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Synthesis and anti-fibrotic effects of santamarin derivatives as cytotoxic agents against hepatic stellate cell line LX2

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ABSTRACT

Liver fibrosis is a final result of extensive deposition of extracellular matrix (ECM) and starts with the activation and proliferation of hepatic stellate cells (HSCs). Our previous study showed that eudesmane sesquiterpenoid santamarin had cytotoxicity against hepatic stellate cell line LX2 (HSC-LX2) with IC_{50} values of 16.5 ± 0.7 μM. To explore the structure–activity relationships, twenty-six derivatives were synthesized by modifying the hydroxyl group, double-bond and unsaturated lactone. Cytotoxicity evaluation suggested that eight derivatives (**6, 9, 13, 17, 20** and **25–27**) increased activity against HSC-LX2. Especially, derivatives **17**, **20** and **25** displayed obvious cytotoxicity with IC_{50} values of 6.4 ± 0.4, 4.6 ± 0.1, and 3.5 ± 0.1 μM, which were 3 to 5-fold higher than santamarin. Preliminary mechanisms study revealed that the active compound **20** exhibited more than 8-fold and 6-fold enhancement of inhibitory effect on the deposition of human hyaluronic acid (HA) and human laminin (HL) with IC_{50} values of 7.6 ± 0.6 and 3.3 ± 1.2 μM.

Introduction

Liver fibrosis is the progressive pathological result of chronic liver injury that causes by many liver diseases like chronic viral hepatitis, alcoholic or nonalcoholic steatohepatitis, etc.[1,2] The pathological process originates from the activation and proliferation of hepatic stellate cells (HSCs), which *trans*-differentiate into myofibroblasts as a repair response to chronic injury.[3,4] The extensive deposition of collagen-based extracellular matrix (ECM) produced by myofibroblasts leads to liver fibrosis.[5] Without effective treatment, liver fibrosis will generally progress to cirrhosis or hepatocellular carcinoma (HCC) which causes two million deaths annually.[6] In recent years, significant therapeutic progress has been achieved, and more than 20 candidate drugs are in clinical trials, but none of them have been approved for the treatment of liver fibrosis.[7–9] Therefore, there is still an urgent need to develop highly effective and specific anti-fibrotic medicants.

Natural products and their derivatives played an important role in the drug discovery due to their diverse structural skeletons and various bioactivities.[10,11] Many natural products such as glycyrrhetinic acid,[12] saikosaponin D,[13] tetrandrine,[14] resveratrol,[15] silybin[16] and cucurbitacin B[17] have been reported as active ingredients against liver fibrosis. It is reported that the inhibition of HSCs proliferation is one of the effective anti-fibrotic strategies.[2,18,19] Our previous study demonstrated that santamarin (STM, **1**), a eudesmane sesquiterpenoid from *Artemisia lavandulaefolia,* showed obvious cytotoxicity against HSC-LX2 with IC_{50} value of 16.5 ± 0.7 μM, and inhibited the deposition of human collagen type I (Col I) and human laminin (HL) in HSC-LX2 with IC_{50} values of 7.3 ± 1.7 μM and 18.6 ± 1.4 μM.[20] Furthermore, it was also reported that STM had various biological activities including anti-tumor, anti-inflammatory and anti-bacterial and anti-ethanol activities.[21–26] For example, STM inhibited the growth of L1210, CCRF-CEM, KB, LS174T and MCF-7 cells *in vitro* with IC_{50} in the range of 0.16–0.92 μg/mL. Mechanism study revealed its cytotoxicity might be related to suppression of microtubular proteins, induction of cell cycle blockage in the G2/M phase, and induction of apoptosis via activation of caspase 3. Although various pharmacological properties of STM had been revealed,

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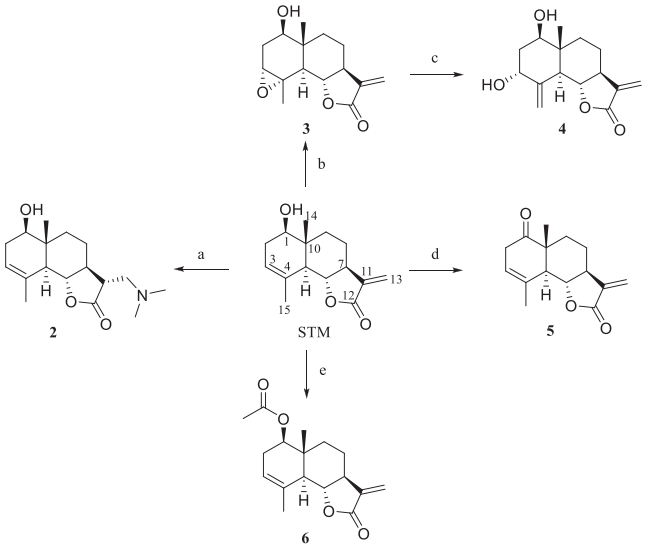
no investigation of chemical modified STM derivatives had been conducted for their anti-fibrotic effects.

Structurally, STM possesses a 6,6,5-tricyclic ring system bearing functional groups of exomethylene group conjugated with carbonyl γ-lactone, 1β-hydroxyl group, olefinic double bond C3═C4, which made it to be perspective for chemical modification. In order to explore the structure–activity relationships (SARs) and possibly develop more potent anti-fibrotic agents, 26 analogues of STM were synthesized and evaluated for cytotoxicity against HSC-LX2.

Investigations have shown that the presence of α-methylene-γ-lactone moiety is important for sesquiterpene lactones to achieve good cytotoxicity. It is validated that the terminal double-bond between C-11 and C-13 is essential. Thus, changing of the α, β -unsaturated lactone system via an aza-Michael addition was conducted (Scheme 1). Reaction of compound **1** with dimethylamine yielded nitrogen-containing compounds **2** as a single diastereomer.[27] To clarify the role of C3-C4 double bond, epoxide **3** was obtained in 81% yield by *m*-CPBA oxidation, subsequent $HClO_4$ mediated epoxy isomerization delivered allylic alcohol **4** in moderate yield.[28] In order to evaluate the function of hydroxy group at C-1 for cytotoxicity, the 1β-hydroxyl group was oxidized to ketone via Dess-Martin periodinane to deliver compound **5,** acetylation of hydroxyl group yielded compound **6**. As shown in Table 1, acetylation product **6** showed an increase in the cytotoxicity with an IC_{50} value of 12.3 ± 0.4 μM. This result prompted us to conduct the further modification focused on introducing different substituents to the hydroxy group (Scheme 2).

In order to clarify the influence of ester side chain on their cytotoxicity against HSC-LX2, the esterification products **7**–**22** were synthesized through the condensation of STM with different carboxylic acids, including aliphatic, alicyclic, aromatic and cinnamic acids. Treatment of STM with acids (two equivalents) in CH_2Cl_2 in the presence of EDCI and DMAP for 2 to 24 h at room temperature afforded target compounds in yield ranging from 43% to 87%.

Pirfenidone is an anti-fibrotic agent approved for the treatment of idiopathic pulmonary fibrosis.[29–30] It also has demonstrated activity in reduce liver fibrosis, which is able to inhibit proliferation of HSCs induced by numerous growth factors, and can decrease collagen deposition in a variety of animal models *in vivo*. With the aim of enhancing the activity, it was designed to synthesize two STM-drug hybrids as novel anti-fibrotic agents by incorporating the pharmacophores of pirfenidone into the core skeleton of STM with different linkers. The target compounds **23** and **24** were synthesized in three steps as depicted in Scheme 3. CuI-catalyzed coupling reaction of pyridin-2-ones with aromatic halides afforded 1,5-disubstituted-pyridin-2(1H)-ones **S1** and **S2** in good yields. Hydrolysis of **S1**, followed by esterification of resulting carboxylic acid with STM, yielded compound **23**. Hydronidone **S4** was prepared from **S3** via BBr_3-mediated demethylation and was then condensed with compound **8** to provide the desired hybrid **24**, in which two pharmacophores were hybridized via ester-ester bonds (Scheme 4).



Scheme 1. Reagents and conditions: (a) Me_2NH, EtOH, r.t., 73%; (b) *m*-CPBA, CH_2Cl_2, r.t., 81%; (c) 6% $HClO_4$ aqueous, DME, r.t., 66%; (d) Dess-Martin periodinane, CH_2Cl_2, r.t., 71%; (e) Ac_2O, DMAP, dry CH_2Cl_2, r.t., 69%.

Inspired by the interesting result that a lot of dimeric sesquiterpene lactones are more potent than their monomers in the bioactivity,[31] santamarin-derived dimers **25**–**27** containing different ester-linker to connect the two monomers were prepared by reacting STM with corresponding diacids in the precense of DMAP and DIC.

All synthesized compounds were characterized by spectral analyses of ^1H NMR, ^{13}C NMR and HRESIMS. The cytotoxicity of all STM derivatives against HSC-LX2 were tested *in vitro* using the MTT method with silybin as the positive control. Twenty-six derivatives were tested at concentrations of 100.0 and 50.0 μM (Table S1, Supporting Information), of which 25 derivatives showed inhibitory ratios higher than 50% at 100.0 μM and their IC_{50} values were further measured for their dose-dependent effects (Table 1).

As expected, compound **2** showed almost no cytotoxicity against HSC-LX2 at the concentration of 100 μM, which suggests that α-methylene-γ-lactone moiety is an essential pharmacophore. Modification on the double bond ($\Delta^{3,4}$) led to significantly decrease in the cytotoxicity, indicating that the double bond was important for maintaining activity. Oxidation of the 1β-hydroxyl group at C-3 of STM delivered the ketone **5**, which exhibited low cytotoxic activity relative to that of STM.

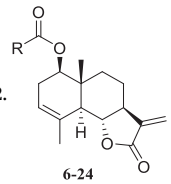
Compounds **6**–**24** with diverse acyloxy group at C-1 showed different activities with IC_{50} values ranging from 4.6 to 92.0 μM, suggesting that ester side chains had significant influence on the cytotoxicity. Compound **6** (IC_{50}, 12.3 ± 0.4 μM) with an acetyl at C-1 displayed higher activity than compound **7** (IC_{50}, 32.4 ± 3.2 μM) with an octanoyl group, which indicated that extending the length of ester side chain was unfavorable. Compound **8** containing an additional carboxyl in the acyloxy was the weakest compound in this series, suggesting the incorporating an additional carboxyl group was unfavorable toward cytotoxicity. Of the aromatic derivatives, 3-*O*-benzoyl and 3-*O*-cinnamoyl analogues **10** and **14** showed similar cytotoxicity with STM. Derivatives **11**, **12**, **15** with nitrogen or sulfur heteroatomic rings did not improve the activity. Comparison of compounds **13** and **16**–**18** with trifluoromethyl substituent at the aromatic ring, the *meta*-substituted compounds **13** and **17** showed better activity than those of *ortho*- and *para*-substituted (**16** and **18**). Compound **17** exhibited cytotoxicity of nearly 3-fold more potent than STM with IC_{50} value of 6.4 ± 0.4 μM. In contrast to 4′-fluorocinnamoyl analogue **19**, the pentafluorocinnamyl analogue **20** enhanced 4-fold cytotoxicity with IC_{50} value of 4.6 ± 0.1 μM, indicating that polyfluorinated substituent was favorable. When the 4-formylcinnamyl and acetoxyl cinnamyl groups were introduced into the substrate, the obtained compounds **21** and **22** exhibited weaker cytotoxicity. A dramatic reduction in cytotoxicity in STM-hydronidone hybrids **23** and **24** was observed, it means that the STM and hydronidone are not compatible towards cytotoxicity against HSC-LX2.

Among dimeric products, compound **25** with a two carbons bridge displayed the most potent activity against HSC-LX2 with an IC_{50} value of 3.5 ± 0.1 μM, which is 5-fold higher than STM. Compound **26** with a three carbons bridge showed cytotoxicity of nearly 3-fold less potent than compound **25**. Replacing its linker with aromatic fragment (**27**) also resulted in an obvious decrease in cytotoxicity, suggesting a different effect of the linker on the toxicity of the dimers.

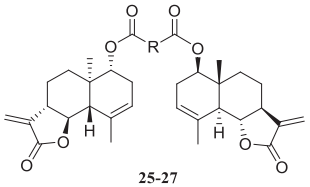
Derivatives **17**, **20** and **25** showed potent cytotoxicity against HSC-LX2 with IC_{50} values of 6.4 ± 0.4, 4.6 ± 0.1, and 3.5 ± 0.1 μM, indicating 3–5 fold higher than STM (IC_{50}, 16.5 ± 0.7 μM). Furthermore, inhibitory effects of the most active compounds **17**, **20** and **25** on the deposition of human hyaluronic acid (HA), HL and Col I in HSC-LX2 were performed to explore the possible mechanisms. As depicted in Table 2, compounds **17**, **20** and **25** all showed better inhibitory effects

Table 1

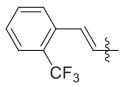
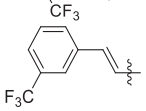
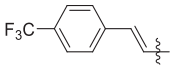
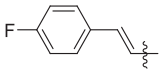
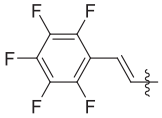
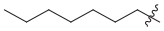
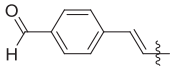
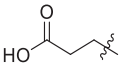
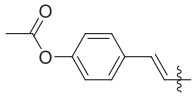
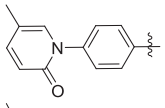
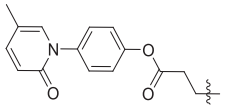
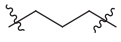
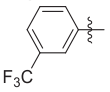
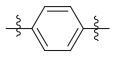
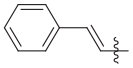
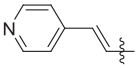
Cytotoxicity of compounds **3**–**27** and against HSC-LX2.



6-24



25-27

Compd.	R	IC_{50} (μM)[a]	Compd.	R	IC_{50} (μM)[a]
STM	–	16.5 ± 0.7	**16**		16.5 ± 1.7
3	–	47.4 ± 5.9	**17**		6.4 ± 0.4
4	–	43.3 ± 2.0	**18**		16.3 ± 0.5
5	–	44.4 ± 3.3	**19**		21.0 ± 0.5
6		12.3 ± 0.4	**20**		4.6 ± 0.1
7		32.4 ± 3.2	**21**		45.5 ± 2.0
8		92.0 ± 11.4	**22**		21.5 ± 1.1
9		11.9 ± 1.2	**23**		55.9 ± 0.1
10		16.9 ± 0.5	**24**		55.9 ± 3.3
11		27.6 ± 1.6	**25**		3.5 ± 0.1
12		31.3 ± 0.2	**26**		11.1 ± 0.7
13		11.5 ± 0.5	**27**		9.0 ± 0.4
14		17.6 ± 0.3	Silybin[b]	–	151.1 ± 3.3
v		16.1 ± 1.8			

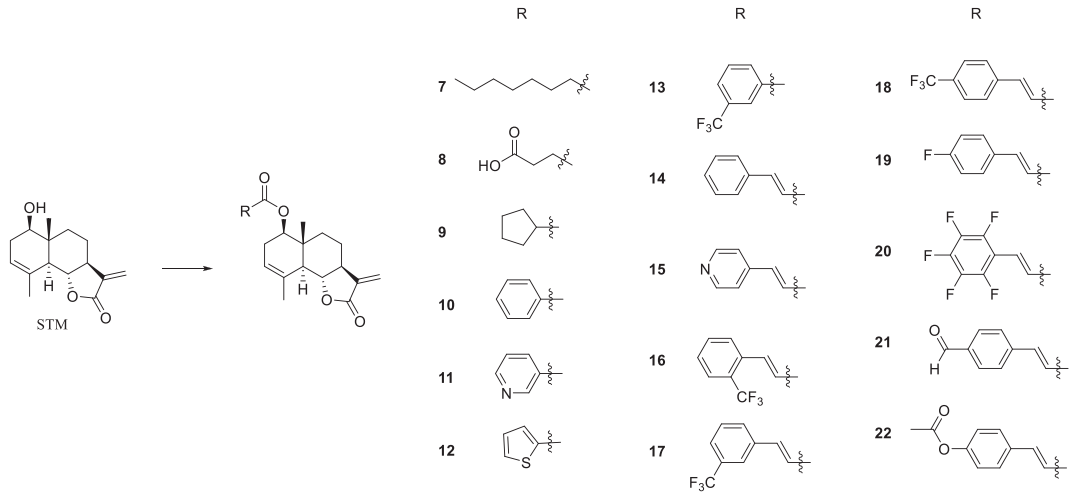
[a] Data were expressed as means ± SD (n = 3).
[b] Silybin was used as the positive control.

on the deposition of ECM by comparing with silybin, and significantly increased inhibitory effect on the deposition of HA and HL, which were 3 to 8-fold more potent than STM. Especially, compound **20** not only showed similar efficacy on Col I deposition as STM, but also manifested more than 8-fold and 6-fold enhancement of inhibitory effect on the deposition of HA and HL with IC_{50} values of 7.6 ± 0.6 and 3.3 ± 1.2 μM.

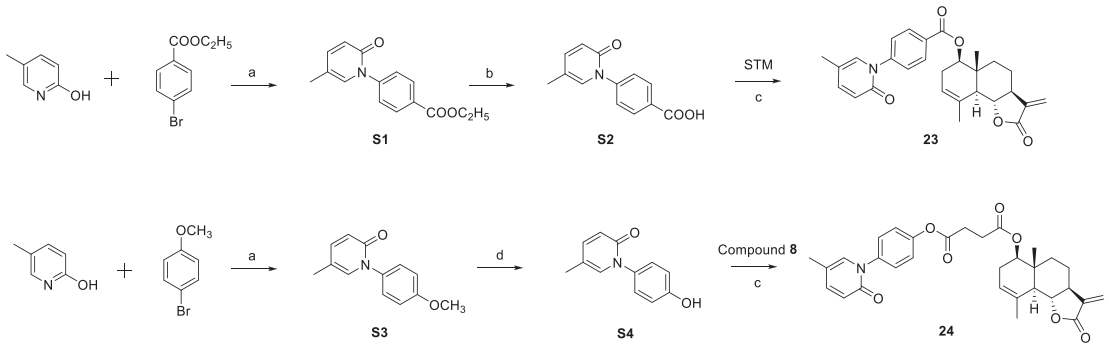
In summary, 26 derivatives of STM were synthesized and evaluated for their cytotoxic activity against HSC-LX2. Eight derivatives showed higher activity, and compound **25** was approximately 5-fold stronger than that of STM. Compound **20** exhibited more than 8-fold increase in inhibitory activity on the deposition of HA and 6-fold increase in HL with IC_{50} values of 7.6 ± 0.6 and 3.3 ± 1.2 μM. The SARs can be concluded as: (a) the *α, β*-unsaturated lactone is crucial pharmacophore to maintain the activity; (b) change of the double-bond leads to decrease activity; (c) oxidation of the hydroxyl group is unfavorable; (d) the acyloxys at C-1 position play a crucial role in the activity. These results demonstrated that compounds **20** and **25** as promising anti-fibrotic candidates showed obvious inhibitory activity on the proliferation of HSC-LX2 and secretion of Col I, HA and HL.

Declaration of Competing Interest

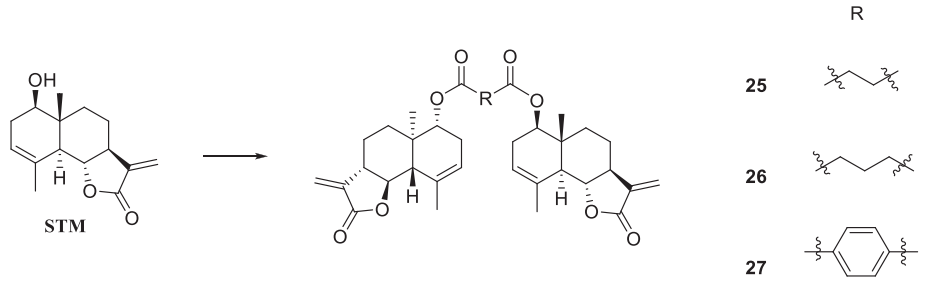
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence



Scheme 2. Reagents and conditions: EDCI, DMAP, appropriate carboxylic acid, dry CH_2Cl_2, r.t., **7** (84%), **9** (80%), **10** (66%), **11** (74%), **12** (87%), **13** (60%), **14** (73%), **15** (43%), **16** (74%), **17** (74%), **18** (67%), **19** (82%), **20** (83%), **21** (81%), **22** (71%) or succinic anhydride, DMAP, dry CH_2Cl_2, r.t., **8** (63%).



Scheme 3. Reagents and conditions: (a) CuI, dry DMF, Ar, 160 °C, **S1** (77%), **S3** (79%); (b) 1 M LiOH aqueous solution, 1,4-dioxane, r.t., 91%; (c) EDCI, DMAP, dry CH_2Cl_2, r.t., **23** (81%), **24** (75%); (d) BBr_3, dry CH_2Cl_2, Ar, −78 °C, 79%.



Scheme 4. Reagents and conditions: EDCI, DMAP, appropriate carboxylic diacid, dry CH_2Cl_2, sealed tube, 50 °C, **25** (60%), **26** (40%), **27** (32%).

Table 2
Inhibitory effect of compounds **17**, **20** and **25** on HA, HL and Col I deposition in HSC-LX2.

Compd.	IC_{50} (μM)[a]		
	HA	HL	Col I
STM	>60.0	18.6 ± 1.4	7.3 ± 1.7
17	21.2 ± 1.3	7.3 ± 2.1	14.7 ± 1.8
20	7.6 ± 0.6	3.3 ± 1.2	6.1 ± 1.9
25	17.9 ± 1.8	6.6 ± 2.9	16.2 ± 4.1
Silybin[b]	164.4 ± 1.4	83.7 ± 2.5	51.5 ± 11.1

[a] Data were expressed as means ± SD (n = 3).
[b] Silybin was used as the positive control.

the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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References

1 Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. *Annu Rev Pathol Mech Dis*. 2011;6:425–456.
2 Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol*. 2017;14:397–411.
3 Barry AE, Baldeosingh R, Lamm R, et al. Hepatic stellate cells and hepatocarcinogenesis. *Front Cell Dev Biol*. 2020;8:709–725.
4 Sokolović A, Sokolović M, Boers W, Elferink RO, Bosma PJ. Insulin-like growth factor binding protein 5 enhances survival of LX2 human hepatic stellate cells. *Fibrogenesis Tissue Repair*. 2010;3:3–16.
5 Ellis EL, Mann DA. Clinical evidence for the regression of liver fibrosis. *J Hepatol*. 2012;56:1171–1180.
6 Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. *J Hepatol*. 2019;70:151–171.
7 Trautwein C, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: concept to treatment. *J Hepatol*. 2015;62:S15–S24.
8 Bansal R, Nagórniewicz B, Prakash J. Clinical advancements in the targeted therapies against liver fibrosis. *Mediators Inflamm*. 2016;2016:1–16.
9 Zhang WD, Wang RF, Wu HM, Yang H, Wang GC. Research progress in drugs for anti-hepatic fibrosis. *Acta Pharm Sin*. 2018;53:667–675.
10 Koehn FE, Carter GT. The evolving role of natural products in drug discovery. *Nat Rev Drug Discovery*. 2005;4:206–220.
11 Kingston DGI. Modern natural products drug discovery and its relevance to biodiversity conservation. *J Nat Prod*. 2011;74:496–511.
12 Wang JY, Zhang QS, Guo JS, Hu MY. Effects of glycyrrhetinic acid on collagen metabolism of hepatic stellate cells at different stages of liver fibrosis in rats. *World J Gastroenterol*. 2001;7:115–119.
13 Dang SS, Wang BF, Cheng YA, Song P, Liu ZG, Li ZF. Inhibitory effects of saikosaponin-d on CCl_4-induced hepatic fibrogenesis in rats. *World J Gastroenterol*. 2007;13:557–563.
14 Hsu YC, Chiu YT, Cheng CC, Wu CF, Lin YL, Huang YT. Antifibrotic effects of tetrandrine on hepatic stellate cells and rats with liver fibrosis. *J Gastroenterol Hepatol*. 2007;22:99–111.
15 Chávez E, Reyes-Gordillo K, Segovia J, et al. Resveratrol prevents fibrosis, NF-κB activation and TGF-β increases induced by chronic CCl_4 treatment in rats. *J Appl Toxicol*. 2008;28:35–43.
16 Loguercio C, Festi D. Silybin and the liver: from basic research to clinical practice. *World J Gastroenterol*. 2011;17:2288–2301.
17 Sallam AM, Esmat A, Abdel-Naim AB. Cucurbitacin-B attenuates CCl_4-induced hepatic fibrosis in mice through inhibition of STAT-3. *Chem Biol Drug Des*. 2018;91:933–941.
18 Bangen JM, Hammerich L, Sonntag R, et al. Targeting CCl_4-induced liver fibrosis by RNA interference-mediated inhibition of cyclin E1 in mice. *Hepatology*. 2017;66:1242–1257.
19 Qu Y, Zhang QD, Cai XB, et al. Exosomes derived from miR-181-5p-modified adipose-derived mesenchymal stem cells prevent liver fibrosis via autophagyactivation. *J Cell Mol Med*. 2017;21:2491–2502.
20 Shen C, Huang XY, Geng CA, et al. Cytotoxic sesquiterpenoids against hepatic stellate cell line LX2 from *Artemisia lavandulaefolia*. *Bioorg Chem*. 2020;103, 104107.
21 Matsuda H, Shimoda H, Uemura T, Yoshikawa M. Preventive effect of sesquiterpenes from bay leaf on blood ethanol elevation in ethanol-loaded rat: Structure requirement and suppression of gastric emptying. *Bio Med Chem Lett*. 1999;9:2647–2652.
22 Matsuda H, Kagerura T, Toguchida I, Ueda H, Morikawa T, Yoshikawa M. Inhibitory effects of sesquiterpenes from bay leaf on nitric oxide production in lipopolysaccharide-activated macrophages: structure requirement and role of heat shock protein induction. *Life Sci*. 2000;66:2151–2157.
23 Sun CM, Syu WJ, Don MJ, Lu JJ, Lee GH. Cytotoxic sesquiterpene lactones from the root of *Saussurea lappa*. *J Nat Prod*. 2003;66:1175–1180.
24 Ma G, Chong L, Li Z, Cheung AH, Tattersall MH. Anticancer activities of sesquiterpene lactones from *Cyathocline purpurea* in vitro. *Cancer Chemother Pharmacol*. 2009;64:143–152.
25 Mehmood T, Maryam A, Tian X, Khan M, Ma T. Santamarine inhibits NF-κB and STAT3 activation and induces apoptosis in HepG2 liver cancer cells via oxidative stress. *J Cancer*. 2017;8:3707–3717.
26 Wu X, Zhu H, Yan J, Khan M, Yu X. Santamarine inhibits NF-kappaB activation and induces mitochondrial apoptosis in A549 lung adenocarcinoma cells via oxidative stress. *Biomed Res Int*. 2017;4734127.
27 Matsuda H, Kageura T, Inoue Y, Morikawa T, Yoshikawa M. Absolute stereostructures and syntheses of saussureamines A, B, C, D and E, amino acid–sesquiterpene conjugates with gastroprotective effect, from the roots of *Saussurea lappa*. *Tetrahedron*. 2000;56:7763–7777.
28 Ruiz-Cancino A, Cano AE, Delgado G. Sesquiterpene lactones and flavonoids from *Artemisia ludoviciana* ssp. *mexicana*. *Phytochemistry*. 1993;33:1113–1115.
29 García1 L, Hernández I, Sandoval A, et al. Pirfenidone effectively reverses experimental liver fibrosis. J Hepatol 2002;37:797-805.
30 Sario AD, Bendia E, Baroni GS, et al. Effect of pirfenidone on rat hepatic stellate cell proliferation and collagen production. *J Hepatol*. 2002;37:584–591.
31 Ma LF, Chen YL, Shan WG, Zhan ZJ. Natural disesquiterpenoids: an update. *Nat Prod Rep*. 2020;37:999–1030.