the above plate at 1 μL per well, the highest final concentration in the experiment is 4 μM (200 fold dilution). TDF (tenofovir dipiroxil fumarate, Selleck, Cat S1400) has a highest concentration of 4 μM as a positive control. 1 μL of DMSO was added in to the positive control well at a final concentration of 0.5%, TDF was added in to the positive control well at a final concentration of 1μM.

Detection of viral genomic DNA by qPCR

[00399]. Primer: HBV-For-202, CAGGCGGGGTTTTTCTTGTTGA; HBV-Rev-315, GTGATTGGAGGTTGGGGACTGC. Copies of virus can be calculated using a standard curve plotted by using plasmid containing HBV genome and using SYBR Premix Ex Taq II – Takara DRR081S kit and 1 μL cell culture supernatant as a template. EC50 values of the compound on viral replication were calculated by a four parametric nonlinear regression model using Graphpad Prism 5 software to manage concentration - viral copy number. The results were shown as table 3.

Table 3 EC50 values of the compound of the invention against HBV replication

Example	EC_{50}(nM)
Example 7	56
Example 11	32
Example 12	37
Example 13	4
Example 14	7
Example 15	5
Example 16	14
Example 17	4
Example 18	4
Example 19	3
Example 20	5
Example 21	22
Example 22	5
Example 23	7
Example 24	4
Example 25	5
Example 26	8
Example 27	7
Example 28	5
Example 29	6

cytotoxicity of the compound was detected using CellTiter Glo agent.

[00403]. The cytotoxicity of the compound was calculated using the following formulate, cytotoxicity(%) = 100-(detection value/mean of DMSO control wells values × 100). CC50 values were calculated by a four parametric nonlinear regression model using Graphpad Prism 5 software to manage concentration - cytotoxicity(%). CC50 values more than 50 shows that the cytotoxicity is low. The results were shown as table 4.

Table 4 CC50 values of cytotoxicity of the compounds of the invention

Example	CC_{50}(μM)
Example 13	>150
Example 14	>150
Example 15	>150
Example 16	>150
Example 17	>150
Example 18	>150
Example 19	>150
Example 20	>150
Example 21	110
Example 22	>150
Example 23	>150
Example 24	>150
Example 25	>150
Example 26	>150
Example 27	>150
Example 28	>150
Example 29	>150
Example 30	133
Example 31	>150
Example 32	>150
Example 33	>150
Example 34	>150
Example 36	>150
Example 37	>150
Example 38	>150
Example 39	>150
Example 40	>150
Example 44	>150
Example 45	>150

the reference gene equal to ΔCt, *i.e.* Cttarget gene – Ct18S = ΔCt. The ΔCt value of the treatment group minus the ΔCt value of the blank control equal to ΔΔCt, *i.e.* ΔCttreatment group –ΔCtblank control = ΔΔCt. The changes of multiple between the treatment group and the blank control were compared by the statistical analysis by the 2-ΔΔCt method.

Calculation of enzyme activity data

[00442]. The amount of enzyme metabolite production of CYP1A2, CYP2B6 and CYP3A4 were shown as the data of the experiment. The changes of the enzyme activity were represented by comparison of induction multiples of the corresponding cytochrome enzyme in the presence or absence of a test compound. The calculation method of induction multiple and the calculation method of induction ratio to the control compound were shown as follows:

[00443]. induction multiple = enzyme activity of the sample treated with the test compound / nzyme activity of the sample treated with control group

[00444]. induction ratio to the control compound = (induction multiple of the sample treated with the test compound - 1) / (induction multiple of the sample treated with the control compound - 1) × 100%

[00445]. The results of liver drug enzyme induction effect test are shown as table 7:

Table 7 The data of liver drug enzyme induction effect test

Example	Induction ratio of the control compound (10 μM, rifampicin)		
	CYP1A2	CYP2B6	CYP3A4
Example 11	2.5%	2.4%	17.0%
Example 13	-2.7%	-5.3%	-3.7%
Example 14	-0.8%	-1.6%	-0.9%
Example 15	-0.8%	-2.7%	-1.04%
Example 16	-0.8%	0.03%	-1.21%
Example 17	-2.0%	-3.9%	-5.1
Example 18	1.8%	1.3%	0.9%
Example 19	3.7%	1.9%	1.6%
Example 20	-0.8%	-2.1%	1.4%
Example 21	0.3%	1.6%	10.3%
Example 22	2.1%	1.5%	1.0%
Example 23	-2.5%	-3.9%	-1.6%
Example 24	-2.8%	-5.7%	-3.9%
Example 25	-2.9%	-5.6%	-3.2%
Example 26	1.6%	1.4%	0.5%
Example 27	-1,2%	-1.3%	-0.8%
Example 28	2.3%	1.2%	1.4%