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REVIEW ARTICLE

NATURAL ANTI-HCV AGENTS

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ABSTRACT

Over 4 million people in the United States and 200 million individuals worldwide are infected with hepatitis C virus (HCV). The incidence in the U.S. has been decreasing since 1992, when blood product screening for HCV became standard. Nevertheless, in the U.S. 19,000 persons were infected in 2006, current therapies are not effective against specific HCV genotypes and over 8,000 subjects die each year. Globally, 50% of those chronically infected with HCV will not respond to current therapies; therefore, chronic liver infection caused by HCV will continue to increase, as will the costs for caring for these individuals. Efficient treatment strategies to manage HCV-associated hepatitis are thus urgently needed. There are currently over 70 phase I and II interventional studies enrolling participants (clinicaltrials.gov, accessed March 2009). HCV vaccines for prevention and treatment remain elusive, so there has been a revitalization of efforts to discover new chemical entities (NCEs) through natural product chemistry. Here we present an update on the studies performed in this search, including 45 small-molecule natural anti-HCV agents obtained during the past 2 decades, along with their chemical structures, pharmacology, preclinical studies and clinical trials.

INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded positive-sense RNA virus from the Flaviviridae family characterized by six genotypes. The virus was first identified in 1989 as a major causative agent of non-A, non-B (NANB) hepatitis and cirrhosis. HCV has been proven to be strongly associated with the development of hepatocellular carcinoma (HCC) and is the leading cause of chronic liver disease. Nowadays, over 4 million people in the United States and 200 million individuals worldwide are infected with HCV (1-5). Data suggest that transmission primarily occurs during illicit intravenous drug use from infected blood (6-8). In the past, blood transfusions were a prominent culprit; however, transmission from these sources has been dramatically reduced because blood and organ donations have been screened for HCV since 1992. Nevertheless, in the U.S. 19,000 persons were infected in 2006 alone, current therapies are not effective against specific HCV genotypes and over 8,000 subjects die each year. Globally, 50% of those chronically infected with HCV will not respond to current therapies, so chronic liver infections caused by HCV continue to increase. Therefore, the urgency for new treatments is intensifying.

Rapid and robust in vivo replication of the HCV virus occurs within hepatocytes and lymphocytes during a period of 35-60 days after incubation (9). Liver function tests can detect HCV infection during this acute phase. However, many people are unaware that they are infected, so they do not seek treatment due to the subtlety of the initial symptoms, which can include abdominal pain, fatigue, pruritus, jaundice and generalized malaise (10-12). The progression of HCV is varied; 15-25% of infected individuals spontaneously clear the virus, while 75-85% develop life-long chronic disease (13-30). Of those with chronic HCV, approximately 20% will develop cirrhosis, with 10% per year progressing to either end-stage liver disease (ESLD) or HCC, with a combined 3-4% death rate per year (14). Consequently, chronic HCV is the most common reason for liver transplant in the U.S. (8). Comorbidities such as hepatitis B virus (HBV) infection, ESLD and alcoholism hasten HCV progression, and HIV management is severely compromised by HCV infection (14, 21, 22).

The HCV patient population comprises the majority of persons with chronic liver disease in the U.S. Cirrhosis, HCC and liver transplant caused by chronic HCV infection will cost over $1 billion per year in direct medical care costs and $2 billion per year for disabilities by 2010 (23). Worldwide, about 2-3% of the population is infected with HCV (12, 24). Currently, there is no vaccine available against HCV (25) and no effective therapy for HCV-associated chronic hepatitis. The standard of care is interferon (IFN) with ribavirin for 24-48 weeks, depending on genotype. However, IFN treatment is hampered by low tolerance, high cost (especially for low-income countries), variability in efficacy and the development of resistance (26-30). The efficacy of IFN treatment can vary greatly among individuals, ranging from 30% to 80% depending on the patient's age, cirrhosis stage, liver transplant status and racial/ethnic group, as well as the HCV genotype (13, 31, 32).

The clear unmet needs for HCV treatment have prompted the FDA to refocus its support for the development of potential treatments (9). In 2006, 15 current Investigational New Drug (IND) holders of HCV therapies in development provided input to the FDA in an advisory meeting on drug development issues specifically related to patient populations, controls for phase III studies, study designs, efficacy endpoints and long-term follow-up. Based on this meeting, the FDA provided new guidance for the development of new chemical entities (NCEs) or biologics targeting different mechanisms of action, with the ultimate goal of preventing the progression of liver disease, improving quality of life and reducing the incidence of HCC. The FDA suggested that new products must inhibit viral replication or other virus-specific functions with a defined mechanism of action. A comprehensive understanding of the development of resistance for any new product is also a prerequisite.

In addition, it was clearly stated at the 2006 advisory committee meeting that the FDA and HCV experts would like to end the use of IFN and ribavirin to decrease adverse events while increasing compliance. This exemplifies the tremendous opportunity that exists for NCEs targeting HCV. They proposed emulating the successful HIV campaign characterized by eras of aggressive development of HIV therapeutics and mandatory combination therapy designed to prevent resistance to therapies (9). An approach using combination therapy with drugs exhibiting different mechanisms of action is already being pursued by Schering-Plough, which is currently conducting clinical trials with IFN and glycyrrhizin, a natural product discussed in this review.

More effective treatment strategies to cure HCV-associated hepatitis are being developed (18, 29, 33-37). Recently, a number of advances have been published on small-molecule inhibitors of HCV replication (17, 19, 28, 38-50), medicines for liver disease (including anti-HCV drugs) (51, 52) and therapies for chronic HCV (29, 53). The development pipeline for new HCV medicines includes polymerase inhibitors, protease inhibitors, ciclosporin analogues, Toll-like receptor (TLR) agonists, antisense oligonucleotides and vaccines. The current polymerase and protease inhibitors still require IFN to offset resistance (in vitro and early clinical data). It is suggested that more clinical data are required to evaluate vaccine treatments because viral kinetics appear to react atypically with immune-based therapy (9).

It has been well documented that natural products have served as a major source for drugs (54). Today, medicinal herbs are widely prescribed for HCV in many countries (29), especially for patients who are not eligible for IFN/ribavirin or who fail to respond to IFN (30). Several botanicals and active compounds as potential treatments for liver disease have been summarized in previous reviews (30, 55-65). This review examines 45 small-molecule natural products and describes both clinical and preclinical studies. In some cases, the mechanisms of action are defined and in others they require further elucidation. The studies revealing these mechanisms have the potential to meet FDA requirements and recommendations for virological clearance strategies and disease suppression strategies, including compounds that decrease replication by inhibiting protease and/or helicase and creating a cytokine-dominant environment; or those with hepatoprotective effects that prevent liver fibrosis by lowering serum alanine transaminase (ALT) and aspartate transaminase (AST) levels, reducing chronic inflammation in the liver and inducing hepatocyte proliferation/liver regeneration.

The growing and significant population not responding to IFN is heterogeneous and challenging, thus creating a tremendous unmet need. The FDA's desire to supplant the current standard of care and use combination therapy, in addition to the refractory nature of HCV, demand attention from natural product chemists to seize these opportunities for strategic advantage. Our review includes potential small-molecule natural anti-HCV products published in the literature from January 1989 to February 2009. Herbal mixtures (66) and natural products used in combination with IFN or ribavirin (67) are not included in this review.

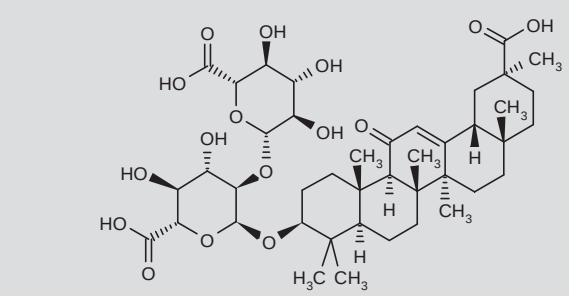
NATURAL ANTI-HCV AGENTS

Glycyrrhizin

Glycyrrhizin (**1**), an active compound extracted from licorice roots (68), is a conjugate of one molecule of glycyrrhetinic acid and two molecules of glucuronic acid. It has been used for many centuries as an antiallergic agent in traditional Chinese medicine (57). Glycyrrhizin has antioxidant, immunosuppressive and antiinflammatory effects. In Japan, glycyrrhizin has been accepted as a treatment for chronic hepatitis for more than 20 years (57). It is used as a constituent of Stronger Neo Minophagen C (SNMC) (comprised of 0.2% glycyrrhizin, 0.1% cysteine and 2.0% glycine in physiological saline solution) for patients with acute and chronic hepatitis (69). Currently, 17 million ampoules containing 40 mg of glycyrrhizin are used in Japan per year for the treatment of chronic hepatitis.

Several randomized clinical trials of glycyrrhizin (administered as SNMC) have been described in the literature. The first randomized, double-blind, placebo-controlled trial in 133 chronic hepatitis patients was published by Suzuki et al. in 1977 in Japanese (70), and subsequently in English in 1983 (71). In this study glycyrrhizin was given as SNMC; 67 patients received 40 mL/day SNMC and 66 received placebo for 4 weeks. Serum ALT level was used as a sign of improved liver function. The patients' serum ALT decreased during treatment with i.v. SNMC, and a significant improvement in liver function was noted in the SNMC group ($P < 0.001$). However, after discontinuation of SNMC, the serum ALT levels rebounded (70).

In 1984, another report showed that oral administration of glycyrrhizin could significantly improve liver function. In this study 7.5



Glycyrrhizin (**1**)

mg glycyrrhizin as capsules was given twice a day for 30 days for acute hepatitis and for 90 days for chronic hepatitis. No side effects were reported (72). Two years later, a higher dose of SNMC (100 mL/day for 8 weeks) improved patients' serum ALT levels; the pathological features of liver biopsy following treatment compared to before treatment were also discussed (73, 74).

Following 12 years of open uncontrolled studies, Wildhirt concluded that the biochemical and histological improvements after SNMC were better than those obtained with IFN (75). In 1997, the effects of glycyrrhizin on liver function, HCV RNA levels and viral complexity in HCV-infected patients were reported. SNMC (60 mL) was given three times a week for 16 weeks. The serum ALT levels fell by half in 74.1% of treated patients. No significant differences in HCV RNA levels and viral complexity of HCV RNA were observed (76).

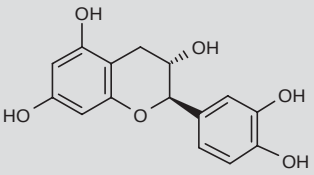
The long-term preventive effect of i.v. injections of SNMC on HCC development in Japanese patients was investigated by Arase et al. in 1997. In this study, the incidence of HCC in an SNMC-treated group (84 patients) was significantly lower than in a group (109 patients) not treated with SNMC but given a mixture of different herbal treatments including vitamin K. Their results suggested that long-term administration of SNMC for chronic HCV was effective in preventing HCC development. However, the mechanism for this reduction in HCC rate was unclear (69).

In order to evaluate the effect of glycyrrhizin on serum ALT and HCV RNA and its safety in European patients, a randomized, double-blind, placebo-controlled trial was conducted. Compared with placebo, there were reductions in ALT levels during treatment, but this was not sustained after cessation of treatment, and there were no significant effects on HCV RNA levels (77).

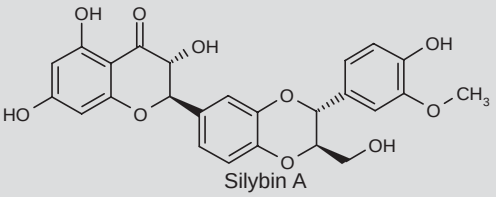
In the same year (1999), results for combination therapy using glycyrrhizin plus ursodeoxycholic acid (UDCA) for chronic HCV infection were reported. The results showed that the combination therapy was effective in improving liver-specific enzyme abnormalities. However, there was no placebo group in this trial; the comparison treatment was a combination of SNMC and UDCA (78).

(+)-Cyanidan-3-ol

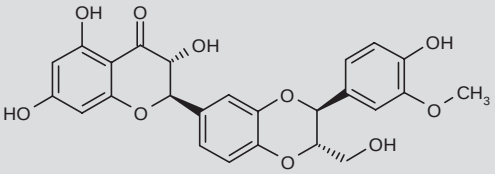
Catechins are a class of flavonoids with hepatoprotective activity. Among this class of natural products, (+)-cyanidan-3-ol (**2**), sold as Catergen, Kanebo or Zyma, was derived from *Uncaria gambir* (61). In 1977, the effect of (+)-cyanidan-3-ol on the course of acute hepatitis was examined in 35 patients in a double-blind study. During the first weeks, the patients treated with (+)-cyanidan-3-ol showed a noticeable reduction in transaminase activity in the blood, in contrast to the placebo group (79). In 1983, another randomized, double-blind trial was reported in 124 patients with acute viral hepatitis (hepatitis A, n = 35; hepatitis B, n = 58; and hepatitis C, n = 31); 58 patients were treated with (+)-cyanidan-3-ol (3 mg/day) and 66 were treated with placebo for 50 days. AST, ALT and serum bilirubin were tested every 5 days. There was a significant difference in efficacy depending on the type of hepatitis being treated. For patients with HCV, 14 treated and 17 controls, the ALT and AST levels were both significantly lower in the cyanidanol group than the placebo group after 40 and 45 days. There were moderate but significant differences in the HBV group in favor of catechin and no significant difference in the HAV group (80).



(+)-Cyanidan-3-ol (**2**)



Silybin A



Silybin B

Silybin (**3**)

Silybin

Silybum marianum, from the fruits of the milk thistle, has been used as a treatment for hepatobiliary diseases since the 16th century. Its active ingredients include flavonoids (predominantly silybin) (81). Milk thistle is one of the most popular herbal remedies used by patients with liver disease. A survey of patients at a hepatology clinic found that 31% reported using herbal supplements to treat their liver disease, of which milk thistle was the most common (82). Jacobs et al. reviewed the use of milk thistle for the treatment of liver disease; it appeared to be safe and well tolerated (83).

Silymarin, a flavonolignan extracted from the seeds of milk thistle, has been most widely studied as a remedy for liver diseases. It is a mixture of mainly three flavonolignans, silybin (**3**), a mixture of the diastereomers silybin A and silybin B and also known as silibinin, silidianin and silychristine, with silybin being the most active (84). For acute hepatitis, studies have shown mixed results (85, 86). The beneficial effects of silymarin were most often seen in patients who had cirrhosis as a result of alcohol abuse. Silymarin has a good safety record and only rare case reports of gastrointestinal disturbances and allergic skin rash have been described (87).

In 1993, a small, short-term trial in 20 patients comparing silybin and placebo showed significant reductions in serum ALT, AST, γ-glutamyltransferase (GGT) and total bilirubin; silybin might therefore have liver-protective effects (88). However, this trial included only 20 patients treated for 1 week and no follow-up assessment (59, 83). Subsequently, a pilot study of silymarin in patients with primary biliary cirrhosis (PBC) who had a suboptimal response to UDCA reported that silymarin was well tolerated, but there was no significant change

in liver enzymes (89). More recently, the response of pedigreed nonresponders to full-dose pegylated interferon/ribavirin (PegIFN/RBV) to silybin was studied by Ferenci et al. The first group (16 patients with chronic HCV) received 10 mg/kg/day i.v. silybin (Legalon Sil; Madaus, Köln, Germany) for 7 days. In a subsequent dose-finding study, the second group (20 patients) received 5, 10, 15 or 20 mg/kg/day for 14 days. The results showed that silybin was well tolerated and had a substantial antiviral effect against HCV in nonresponders (90).

Sch-68631, Sch-351633, Sch-644342 and Sch-644343

The polyprotein of the HCV RNA virus is co- and post-translationally processed by cellular and virally encoded proteases to produce the mature structural and nonstructural (NS) proteins. Among the NS proteins, the NS3 serine-like protease and the RNA-dependent RNA polymerase (NS5B) are essential for viral maturation and replication, and therefore represent important targets (28). During the search for new HCV protease inhibitors, a novel secondary metabolite, Sch-68631 (**4**), was isolated from the fermentation culture broth of *Streptomyces* spp. (culture 94-02747) in 1996. The structure of Sch-68631 was elucidated by analysis of spectroscopic data as a phenanthrenequinone derivative. Sch-68631 demonstrated an IC_{50} of 2.5 µg/mL in an in vitro assay for HCV NS3 serine protease. Several quinone (common entities in various natural products) analogues were also tested in the HCV protease assay along with Sch-68631; the results showed that quinones such as sakyomicin A are inactive in the HCV assay and were thus not regarded as the key functionalities responsible for the HCV protease-inhibitory activity (91). Three years later, a new HCV protease inhibitor, a fungal metabolite designated Sch-351633 (**5**), was isolated from the fungus *Penicillium griseofulvum* (culture Mer-0442, designated as Schering Culture Collection Fungi, SCF-1704) by the same research group. The microorganism was isolated from a soil sample collected from a desert terrain in Arizona. The structure of Sch-351633 was determined by analysis of spectroscopic data to be a bicyclic hemiketal lactone. It exhibited inhibitory activity in the HCV protease assay with an IC_{50} value of 3.8 µg/mL (92).

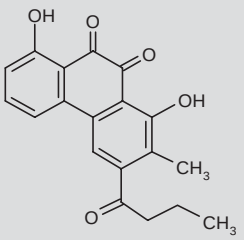
At the same research institute (Schering-Plough Research Institute), two novel oligophenolic compounds, Sch-644342 (**6**) and Sch-644343 (**7**), were isolated from the Peruvian plant *Stylogne cauliflora* in 2003 and identified as inhibitors of HCV NS3 protease. Both compounds were active in the HCV NS3 protease assay, with IC_{50} values of 0.8 and 0.3 µM respectively (93).

Oxymatrine

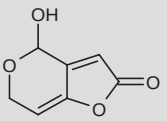
Oxymatrine (**8**) is the major alkaloid extracted from the root of species of the Chinese herb *Sophora*, including *Sophora alopecuraides*, *S. flavescens, S. subprostrata* and *S. japonica* (30, 59, 94-98).

Numerous pharmacological activities have been reported for oxymatrine, including anti-HBV activity and improvement in liver function (59, 95, 96, 99-102), antibacterial and antiparasitic activity (103-105), regulation of the immune response (106), induction of cytochrome P450 production (107), antiviral activity (108, 109) and antifibrotic activity (59, 94, 96-98, 110, 111).

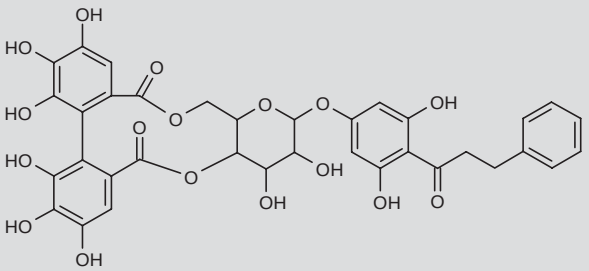
Most previous clinical trials indicated that oxymatrine might be effective in normalizing serum ALT levels and clearing HBV virus (96, 102, 112). In recent years, oxymatrine has been reported to have anti-HCV activity in cell culture (113), animal and human studies (59, 95). Several clinical trials have examined the effect of oxymatrine in



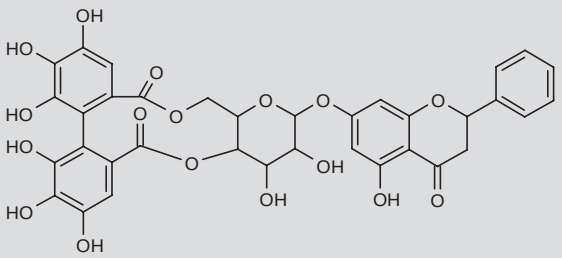
Sch-68631 (**4**)



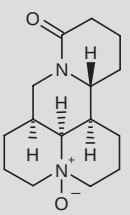
Sch-351633 (**5**)



Sch-644342 (**6**)



Sch-644343 (**7**)



Oxymatrine (**8**)

chronic HCV patients (59, 94, 114). Results showed that oxymatrine was effective in inhibiting the proliferation of HCV, preventing liver fibrosis and balancing host immunity. These data suggest that it may be a safe and effective treatment for chronic HCV (94, 114).

An animal study showed that oxymatrine had some hepatoprotective activity, as indicated by its ability to decrease allyl alcohol toxicity, but not CCl_4^-, acetaminophen- or cadmium chloride-induced acute hepatitis (115). Li et al. demonstrated in an open, randomized, parallel-group clinical trial in human subjects infected with HCV that oxymatrine significantly lowered soluble interleukin-2 receptor (IL-2R) levels, as well as serum collagen type IV (94). However, additional information regarding the safety of oxymatrine was not forthcoming.

In 2004, 40 patients were randomized to receive either an i.v. injection of 600 mg/day oxymatrine or other products, such as vitamin A, administered orally as a control. After 3 months of treatment, 47.1% of the cases showed HCV clearance compared to only 5.6% in the control group. The treated group had a significantly higher serum ALT normalization rate than the control group in the first 2 months; however, there was no significant difference at the end of the third month of treatment. Moreover, the trial was not blinded (114).

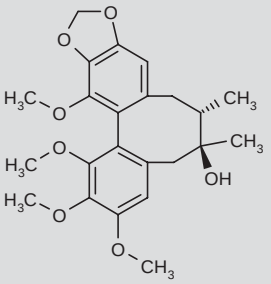
In the same year, another double-blind trial aiming to assess the effect of oxymatrine on liver fibrosis in both HCV and HBV chronically infected patients was reported. The treated group received a single 300-mg oxymatrine capsule and a complex of vitamins B and C orally 3 times a day, while controls received an empty capsule in lieu of oxymatrine, as well as the vitamins. The results showed significant improvement in liver fibrosis, as measured by a series of indicators, including serum hyaluronic acid and type III procollagen peptide. The authors also reported significant improvements in ALT (P = 0.0007) and AST (P = 0.0025) levels in the oxymatrine-treated group relative to the control group (111).

Gomisin A

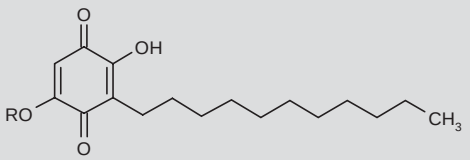
TJ-108 (Ninjin-yoei-to) is a Japanese Kampo mixture with antioxidant, antiinflammatory (116) and hepatoprotective properties in both hepatocyte cell culture (117) and rat models (118).

To evaluate the effect of the Kampo medicine on chronic HCV, two uncontrolled clinical treatments were assessed both short term and long term and it was shown that TJ-108 had anti-HCV activity in vitro. Based on these findings, the researchers performed cell culture and animal studies to isolate the antiviral component of TJ-108, which was identified as gomisin A (TJN-101; **9**) from the *Schisandra* fruit. Gomisin A was then studied using human acute lymphoblastic leukemia MOLT-4 cells and an animal model of immunologically induced acute hepatic failure (119).

*Schisandr*a has been shown to improve liver function, as measured by serum ALT and AST. Several laboratory studies have reported gomisin A to be hepatoprotective (119). It has been shown to improve necrosis of hepatocytes in acetaminophen-treated mice by inhibiting lipid peroxidation (120, 121), enhance liver regeneration by mediating hepatocyte cell proliferation (122), and improve bile flow and liver function in rats (123, 124). Gomisin A has also been reported to improve survival in lipopolysaccharide (LPS)-induced acute hepatic failure (119). However, more studies are needed to confirm these findings.



Gomisin A (TJN-101; **9**)



Embelin (**10**), R =H
5-*O*-Methylembelin (**11**), R = CH_3

In 2005, a study in 50 chronic HCV patients treated with a combination of *Schisandra* and six other antioxidant oral preparations (glycyrrhizin, silymarin, ascorbic acid, lipoic acid, L-glutathione and α-tocopherol) showed a 1 log decrease in viral load in 25%, histological improvement in 36% and normalization of serum enzymes in 44% of patients who had elevated pretreatment serum ALT levels (30, 125). Improvements were maintained 20 weeks after the end of treatment. While the study had no placebo control, it was unlikely that these improvements were spontaneous. Thus, further controlled studies of both *Schisandra* and gomisin A are needed to evaluate their hepatoprotective properties (30).

Embelin and 5-*O*-methylembelin

In 2000, Hattori et al. described an in vitro screening of Sudanese plant extracts (152 methanol and water extracts from different parts of 71 plants) for their inhibitory effects on HCV protease. The plants studied were selected as the most commonly used in Sudanese traditional medicine. Thirty-four extracts showed significant inhibitory activity (≥ 60% inhibition at 100 µg/mL). From the methanol extract of *Embelia schimperi* (fruit), two benzoquinones, embelin (**10**) and 5-*O*-methylembelin (**11**), were isolated and found to show significant activity against HCV protease, with IC_{50} values of 21 and 46 µM, respectively. Moreover, the inhibitory activity of derivatives of embelin against HCV protease, as well as their effects on other serine proteases, were investigated. For these compounds, the alkyl side-chain tended to enhance the anti-HCV protease activity, whereas methylation and acetylation of embelin had no appreciable effects (126).

Hypericin

Hypericin (**12**), a naturally occurring compound isolated from the stems and petals of the genus *Hypericum*, including the common St. John's wort plant (*Hypericum perforatum*) (127), has shown broad-spectrum antiviral activity (52, 128-134). This compound is an aro-

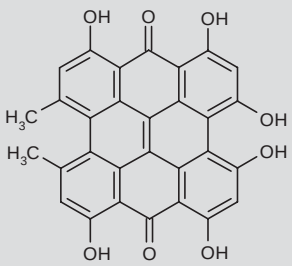
matic polycyclic anthrone, a class of pigmented chemical substances that have photosensitizing activity. In 2001, Jacobson et al. conducted a phase I dose-escalation study to determine the safety and antiviral activity of hypericin in patients with chronic HCV infection. They found that hypericin had in vitro activity against several viruses, including bovine viral diarrhea virus (BVDV), a pestivirus with structural similarities to HCV. However, there was no detectable anti-HCV activity in patients with chronic HCV infection given oral doses of 0.05 and 0.10 mg/kg/day of hypericin (135).

Mellein

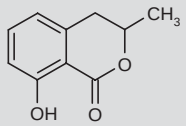
The chloroform extract obtained from the culture broth of the fungus *Aspergillus ochraceus* was found to inhibit the final stage of polyprotein processing during HCV replication. Mellein (also known as ochracin; **13**) was isolated from this extract as the active principle. It exhibited inhibitory activity in the HCV NS3 protease assay, with an IC_{50} value of 35 μM (136).

Brevifolin, brevifolin carboxylic acid, ellagic acid, corilagin and phyllanthusiin U

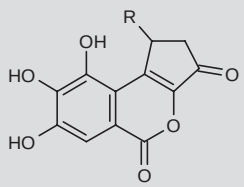
In recent research, HCV NS3/NS4A protease has been used as an enzyme target for anti-HCV compounds (137, 138). Five polyphenol compounds, brevifolin (**14**), brevifolin carboxylic acid (**15**), ellagic acid (**16**), corilagin (**17**) and phyllanthusiin U (**18**), isolated from the extract of *Phyllanthus urinaria* L., had inhibitory activity against HCV NS3/NS4A protease, with IC_{50} values of 0.1, 0.03, 0.003, 0.009, 0.001 mg/mL, respectively (139).



Hypericin (**12**)



Mellein (**13**)

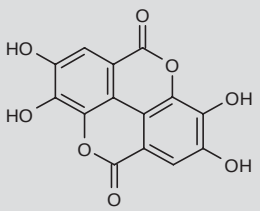


Brevifolin (**14**), R = H
Brevifolin carboxylic acid (**15**), R = COOH

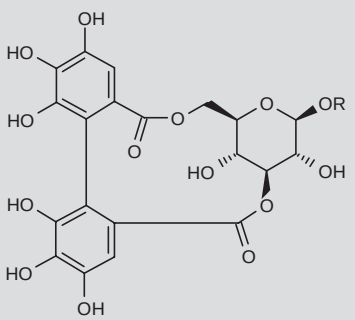
Iso-α-acids and xanthohumol

In 2004, Buckwold and colleagues investigated whether crude hop extracts and purified hop components, including *iso*-α-acids (**19a, 19b**) and xanthohumol (**20**), had antiviral activity. The results showed that pure *iso*-α-acids and xanthohumol, a prenylchalcone flavonoid, demonstrated low to moderate antiviral activity against BVDV as a surrogate model of HCV, with IC_{50} values in the low μg/mL range. *Iso*-α-acids and xanthohumol may therefore serve as interesting lead compounds from which more active anti-HCV agents could be synthesized (140).

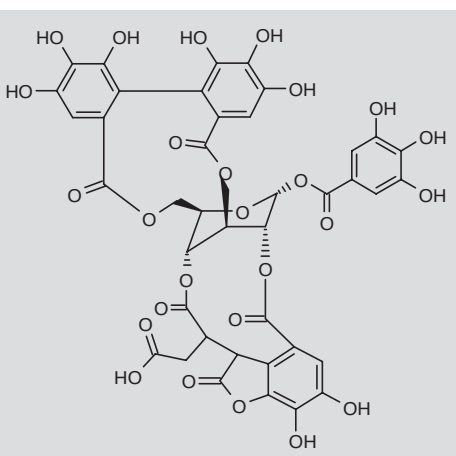
Iso-α-acids are isomerized forms of the α-acids (humulones) originating from the hop plant (*Humulus lupulus*) (141). The α-acids dis-



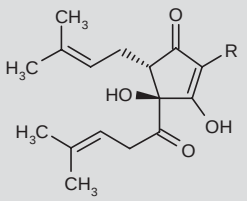
Ellagic acid (**16**)



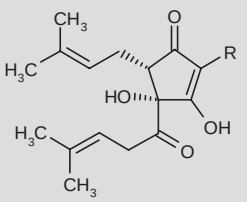
Corilagin (**17**), R = galloyl



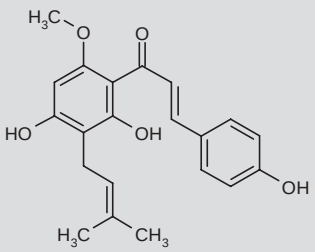
Phyllanthusiin U (**18**), R = galloyl



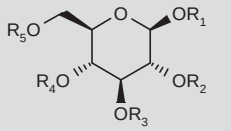
Iso-α-acids (**19a**)
R = $COCH_2CH(CH_3)_2$
R = $COCH(CH_3)_2$
R = $COCH(CH_3)CH_2CH_3$



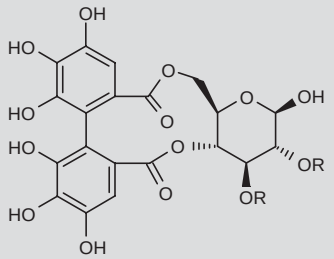
Iso-α-acids (**19b**)
R = $COCH_2CH(CH_3)_2$
R = $COCH(CH_3)_2$
R = $COCH(CH_3)CH_2CH_3$



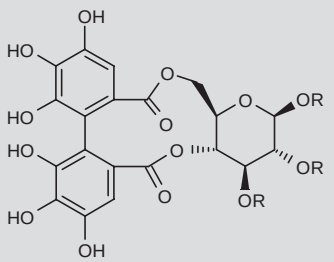
Xanthohumol (**20**)



21, $R_1 = R_2 = R_5$ = galloyl, $R_3 = R_4 = H$
22, $R_1 = R_2 = R_3 = R_5$ = galloyl, $R_4 = H$
23, $R_1 = R_2 = R_3 = R_4 = R_5$ = galloyl



Tellimagrandin I (**24**), R = galloyl



Tellimagrandin II (**25**), R = galloyl

play six different structure styles: *cis*- and *trans*-isohumulone, *cis*- and *trans*-isocohumulone and *cis*- and *trans*-isoadhumulone. Xanthohumol, also from *H. lupus*, was found to be a more potent antiviral agent against several viruses, including HCV, than the isomer *iso*-xanthohumol (140). Xanthohumol has also been reported to have anti-HIV (142), anticancer (143) and antioxidant activities (144).

1,2,6-Tri-*O*-galloyl-β-D-glucose, 1,2,3,6-tetra-*O*-galloyl-β-D-glucose, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, tellimagrandin I and tellimagrandin II

Over 100 traditional Chinese medicines have been screened for activity as natural HCV inhibitors. Results from enzyme-linked immunosorbent assay (ELISA)-guided fractionation suggested that the AcOEt extract fraction from *Galla chinensis* was efficient in inhibiting the NS3 protease activity of HCV. Using a bioassay, three polyphenol compounds, 1,2,6-tri-*O*-galloyl-β-D-glucose (**21**), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**22**) and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**23**), were purified from the EtOAc extract fraction of *G. chinensis* in 2004. They inhibited HCV NS3 protease with IC_{50} values of 1.89, 0.75 and 1.60 μM, respectively. Compound **22** belongs to the gallotannin or polyphenol class of natural products (145). It has been reported to have inhibitory effects against some tumor cells, lipid peroxidation and blood platelet aggregation in vitro (146, 147).

In 2005, compound **23** was reported as a natural anti-HCV agent from Chinese herbal medicines. In that study, bioactivity-guided extraction and isolation methods were performed. Active pure compounds were obtained from ethanolic extracts of *Saxifraga melanocentra* Franch. and their in vitro inhibitory activities (IC_{50}) against HCV NS3 serine protease were tested by ELISA. Eighteen polyphenols representing active compounds were isolated and identified. A broad degree of anti-HCV activity was observed among these compounds in the following order: gallated esters of D-glucose and rutin (0.68-4.86 μM) > flavonoids (33.11-370.37 μM) > gallic acid and its methyl and ethyl esters, bergenin and others (> 1000 μM). The most active compound was **23** (0.68 μM). The results showed that polyphenols were responsible for the anti-HCV properties (148).

In 2006, Xu et al. found that both the AcOEt and *n*-BuOH extract fractions from dried fruits of *Cornus officinalis* Sieb. et Zucc. had inhibitory activity against HCV NS3 protease, with IC_{50} values (mg/mL) of 0.02 and 0.052, respectively. Under bioassay-guided fractionation, the four active tannin compounds **22**, **23**, tellimagrandin I (**24**) and tellimagrandin II (**25**) were isolated from the AcOEt fraction of *C. officinalis*. The four compounds had inhibitory activity against HCV NS3 protease in vitro, with IC_{50} values of 6.98, 5.11, 7.0 and 4.8 μmol/L, respectively (149).

Upon analysis of inhibitors of HCV protease from natural plants, many of them were found to be hydrolyzable tannin compounds (139, 145, 149), suggesting that hydrolyzable tannins might be effective components for the treatment of HCV infection and indicating a core structure for inhibitors of HCV protease. Efforts should be directed on the continued search for natural products, especially polyphenols, with anti-HCV activity (149).

Parthenolide

In an effort to discover novel anti-HCV agents, Hsu et al. identified parthenolide (**26**), an active component in feverfew, a popular remedy for fever and migraine, from a library of 720 pure natural products and their derivatives. The natural products library provided a unique set of compounds with extensive chemical diversity (150).

Parthenolide, a sesquiterpene lactone, is the putative active ingredient of feverfew (*Tanacetum parthenium*), one of the most commonly used medicinal herbs in the U.S. (151). It was able to potentiate the anti-HCV effect of interferon alfa. In one study it was found to have an EC_{50} value of 2.21 μM in an HCV replicon assay (150).

In order to discern the structure–activity relationships (SAR), several commercially available analogues of parthenolide and a series of synthetic Michael-type adducts of parthenolide were also evaluated and exhibited anti-HCV activities at micromolar concentrations. The results showed that the spatial arrangement of the terpenoid skeleton fused with an α-methylene-γ-lactone moiety was critical for anti-HCV activity. Moreover, a series of secondary amino adducts at the β-position also had anti-HCV activity at micromolar concentrations, suggesting that an *exo*-methylene lactone functionality may play an important role in the anti-HCV activity of parthenolide (150).

3,3′-Digalloylproprodelphinidin B2, 3,3′-digalloylprocyanidin B2, (–)-epigallocatechin-3-*O*-gallate and (–)-epicatechin-3-*O*-gallate

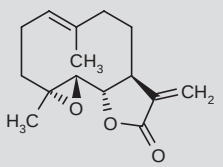
In 2007, bioassay-guided fractionation and isolation were performed on a crude ethanol extract from rhizomes of the Chinese medicinal herb *Rhodiola kirilowii* (Regel.) Maxim.; 12 compounds in the ethyl acetate fraction proved to be the most active. These compounds were tested for in vitro activity against HCV NS3 serine protease. Four (–)-epicatechin derivatives —3,3′-digalloylproprodelphinidin B2 (rhodisin) (**27**), 3,3′-digalloylprocyanidin B2 (**28**), (–)-epigallocatechin-3-*O*-gallate (EGCG) (**29**) and (–)-epicatechin-3-*O*-gallate (ECG) (**30**)— were the most potent, with IC_{50} values of 0.77, 0.91, 8.51 and 18.55 μM, respectively. Methylation and acylation of the hydroxyl groups of these four (–)-epicatechin derivatives caused a decrease in activity. These nonpeptide inhibitors of HCV NS3 serine protease may therefore be potential candidate anti-HCV agents (152).

11-*O*-(4-*O*-Methylgalloyl)bergenin

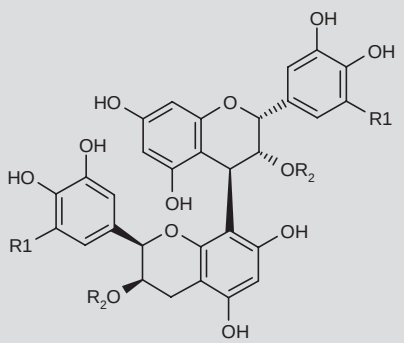
To study the chemical and bioactive characteristics of bergenin derivatives isolated from *Saxifraga melanocentra* Franch., a new gallic acid ester of bergenin, 11-*O*-(4-*O*-methylgalloyl)bergenin (**31**), was purified from the aerial parts of the plant. The compound showed weak inhibitory activity against HCV NS3 serine protease, with an IC_{50} of 0.32 mg/mL (153).

Hispitolides A-E and ambrosanolide-B

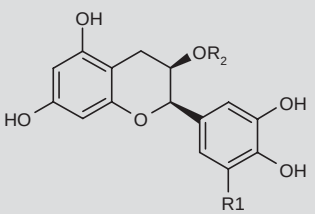
Scientists from Sequoia Sciences screened their compound libraries generated from plants using high-throughput natural product chemistry procedures in an attempt to discover anti-HCV agents. Six C_{14}-oxygenated pseudoguaienolides —hispitolide-A (**32**), hispitolide-B (**33**), hispitolide-C (**34**), hispitolide-D (**35**), hispitolide-E (**36**) and ambrosanolide-B (**37**)— from the plant *Parthenium hispitum* Raf. (Asteraceae) were shown to inhibit HCV replication. Among these, compounds **33**-**35** were found to possess significant in vitro anti-HCV activity, with > 90% inhibition at 2 μM (154).



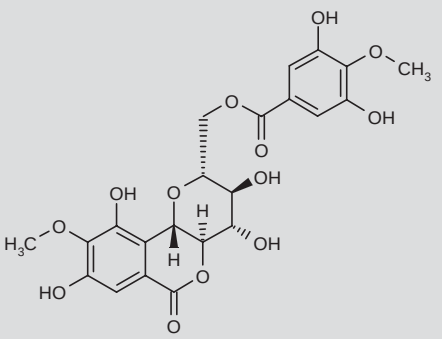
Parthenolide (**26**)



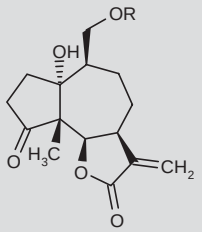
27, R_1 = OH, R_2 = galloyl
28, R_1 = H, R_2 = galloyl



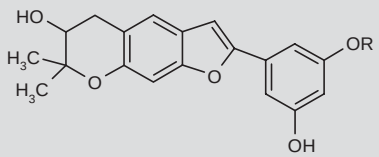
29, R_1 = OH, R_2 = galloyl
30, R_1 = H, R_2 = galloyl



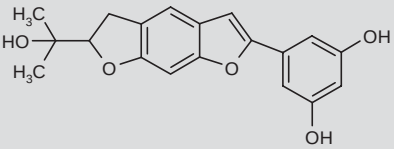
31



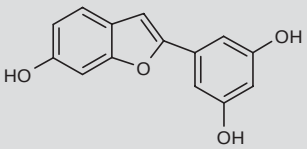
32, R = $COCH_2CH_3$
33, R = $COCH_2CH_2CH_3$
34, R = (*E*)-$COC(CH_3)=CHCH_3$
35, R = $COCH=C(CH_3)_2$
36, R = (*Z*)-$COC(CH_3)=CHCH_3$
37, R = $COCH_2CH(CH_3)_2$



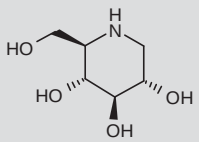
Mulberroside C (**38**), R = β-D-xylopyranoside
Moracin P (**40**), R = H



Moracin O (**39**)



Moracin M (**41**)



1-Deoxynojirimycin (**42**)

Mulberroside C and moracins O, P and M

Medicinal herbs are increasingly used in the search for safe and effective drug candidates for HCV infection. In 2007, the anti-HCV effect of compounds from Mori Cortex Radicis was investigated. During a screen for extracts with anti-HCV affinity from 173 medicinal plants, the methanol extract of Mori Cortex Radicis was selected. Five compounds, mulberroside C (**38**), moracins O (**39**), P (**40**) and M (**41**), and mulberrofuran K were isolated from the ethyl acetate-soluble fraction of this herb. Mulberroside C and moracin M showed significant inhibitory activity, while moracins O and P showed potent inhibitory activity (IC_{50} = 80.8 and 35.6 μM, respectively) in a replicon cell assay, which was confirmed by NS3 helicase-inhibitory activity (IC_{50} = 27.0 and 42.9 μM, respectively). The SAR studies showed that the presence of the D-ring in mulberroside C and moracins O and P was required for potent anti-HCV activity, and the hydroxy group on the C-ring increased anti-HCV activity (155).

1-Deoxynojirimycin

The piperidine alkaloid 1-deoxynojirimycin (1-DNJ; **42**) has been detected in high yields in all parts (root bark, fruits, leaves and silkworms) of the mulberry tree (*Morus* sp.) (156). Compound **42** was evaluated for its in vitro anti-HCV activity using BVDV as a surrogate, and was shown to inhibit virus-induced cell killing with an EC_{50} of 2.96 mM and reduced progeny virus with an EC_{90} of 0.24 mM. Moreover, the silkworm (*Bombyx mori* L.) extract containing various DNJ-derived iminosugars exhibited 1,300-fold greater antiviral effect against BVDV in comparison to **42** (157). The anti-HCV mechanisms of iminosugar derivatives have been studied, implying that this class of natural products may be potential drugs for the treatment of HCV infection (158, 159).

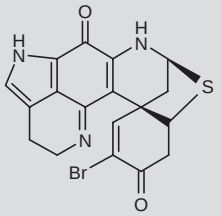
Discorhabdins A and C and 3-dihydrodiscorhabdin C

In 2008, the marine natural products discorhabdins A (**43**) and C (**44**) and 3-dihydrodiscorhabdin C (**45**) were isolated from a new deep-water Alaskan sponge species of the genus *Latrunculia* (class Demospongiae; order Poecilosclerida; family Latrunculiidae). Compounds **43**-**45** were evaluated for their in vitro antiviral activity in the HCV Huh-7 replicon system and displayed EC_{50} values of < 10 μM. However, they were also cytotoxic toward Huh-7 clone B cells (160).

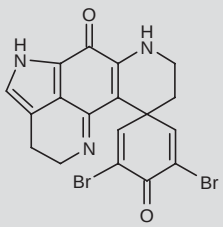
CONCLUSIONS

HCV is a serious public health problem and effective treatment strategies to manage HCV-associated hepatitis are urgently needed. More than 70 phase I and II interventional studies are currently enrolling participants. Vigorous responses by the FDA and pharmaceutical companies have been manifested in guidelines for more appropriate designs of clinical trials (9). The search for novel candidates as potent and specific inhibitors of HCV is evidenced by the many companies and their partners conducting clinical trials. Vertex, Schering-Plough, Roche and InterMune are examining the effects of small molecules as protease inhibitors (i.e., telaprevir) and liver-sparing agents (glycyrrhizin, orlistat and rosiglitazone) and all of these trials are designed with special attention to the parameters suggested by recent FDA discussions.

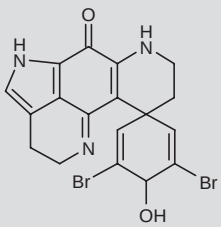
HCV vaccines for prevention and treatment remain elusive, so there is a revitalization of efforts to discover NCEs through natural product



Discorhabdin A (**43**)



Discorhabdin C (**44**)



3-Dihydrodiscorhabdin C (**45**)

chemistry. Natural products could play an important role as sources for anti-HCV agents (145). In the course of the discovery and development of small-molecule natural products that could potentially inhibit HCV, several replicon systems (150, 154, 155, 160) and specific enzyme targets (i.e., NS3 serine protease, NS3 helicase and NS3/NS4A protease) (91-93, 126, 136-139, 145, 149, 152-155) have been examined. Many medicinal herbs are increasingly being used for patients with HCV infection (58). For example, Oka et al. found that Sho-saiko-to (a prescription consisting of Bupleuri Radix, Zizyphi Fructus, Pinelliae Tuber, Ginseng Radix, Zingiberis Rhizoma, Glycyrrhizae Radix and Scutellariae Radix) could inhibit the development of HCC (161). However, very few exceptionally active components have been discovered. To our excitement, more and more attention is focusing on the search for anti-HCV agents from natural products, with 14 natural anti-HCV compounds reported in 2007 alone.

In this review, 45 potential anti-HCV small-molecule natural products are discussed from the following viewpoints: chemical structure, pharmacology, laboratory and animal studies, as well as clinical trials. These anti-HCV bioactive compounds were mostly isolated from plants, with only a few obtained from microorganisms (91), fungi (92, 136) or marine sponges (160). Numerous animal and human studies are being performed to evaluate these natural products in chronic HCV, although studies thus far are inconclusive (30, 58, 59). There is an opportunity for continuation of basic and clinical research, including preparation and standardization, because diverse mechanisms particular to natural product chemistry can be advantageous for treating the varied and virulent characteristics of chronic HCV.

DISCLOSURE

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