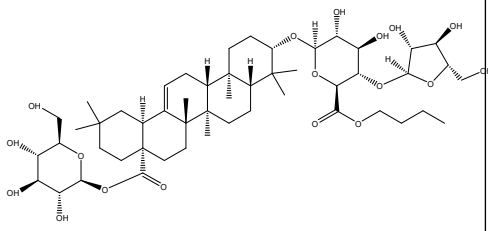
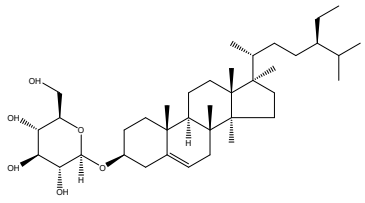
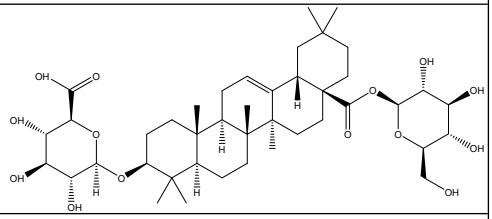
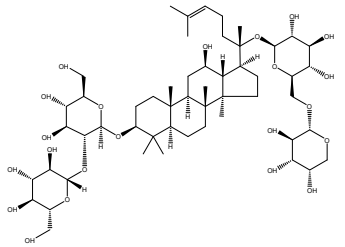
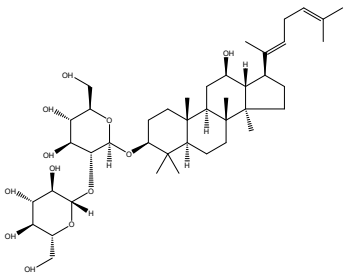
Basic information of natural compounds

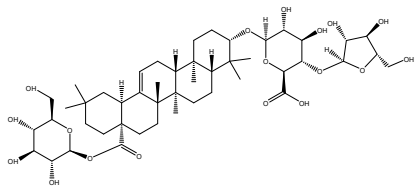
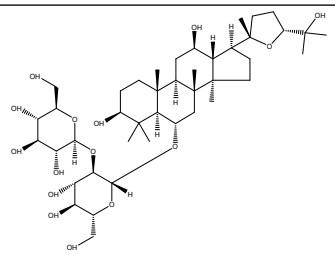
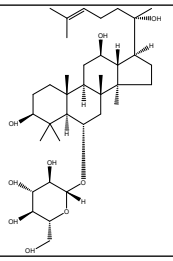
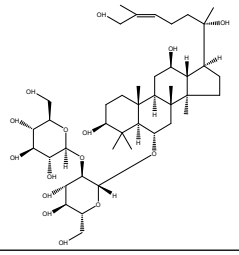
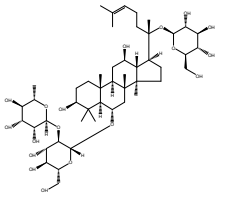
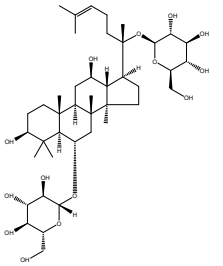
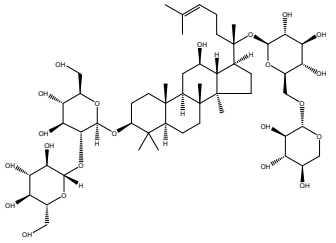
Table S3. Natural Compounds

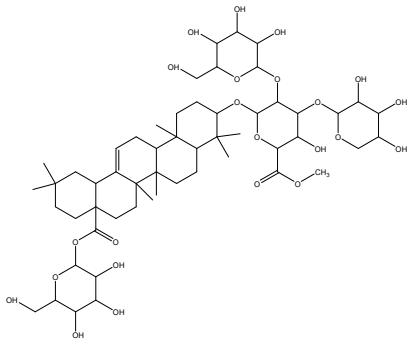
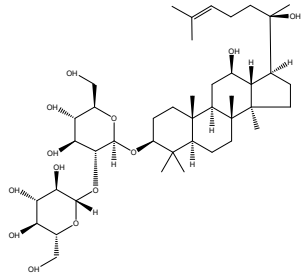
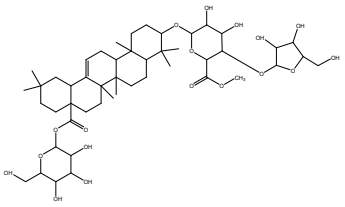
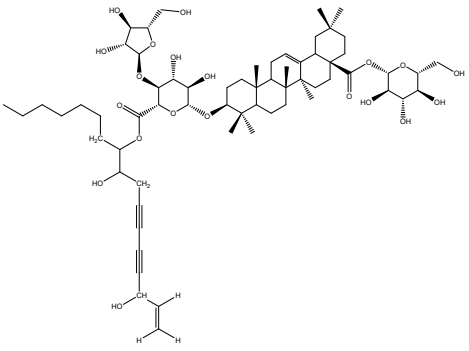
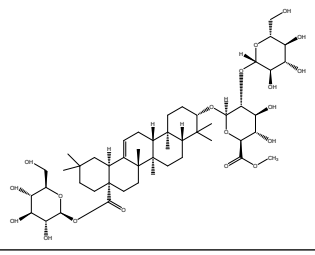
Code	Code Name	Molecular fomula	Chemical Name	Structure
1	XQ-39	$C_{30}H_{48}O_3$	Oleanolic acid	

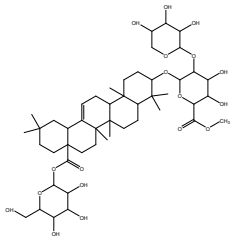
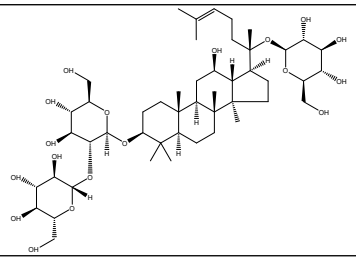
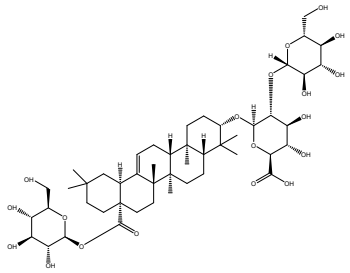
2	XQ-33	$C_{36}H_{56}O_9$	28-Desglucosylchikusetsusaponin IVa	
3	XQ-43	$C_{29}H_{50}O$	β-Sitosterol	
4	XQ-29	$C_{54}H_{86}O_{23}$	β-D-Glucopyranosiduronic acid	
5	XQ-1	$C_{42}H_{72}O_{14}$	Pseudoginsenoside	
6	XQ-25	$C_{59}H_{90}O_{16}$	Baisanqisaponin A	
7	XQ-32	$C_{41}H_{70}O_{13}$	Notoginsenoside R_2	

8	XQ-31	$C_{42}H_{72}O_{14}$	Ginsenoside Rf	
9	XQ-27	$C_{37}H_{58}O_9$	β-D-Glucopyranosiduronic acid	
10	XQ-23	$C_{42}H_{70}O_{12}$	（E）-Ginsenoside F_4	
11	XQ-34	$C_{42}H_{72}O_{13}$	Ginsenoside Rg_3	
12	XQ-35	$C_{36}H_{62}O_9$	Ginsenoside F_1	
13	XQ-37	$C_{54}H_{92}O_{23}$	Ginsenoside Rb_1	

14	XQ-4	$C_{42}H_{72}O_{13}$	20(S)-Ginsenoside Rg2	
15	XQ-12	$C_{43}H_{68}O_{14}$	Chikusetesusaponin IVa methyl ester	
16	XQ-26	$C_{51}H_{82}O_{18}$	Taibaienoside I	
17	XQ-42	$C_{35}H_{60}O_6$	Daucosterin	
18	XQ-3	$C_{42}H_{66}O_{14}$	Chikusetsusponin IVa	
19	XQ-41	$C_{53}H_{90}O_{22}$	Ginsenoside Rb_2	
20	XQ-38	$C_{42}H_{70}O_{12}$	Ginsenoside Rg_5	

21	XQ-2	$C_{47}H_{74}O_{18}$	Chikusetsusaponin IV	
22	XQ-21	$C_{42}H_{72}O_{15}$	24（R）-Majoroside R_1	
23	XQ-7	$C_{36}H_{62}O_9$	Ginsenoside Rh1	
24	XQ-30	$C_{42}H_{72}O_{15}$	Panajaponol A	
25	XQ-19	$C_{48}H_{82}O_{18}$	Ginsenoside Re	
26	XQ-20	$C_{42}H_{72}O_{14}$	Ginsenoside Rg_1	
27	XQ-40	$C_{53}H_{90}O_{22}$	Ginsenoside Rb_3	

28	XQ-5	$C_{54}H_{86}O_{23}$	β-D-Glucopyranosiduronic acid, (3β)-28-(β-D-glucopyranosyloxy)-28-oxoolean-12-en-3-yl O-α-L-arabinopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→2)]-, methyl ester (9CI)	
29	XQ-36	$C_{42}H_{72}O_{13}$	R-Ginsenoside Rg_2	
30	XQ-10	$C_{48}H_{76}O_{18}$	Chikusetsusaponin IV methyl ester	
31	XQ-15	$C_{64}H_{98}O_{20}$	Baisanqisaponin B	
32	XQ-8	$C_{49}H_{78}O_{19}$	Chikusetsusaponin V methyl ester	

33	XQ-9	$C_{48}H_{76}O_{18}$	Cynarasaponin H methyl ester	
34	XQ-6	$C_{48}H_{82}O_{18}$	Ginsenoside Rd	
35	XQ-18	$C_{48}H_{76}O_{19}$	Chikusetsusaponin V	

Reference

[1] Chen Y, Yang CJ, Wu Y, Conlon P, Kim Y, Lin H, Tan W (2008) Light-switching excimer beacon assays for ribonuclease H kinetic study. Chembiochem 9(3):355-359

[2] Lu L, Wang W, Yang C, Kang TS, Leung CH, Ma DL (2016) Iridium(III) complexes with 1,10-phenanthroline-based N^N ligands as highly selective luminescent G-quadruplex probes and application for switch-on ribonuclease H detection. Journal of Materials Chemistry B 4(42):6791-6796

[3] Wu K, Ma C, Liu H, He H, Zeng W, Wang K (2017) Label-free fluorescence assay for rapid detection of RNase H activity based on Tb^{3+}-induced G-quadruplex conjugates. Analytical Methods 9(20):3055-3060

[4] Zhao C, Fan J, Peng L, Zhao L, Tong C, Wang W, Liu B (2017) An end-point method based on graphene oxide for RNase H analysis and inhibitors screening. Analytical Methods 90:103-109

[5] Wang L, Zhou H, Liu B, Zhao C, Fan J, Wang W, Tong C (2017) Fluorescence assay for ribonuclease H based on non-labeled substrate and DNAzyme assisted cascade amplification. Analytical Methods 89:11014-11020

[6] Hu N, Wang Y, Liu C, He M, Nie C, Zhang J, Yu Q, Zhao C, Chen T, Chu X (2020) An enzyme-initiated DNAzyme motor for RNase H activity imaging in living cell. Chemical

Communications 56(4):639-642
[7] Zhang K, Huang W, Huang Y, Li H, Wang K, Zhu X, Xie M (2020) DNA Tetrahedron Based Biosensor for Argonaute2 Assay in Single Cells and HIV-1 Related Ribonuclease H Detection in Vitro. Analytical Chemistry 91(11):7086-7096