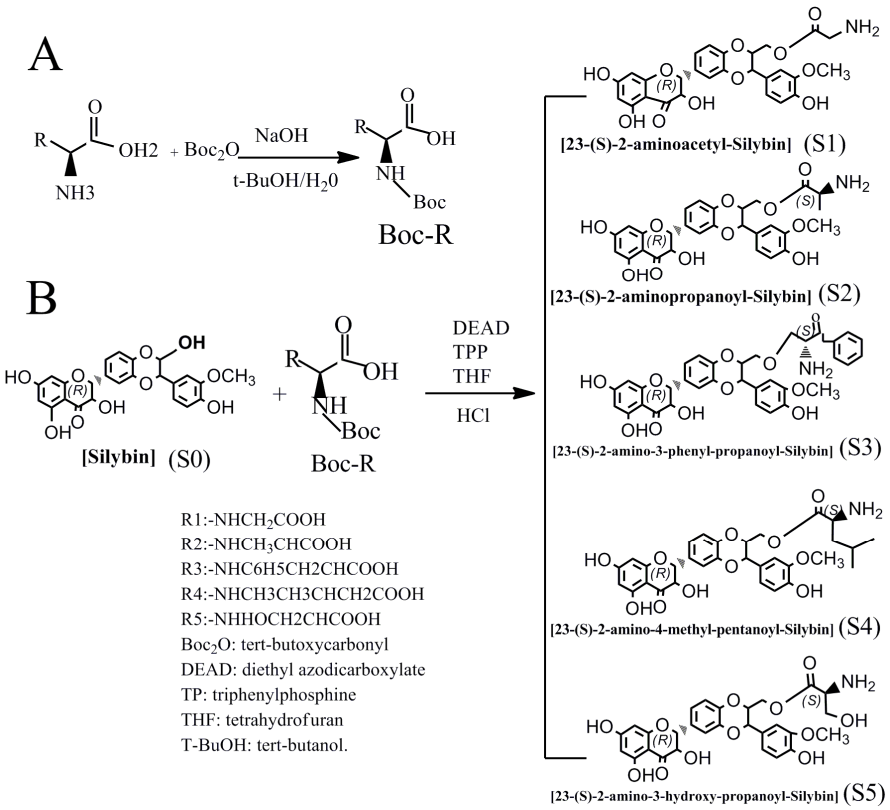


Figure S1. Chemical synthesis of silybin amino acid derivatives. Silybin glycine derivative (S1), silybin alanine derivative (S2), silybin phenylalanine derivative (S3), silybin leucine derivative (S4) and silybin serine derivative (S5) were synthesized as shown in the figure. The process contained two steps. A) In the first step, the amino acids were combined with Boc2O in 10% NaOH solvent (t-BuOH/H2O, v/v=1:1). After purification, we obtained 5 types of N-Boc-amino acids (Boc-R1-5). B) In the second step, silybin (S0) was combined with the different N-Boc-amino acids (Boc-R1-5), hydrolyzed in 6 M HCl. After extraction by ethyl acetate and petroleum ether, we obtained 5 silybin amino acid derivatives.

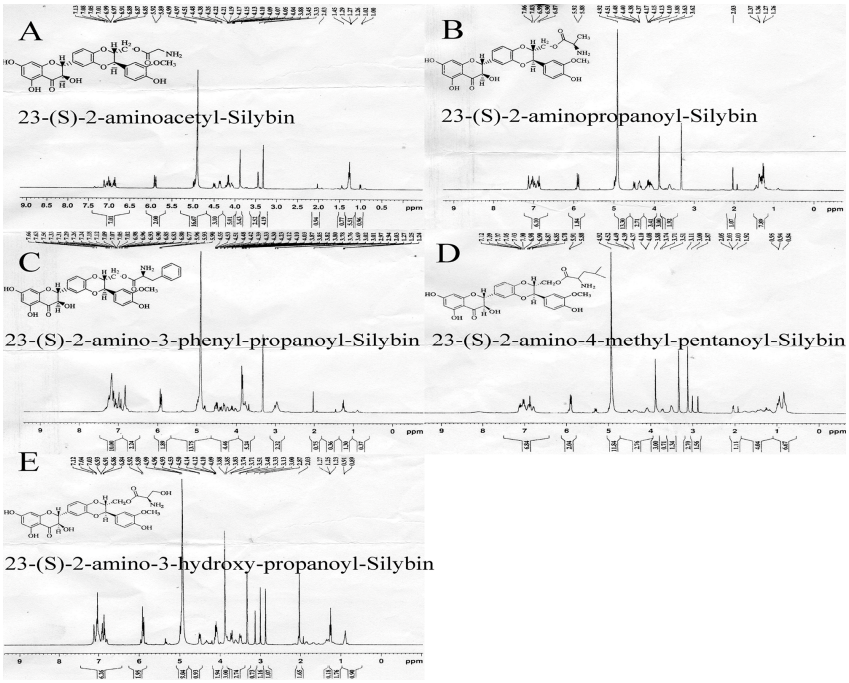


Figure S2. The result of ^{1}H-NMR analysis. ^{1}H-NMR analysis was performed by Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The result was as follows:

(A) 23-(S)-2-aminoacetyl-Silybin (**S1**): (3-(4-hydroxy-3-methoxyphenyl) -6-((2R)- 3,5,7-trihydroxy-4-oxochroman-2-yl) -2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl 2-aminoacetate (400MH, CD_3COCD3) : δ3.821(d, 2H, CH_2), δ1.424(s, 9H, $(CH_3)_3C$-O- CO-)).

(B) 23-(S)-2-aminopropanoyl-Silybin (**S2**): (2S)-(3-(4-hydroxy-3-methoxyphenyl) -6-((2R)-3,5,7- trihydroxy -4 -oxochroman-2-yl) -2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl 2-aminopropanoate (400MH, CD_3COCD_3): δ4.197(s, 1H, CH), δ1.412(s, 9H, $(CH_3)_3C$-O-CO-), δ1.374(d, 3H, CH3).

(C) 23-(S)-2-amino-3-phenyl-propanoyl-Silybin (**S3**): (2S)-(3-(4-hydroxy-3-methoxyphenyl)-6-((2R)-3,5,7-trihydroxy-4-oxochroman-2-yl)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl 2-amino -3- phenylpropanoate (400MH, CD_3COCD_3): δ7.303 (s, 4H, Ar-H), δ7.236(s, 1H, Ar-H), δ 4.464(t, 1H, CH), δ 3.050(dd, 2H, CH_2), δ1.372(s, 9H, $(CH_3)_3C$-O-CO-)).

(D) 23-(S)-2-amino-4-methyl-pentanoyl-Silybin (**S4**): (2S)-(3-(4-hydroxy-3-methoxyphenyl) -6- ((2R) -3,5,7–trihydroxy-4-oxochroman-2-yl)-2,3- dihydrobenzo [b][1,4] dioxin-2-yl) methyl 2-amino-4- methylpentanoate(400MH, CD_3COCD_3): δ4.198(dd, 1H, CH), δ1.747(m, 1H, CH), δ1.569(m, 2H, CH2), δ1.409(s, 9H, $(CH_3)_3C$-O-CO-)), δ0.945(d, 6H, CH_3).

(E) 23-(S)-2-amino-3-hydroxy-propanoyl-Silybin (**S5**): (2S)-(3-(4-hydroxy-3- methoxyphenyl)-6-((2R)-3,5,7– trihydroxy -4-oxochroman -2-yl) -2,3-dihydrobenzo[b][1,4]dioxin-2-yl) methyl2-amino -3- hydroxypropanoate (400MH, CD_3COCD_3): δ4.252(s, 1H,CH), δ3.869 (d, 2H, CH2), δ1.430(s, 9H, $(CH_3)_3C$-O-CO-).

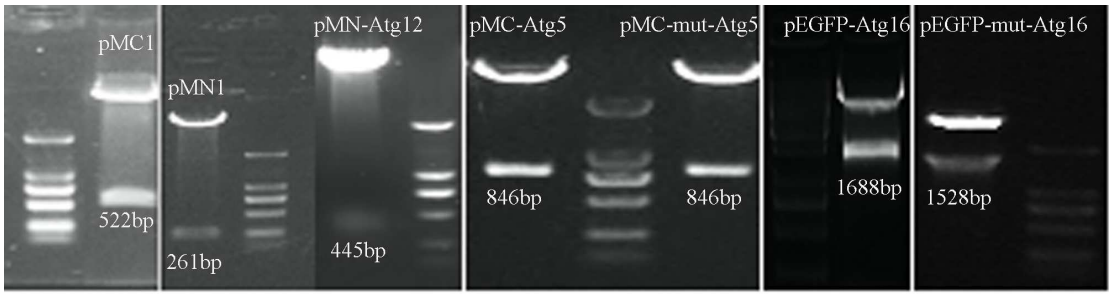


Figure S3. The results of double enzyme digestion of the pMC1, pMN1, pMN-atg12, pMC-Atg5, pMC-mut-atg5, pEGFP-atg16 and pEGFP-mut-atg16 plasmids. The size of each fragment is shown in each graph.

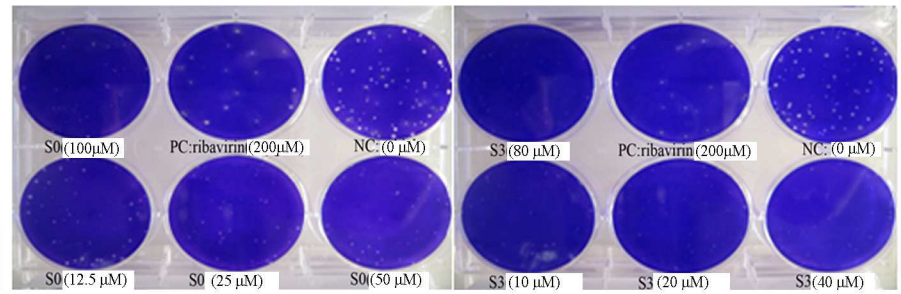


Figure S4. Anti-IAV activities of silybin and its derivatives determined by the classical plaque inhibition assay. The number of plaques (Φ > 1 mm) was counted. Ribavirin and 0.5% DMSO were used as positive and negative controls (PC and NC), respectively.

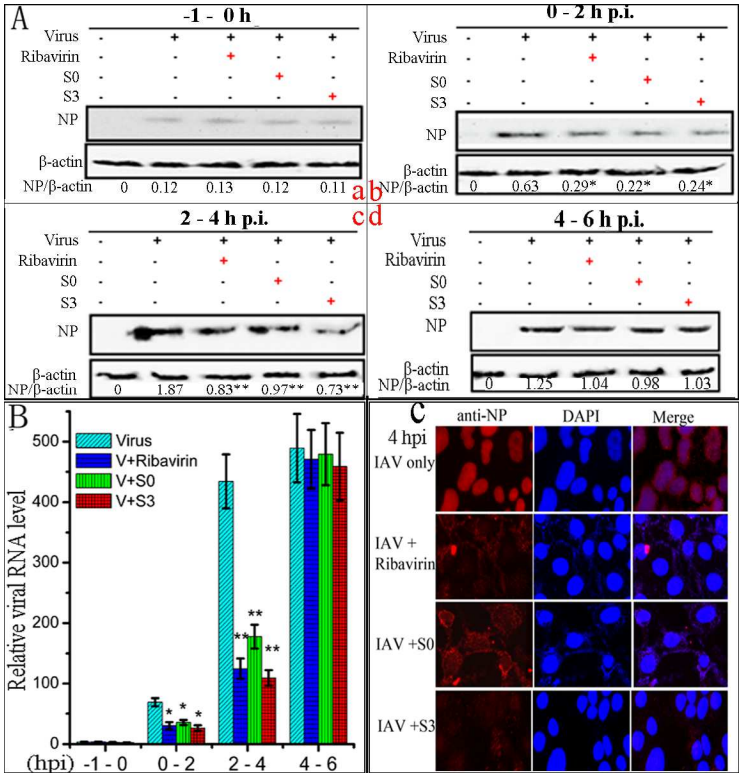
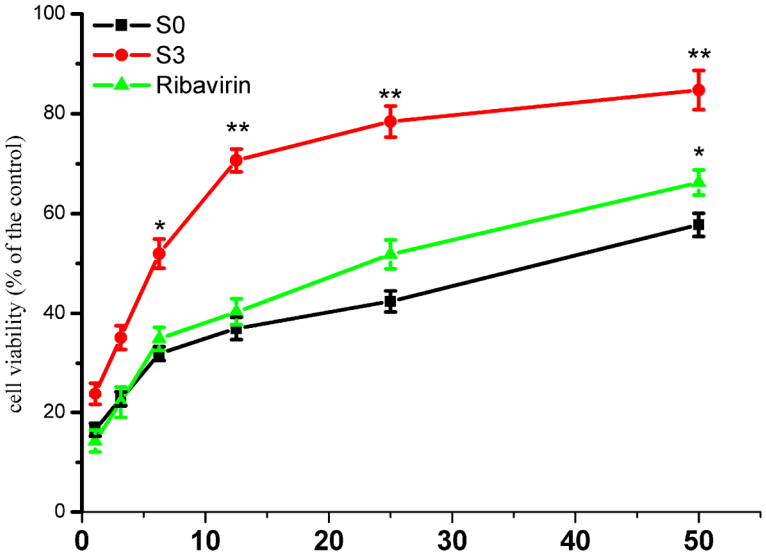


Figure S5. S0 and S3 inhibited IAV proliferation at 1-4 h post infection (p.i.) determined by western blot, quantitative real time RT-PCR (qRT-PCR) and indirect immunofluorescence assays. (**A** and **B)** S0 and S3 inhibited the synthesis of IAV NP protein at 1-4 h p.i. determined by western blot and qRT-PCR. The cells were infected with IAV (MOI = 5.0) and treated with S0, S3 and ribavirin at -1 - 0 (adsorption), 0 - 2, 2 - 4, and 4 - 6 h post infection (p.i.). The zones and average gray values of each band were detected and quantified by BandScan 5.0 software, the multiplication product of zone area and average gray value was calculated, and the results were expressed as the ratio of the target genes to β-actin. (**C)** Indirect immunofluorescence assay. The cells were infected (MOI = 5.0), fixed at 4 h p.i., reacted with anti-NP primary antibody, incubated with a Cy3-conjugated anti-rabbit antibody, and finally stained with DAPI. The concentrations of S0, S3 and ribavirin were 100, 80 and 200 μM, respectively. 0.5% DMSO was used as negative control (virus only group). The data are expressed as means ± SD of 3 experiments, each in duplicate. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs* the negative control.



Compound	CC_{50}(μM)	IC_{50}(μM)	SI
S0	>400	76.88	5.20
S3	>400	8.53	46.90
Ribavirin	>400	81.45	4.91

Figure S6. S0 and S3 inhibited coxsackievirus B3（CVB3）infection, as determined by the SRB method (μM). In the anti-CVB3 assay, the CVB3 strain (Nancy strain) and Vero cells were used. The CC_{50}, EC_{50} and SI (CC_{50}/EC_{50}) are shown below the graphs. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs* the same concentration treatment of ribavirin.

Table S1. The primers for genes cloning and qRT-PCR

Primers	Sequences
Atg5-F	5′-AAAGGTACCATGACAGATGACAAAGATG-′3
Atg5-R	5′-GGTCTCGAGTCAATCTGTTGGCTGTGGGA-′3
Atg12-F	5′-TTAGGTACCGGCCACCATGGCGGAGGAGCCGCAG-′3
Atg12-R	5′-CCGCTCGAGTCCCCACGCCTGAGACTTG-′3
Atg16-F	5′-TTTCTCGAGCTATGGTTCATCACTTTCTGTTTATGGCGTCGGGCCTCCG-′3
Atg16-R	5′- CCTTCTAGATCAGTACTGTGCCCACAGCAC-′3
hLC3-F	5′-AATAGATCTATGCCGTCGGAGAAGACC-′3
hLC3-R	5′-GGCAAGCTTCACTGACAATTTCATCCC-′3
NP-F	5’- GCCAAATCAGCATACAACC-3’
NP-R	5’- CTTGCACTTTCCATCATCC-3’
GAPDH-F	5’-AAG AAG GTG GTG AAG CAG GC-3’
GAPDH-F	5’-TCC ACC ACC CTG TTG CTG TA-3’