Supplementary Material

1. Supplementary Materials and Methods

1.1. Pharmacokinetic study of FZHY decoction in rats

Before the experiment, the rats were fasted overnight but with free access to water. After oral administration of FZHY decoction at 20g raw drug /kg, the rats (n=5 for each time point) were anesthetized and sampled at 0.167 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 11 h, 24 h and 48 h (1.4 g/kg). The hepatic portal vein blood (2 mL) and systemic blood (6-8 mL) were obtained from the portal vein and abdominal aorta, respectively, and the right liver lobe was excised. After centrifuged for 5 min at 10000 rpm, the plasma was obtained. After acetonitrile precipitation, the processed plasma and liver samples were injected to the mass instrument for analysis.

1.2. Instrumentation

The operating parameters were set as follows: spray voltage of 2.5 kv, capillary temperature of 320 °C, source temperature of 350 °C, sheath gas of 35 arbitrary units, auxiliary air of 12 arbitrary units and capillary temperature of 320 °C. The analysis was conducted in full single ion monitoring (SIM) mode. The mass range of m/z 100-1200 was at 1.8-6.35 min in the negative mode and the quantitative ions included 717.14612 for salvianolic acid B [M-H]-, 197.04555 for salvianic acid [M-H]-, 493.11403 for salvianolic acid A [M-H]-, 343.15510 for rosmarinic acid [M-H]-, 1045.55889 for gypenoside XLIX [M-H]-, 1077.58511 for ginsenoside Rb3 [M-H]-. The mass range of m/z 150-400 was at 0-1.8min in the positive mode, and the quantitative ions (m/z) included 494.16567 for amygdalin [M+H]+, 537.21187 for schisantherin A [M+H]+, 433.22209 for schisandrol A [M+H]+, 417.19079 for schisandrol B [M+H]+, 417.22717 for deoxyschizandrin [M+H]+, 401.19587 for schisandrin B [M+H]+, 277.08591 for tanshinone [M+H]+, 297.14851 for cryptotanshinone [M+H]+, 268.10399 adenosine for [M+H]+, 252.10907 for cordycepin [M+H]+. Xcalibur 2.1 workstation was applied for the data analysis and system operation. Chromatographic separation was carried out on an Acquity UPLC BEH column (100 mm × 1.0 mm, 1.7μm, Thermo Scientific, US) at 40 °C. The sample injection and flow rate were set at 5μL and 0.3mL/min, respectively. The mobile phases consisting of solvent A (acetonitrile with 0.1% formic acid) and solvent B (water with 0.1% formic acid) were utilized the following gradient program: 0~1.5min，7% A；1.5~2min，7%~30% A；2~3min，30%~50% A；3~4min，50% A；4~4.5min，50%~70% A；4.5~5.5min，70% A；5.5~6min，70%~90% A；6~9min，90% A；9~9.1min，90%~7% A；9.1~10min，7% A.

1.3. Experimental liver fibrosis models

CCl_4-induced liver fibrosis rat model: Wistar rats were randomly divided into the control (Oil) group (n=8) and the CCl_4 group (n=24). The model group rats were injected subcutaneously with 50% CCl_4 solution in olive oil at a 1mL/kg dose, twice a week for 9 weeks. The control rats were injected subcutaneously with equivalent volume olive oil at a 1mL/kg dose. At the first day of the 7^{th} week, the CCl_4 rats were randomly divided into 3 groups: the CCl_4 group (n=8), the CCl_4 plus JY5 group (n=8) and the CCl_4 plus SORA group (n=8). Rats were respectively administered intragastrically with JY5 (salvianolic acid B: 16mg/kg, amygdalin: 0.5mg/kg,

schisantherin A: 2mg/kg) and SORA (5mg/kg) added to 0.3% CMC-Na suspension at a 10mL/kg dose in drug-treated groups, once a day for 3 weeks. In the control group and CCl_4 group, rats were administered intragastrically with equivalent volume 0.3% CMC-Na suspension at a 10mL/kg dose.

BDL-induced liver fibrosis rat model: SD rats were randomly divided into the control (Sham) group (n=8) and the BDL group (n=24). BDL surgery was performed as previously described (Zhang et al., 2017). At the first day of the second week after BDL, the BDL rats were randomly divided into 3 groups: the BDL group (n=8), the JY5 group (n=8) and the DAPT group (n=8). Rats were administered intragastrically with JY5 (salvianolic acid B: 16mg/kg, amygdalin: 0.5mg/kg, schisantherin A: 2mg/kg) added to 0.3% CMC-Na suspension at a 10mL/kg dose in the JY5 group. The DAPT group rats were injected intraperitoneally with DAPT (30mg/kg) dissolved in DMSO at a dose of 30mg/kg. In Sham group and BDL group, rats were administered intragastrically with equivalent volume 0.3% CMC-Na suspension at a 10mL/kg dose. The rats were treated with drugs once a day for three weeks.

CCl_4-induced liver fibrosis mice model: C57/ BL6 mice were randomly divided into the control (Oil) group (n=8) and the CCl_4 group (n=24). The CCl_4 mice were injected intraperitoneally with 15% CCl_4 solution in olive oil at a 2mL/kg dose, three times a week for 6 weeks. The control mice were injected intraperitoneally with equivalent volume olive oil at a 2mL/kg dose. At the first day of the 4^{th} week, the CCl_4 mice were randomly divided into 3 groups: the CCl_4 group (n=8), the CCl_4 plus JY5 group (n=8) and the CCl_4 plus SORA group (n=8). Mice were respectively administered intragastrically with JY5 (salvianolic acid B: 22.4mg/kg, amygdalin: 0.7mg/kg, schisantherin A: 2.8mg/kg) and SORA (7mg/kg) added to 0.3% CMC-Na suspension at a 10mL/kg dose in drug-treated groups, once a day for 3 weeks. In the control group and CCl_4 group, mice were administered intragastrically with equivalent volume 0.3% CMC-Na suspension at a 10mL/kg dose.

All rats and mice were anaesthetized under sodium pentobarbital i.p. injection, then blood and liver samples were collected, and stored at −80°C for subsequent testing. Partial liver tissues were fixed in 10% neutral-buffered formalin for pathological analysis.

1.4. Histopathological and immunohistochemical analysis

According to corresponding standard protocol, liver sections were stained with Hematoxylin & Eosin (H&E, lot. 20161225, NJBI, Nanjing, China) and Sirius Red (SR). All slides were scanned using the Leica SCN400 slide scanner (Leica Microsystems Ltd., Mannheim, Germany). The whole SR-stained liver sections were analyzed using ImageScope software to calculate the percentage of collagen positive areas (Percent Total Positive × Total Stained Area / Total Analysis Area), which could semi-quantitatively evaluate liver fibrosis better.

IHC staining was performed using the GTVision TM III Detection Kit with peroxidase/DAB, rabbit/mouse (Cat No. GK500705, Gene Tech Co., Ltd, Shanghai, China). Paraffin-embedded sections were dewaxed and rehydrated, subsequently subjected to heat-mediated antigen retrieval in 0.01M sodium citrate buffer, and rinsed in diH2O prior to staining. After cooling to room temperature, the sections were incubated in 3% H_2O_2-methanol mixture solution for 10 minutes to block endogenous peroxidase. Blocking the samples was performed by incubation with 10% goat serum at room temperature for 30 min, before incubation with primary antibody overnight at

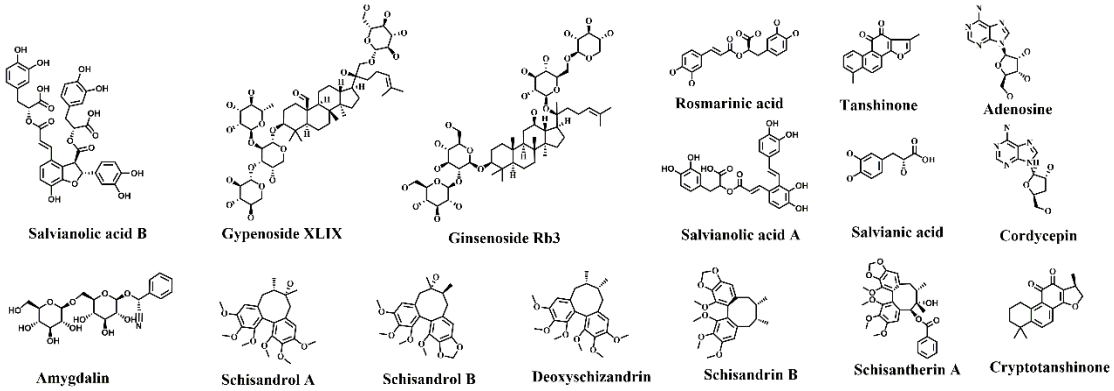
4°C in a wet box. The next day, incubation with the second antibody and chromogen detection was performed using the DAB chromogen by light microscopy. After nuclear counterstaining by hematoxylin, the sections were differentiated, dehydrated and transparentized. Finally, the sections were sealed with neutral gum and scanned using the Leica SCN400 slide scanner to further analysis.

1.5. Luciferase reporter assay

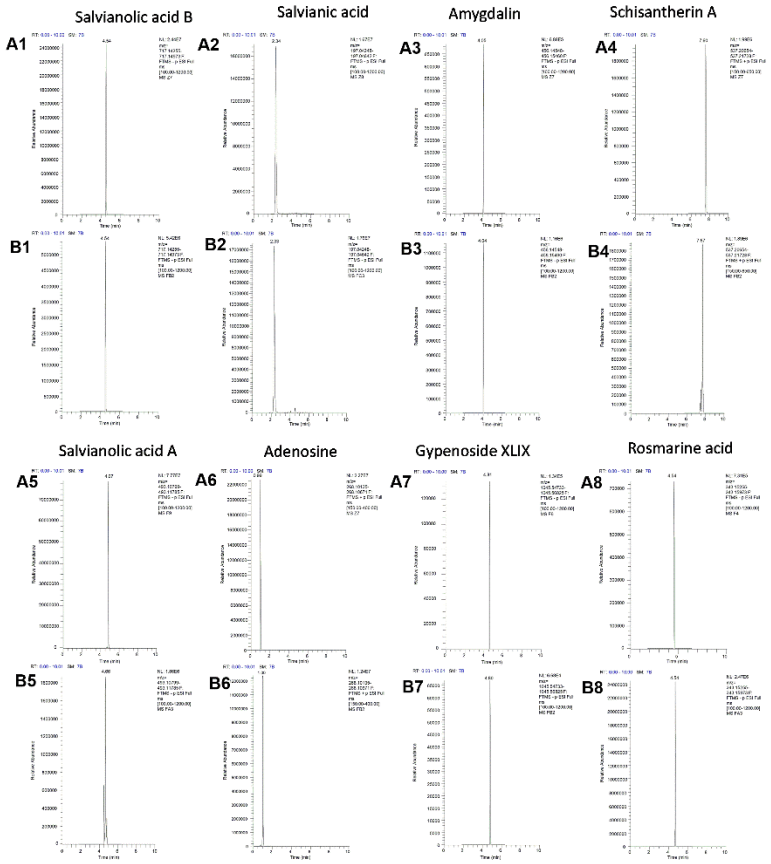
The transcriptional activity of Notch was measured using RBP-κB luciferase reporter plasmid constructed by Shanghai Jikai Gene Chemical Technology Co. Ltd. following the supplier's instructions for use. The transiently co-transfected LX-2 cells with firefly luciferase and renilla luciferase plasmid using Lip8000TM (C0533, Beyotime Biotechnology, China) were treated with TGF-β1(5ng/mL), and simultaneously treated with salvianolic acid B (32μM), amygdalin (1μM), schisantherin A (4μM) or JY5 (37μM) for 24 hours, respectively. RBP-κB luciferase activity was detected by Dual-Lumi™ luciferase reporter gene assay kit (RG088S, Beyotime Biotechnology, China) following the manufacturer's instructions. With renilla luciferase a the internal control in each transfection, the relative luciferase activity was calculated as the ratio of firefly-to-renilla luciferase activity.

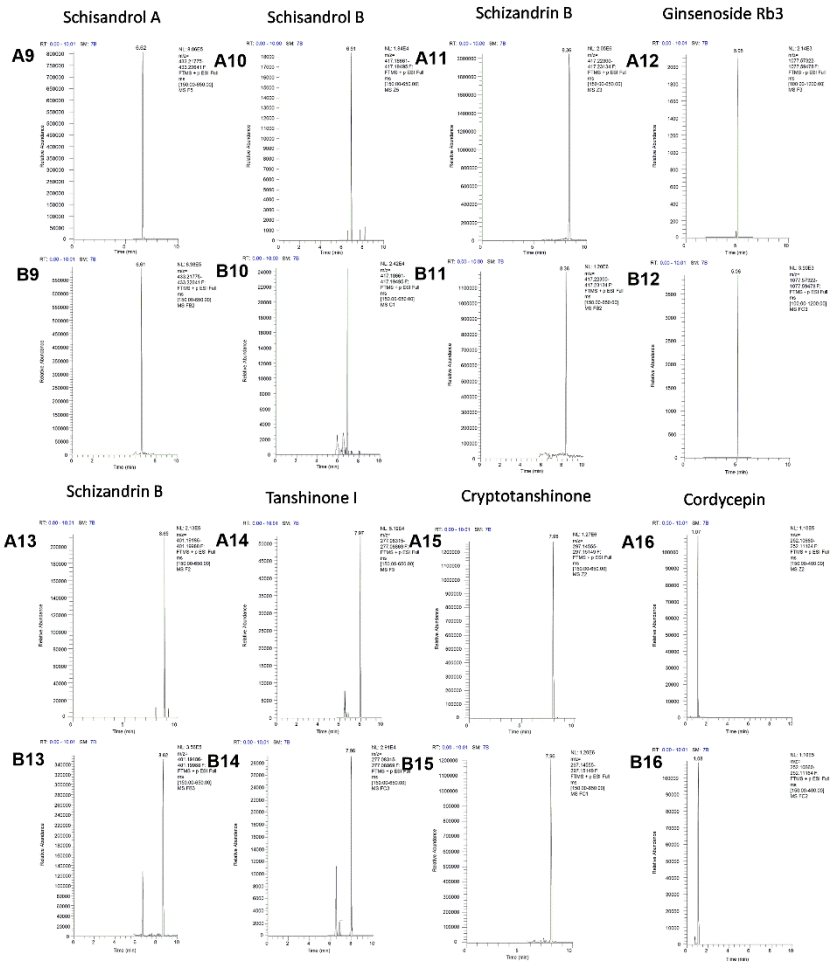
2. Supplementary Figures and Tables

2.1 Supplementary Figures

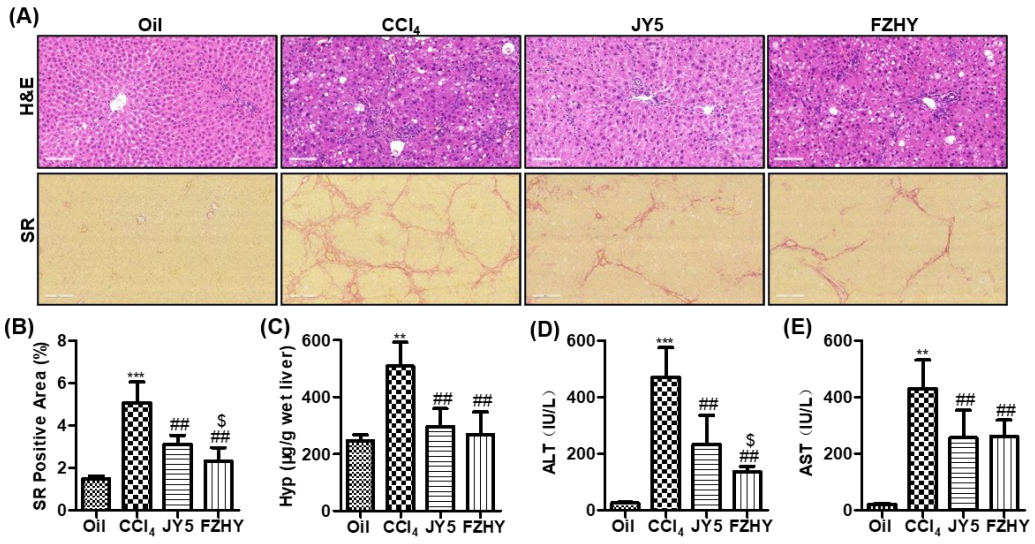


Supplementary Figure 1. The chemicals structure of 16 compounds in FZHY decoction (2g/mL).


Salvianolic acid B
Salvianic acid
Amygdalin
Schisantherin A
A1
A2
A3
A4
B1
B2
B3
B4
Salvianolic acid A
Adenosine
Gypenoside XLIX
Rosmarine acid
A5
A6
A7
A8
B5
B6
B7
B8

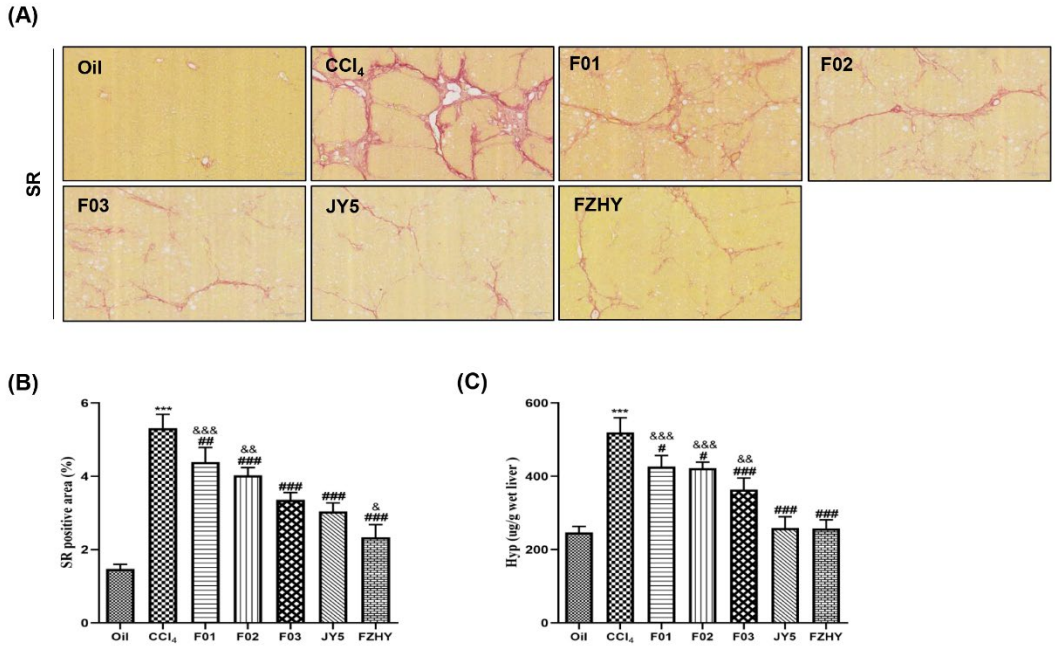


Supplementary Figure 2. The SIM chromatograms of 16 constituents in the standard curves (A) and FZHY decoction by UPLC- Q- trap mass.

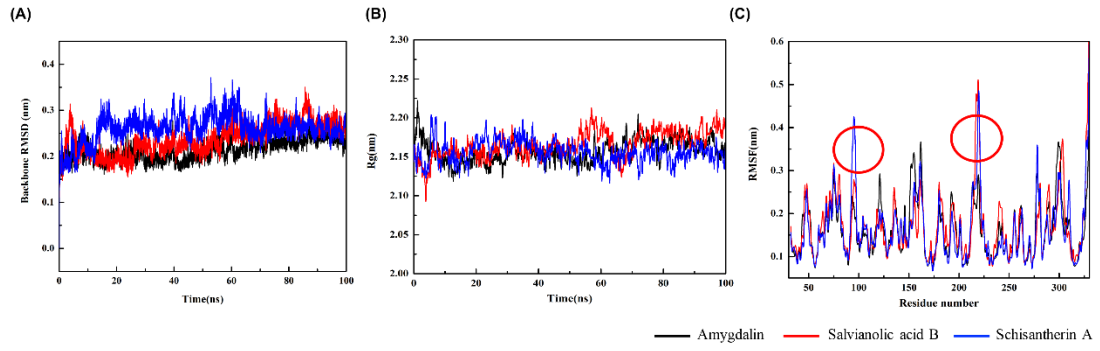


Supplementary Figure 3. JY5 and FZHY significantly alleviates hepatic injury and collagen deposition in CCl_4-induced rat liver fibrosis. H&E (100×) and SR (100×) staining(**A**), and semi-quantitative analysis (**B**) of collagen disposition (%) in

SR-stained liver sections. (**C**). Hydroxyproline content in wet liver tissue was detected. (**D, E**) The levels of serum ALT and AST were measured. *$p < 0.05$, **$p<0.01$, and ***$p<0.001$ vs. the control group, $^{\#\#}p<0.01$ vs. the CCl_4 group, $^{\$}p < 0.05$ vs. the FZHY treated group. Oil, control group.



Supplementary Figure 4. JY5 significantly alleviates liver tissue collagen deposition and Hyp content in CCl_4-induced rat liver fibrosis. SR (100×) staining(**A**), and semi-quantitative analysis (**B**) of collagen disposition (%) in SR-stained liver sections. (**C**). Hyp content in wet liver tissue was detected. ***$p<0.001$ vs. the control group, $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ vs. the CCl_4 group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ and $^{\&\&\&}p < 0.001$ vs. the JY5 treated group. Oil, control group; F01, salvianolic acid B; F02, amygdalin; F03, schisantherin A.



Supplementary Figure 5. Molecular dynamics simulation findings. (A) Stability analysis as inferred by RMSD. (B) Compactness of the protein from Rg. (C) Fluctuations rendered by the RMSF plots.

2.2 Supplementary Tables

Supplementary Table 1. The antibodies used in this study.

Antibody	Species	Company	Cat No	Concentration
Col-I	Rabbit pAb	abcam	ab34710	IHC 1:200
Col-IV	Rabbit pAb	abcam	ab6586	IHC 1:250
α-SMA	Rabbit pAb	abcam	ab5694	IHC 1:200
				WB 1:1000
Desmin	Rabbit pAb	abcam	ab15200	IHC 1:400
Jagged1	Rabbit pAb	Epitomics	Epit3772S	WB 1:1000
Notch2	Rabbit mAb	CST	#4530S	WB 1:1000
Notch3	Rabbit pAb	ABclonal	A13522	WB 1:1000
Notch4	Rabbit pAb	ABclonal	A8303	WB 1:1000
RBP-κB	Rabbit mAb	CST	#5313	WB 1:1000
GAPDH	Mouse mAb	Proteintech	60004-1-Ig	WB 1:5000
Anti-rabbit IgG	Goat	CST	#5366	WB 1:10000
Anti-mouse IgG	Goat	CST	#5257	WB 1:10000

Supplementary Table 2. Primers used in this study.

Name	Rat (5'→3')	Mouse (5'→3')	Human (5'→3')
α-SMA			
Forward	GAGGAGCATCCGACCTTGC	GTCCCAGACATCAGGGAGTAA	TGCAACATGGAAGGTATTGC
Reverse	TTTCTCCCGGTTGGCCTTA	TCGGATACTTCAGCGTCAGGA	TTCACACAGCACCAAGC
Col-I			
Forward	GCCTCCCAGAACATCACCTA	GCTCCTCTTAGGGGCCACT	CCAAGGTGGGATCGTGAGG
Reverse	CCTTCTTGAGGTTGCCAGTC	CCACGTCTCACCATTGGGG	TCGGAAGGATAAAACGCGGTC
Notch2			
Forward	GAGGAAGAAGTGTCTCAA	CCACCTGCAATGACTTCATCGG	CCCAATGGGCAAGAAGTCTA
Reverse	GTGGCATCAGAAACATAT G	TCGATGCAGGTGCCTCCATTCT	CACAATGTGGTGGTGGTGGGATA
Notch3			
Forward	GACAAGGACCACTCCCACTACT	GACTGCTCACTGAACGTGGA	GCATAGGCCAGTTCACCTGT
Reverse	ATCCACATCATCCTCACAACTG	CACACCGGCTGTTGTTGAAG	AATGTCCACCTCGCAATAGG
Notch4			
Forward	TGTCAGGAACCAGTGTCAGAA C	CTCTTGCCACTCAATTTCCCT	TCTCCGGCACCCGATGT
Reverse	CCTGGGCTTCACATTCATCTAT	TTGCAGAGTTGGGTATCCCTG	TCAAAGCCTGGGAGACACTTG
Jagged1			
Forward	CCATCAAGGATTATGAGAAC	CCTCGGGTCAGTTTGAGCTG	CAACCGTGCCAGTGACTATTTCTGC
Reverse	TGGTGCTTATCCATATCA	CCTTGAGGCACACTTTGAAGTA	TGTTCCCGTGAAGCCTTTGTTACAG
Jagged2			
Forward	AAATGAGTGGTCCGTGGCAGA	CAATGACACCACTCCAGATGAG	AACGATACCCCGAATGAGG
Reverse	TGGTTGGAAGCCTTGTCTGCT	GGCCAAAGAAGTCGTTGCG	GCTGCCACAGTAGTTCAGGTCTTTG
Delta-like 1			
Forward	GTGTGCAGATGGTCCTTGCTTC	GCTGGAAGTAGATGAGTGTGCTC	TCCTGATGACCTCGCAACAGA
Reverse	CTGACATCGGCACAGGTAGGAG	CACAGACCTTGCCATAGAAGCC	ACACACGAAGCGGTAGGAGT
Delta-like 3			
Forward	CTGAGGTTACAAGACGGTGCT	CTGGTGTCTTCGAGCTACAAAT	CACTCCCGGATGCACTCAAC
Reverse	GTAAATGGAAGGGGCTGGTATG	TGCTCCGTATAGACCGGGAC	CCCGAGCGTAGATGGAAGGA

Delta-like 4			
Forward	GCAGAACCACACACTGGACTAT	TTCCAGGCAACCTTCTCCGA	GACCACTTCGGCCACTATGT
Reverse	TGGCACCTTCTCTCCTAAACTC	ACTGCCGCTATTCTTGTCCC	CCTGTCCACTTTCTTCTCGC
RBP κB			
Forward	TTGCTTACCTTCAGGCGTGTG	TGGCTACATCCATTACGGGCAG	TCATGCCAGTTCACAGCAGTGG
Reverse	GCCCAATGAGTCTGCTGCAA	GTGGAGTTGTGATACAGGGTCG	TGGATGTAGCCATCTCGGACTG
GAPDH			
Forward	GGCACAGTCAAGGCTGAGAATG	AAGGTCATCCATGACAACTTTGGC	GAAATCCCATCACCATCTTCCAGG
Reverse	ATGGTGGTGAAGACGCCAGTA	ACAGTCTTCTGGGTGGCAGTGAT	GAGCCCCAGCCTTCTCCATG

Supplementary Table 3. The content and source of 16 compounds in FZHY decoction.

Compounds	Source	Concentration (μg/mL)
Salvianolic acid B	*Salvia miltiorrhiza*	3067.86
Danshensu	*Salvia miltiorrhiza*	1939.75
Amygdain	*Peach kernel*	1926.57
Schisantherin A	*Schisandra chinensis*	1400.61
Salvianolic acid A	*Salvia miltiorrhiza*	1399.69
Adenosine	*Cordyceps mycelium*	855.73
Gypenoside XLIX	*Gynostemma pentaphylla*	346.19
Rosmarinic acid	*Salvia miltiorrhiza*	276.23
Schisandrol A	*Schisandra chinensis*	144.88
Schisandrol B	*Schisandra chinensis*	70.95
Deoxyschizandrin	*Schisandra chinensis*	8.65
Ginsenoside Rb3	*Gynostemma pentaphylla*	4.66
Schisandrin B	*Schisandra chinensis*	4.59
Tanshinone I	*Salvia miltiorrhiza*	2.93
Cryptotanshinone	*Salvia miltiorrhiza*	1.39
Cordycepin	*Cordyceps mycelium*	1.21

Supplementary Table 4. The related information of components docked with key targets.

Protein	Component	Binding energy (kcal/mol)
Jagged1	Salvianolic acid B	1.16
Jagged1	Amygdalin	-0.64
Jagged1	Schisantherin A	-2.88
Notch2	Salvianolic acid B	0.19
Notch2	Amygdalin	-1.8
Notch2	Schisantherin A	-3.32
RBP-κB	Salvianolic acid B	-5.7
RBP-κB	Amygdalin	-3.84
RBP-κB	Schisantherin A	-6.7

The binding energy refers to the strength of the binding between the receptor and the ligand; the lower the binding energy, the more stable the docking module.

References

Zhang, X., Xu, Y., Chen, J., Liu, C., Du, G., Zhang, H., et al. (2017). Huang Qi Decoction Prevents BDL-Induced Liver Fibrosis Through Inhibition of Notch Signaling Activation. *Am J Chin Med* 45(1)**,** 85-104. doi: 10.1142/S0192415X17500070.