Biochemical and Biophysical Research Communications 440 (2013) 515–520

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Specific inhibition of hepatitis C virus entry into host hepatocytes by fungi-derived sulochrin and its derivatives

Syo Nakajima [a,b], Koichi Watashi [a,b,*], Shinji Kamisuki [b], Senko Tsukuda [a,c], Kenji Takemoto [b], Mami Matsuda [a], Ryosuke Suzuki [a], Hideki Aizaki [a], Fumio Sugawara [b], Takaji Wakita [a]

[a] Department of Virology II, National Institute of Infectious Diseases, Tokyo 162-8640, Japan
[b] Tokyo University of Science Graduate School of Science and Technology, Noda 278-8510, Japan
[c] Micro-Signaling Regulation Technology Unit, RIKEN Center for Life Science Technologies, Wako 351-0198, Japan

ARTICLE INFO

Article history:
Received 5 September 2013
Available online 5 October 2013

Keywords:
HCV
Entry
Sulochrin
Natural product
Screening
Compound

ABSTRACT

Hepatitis C virus (HCV) is a major causative agent of hepatocellular carcinoma. Although various classes of anti-HCV agents have been under clinical development, most of these agents target RNA replication in the HCV life cycle. To achieve a more effective multidrug treatment, the development of new, less expensive anti-HCV agents that target a different step in the HCV life cycle is needed. We prepared an in-house natural product library consisting of compounds derived from fungal strains isolated from seaweeds, mosses, and other plants. A cell-based functional screening of the library identified sulochrin as a compound that decreased HCV infectivity in a multi-round HCV infection assay. Sulochrin inhibited HCV infection in a dose-dependent manner without any apparent cytotoxicity up to 50 μM. HCV pseudoparticle and trans-complemented particle assays suggested that this compound inhibited the entry step in the HCV life cycle. Sulochrin showed anti-HCV activities to multiple HCV genotypes 1a, 1b, and 2a. Co-treatment of sulochrin with interferon or a protease inhibitor telaprevir synergistically augmented their anti-HCV effects. Derivative analysis revealed anti-HCV compounds with higher potencies ($IC_{50} < 5$ μM). This is the first report showing an antiviral activity of methoxybenzoate derivatives. Thus, sulochrin derivatives are anti-HCV lead compounds with a new mode of action.

1. Introduction

Hepatitis C virus (HCV) infection is a major causative agent of chronic liver diseases such as liver cirrhosis and hepatocellular carcinoma [1]. The standard anti-HCV therapy has been a co-treatment with pegylated-interferon (IFN)α and ribavirin, but this therapy is limited by less efficacy to certain HCV genotypes, poor tolerability, serious side effects, and high cost [2,3]. In addition to the newly approved protease inhibitors, telaprevir and boceprevir, a variety of anti-HCV candidates are under clinical development. Although these drugs improve the virological response rate, the emergence of drug-resistant virus is expected to be a significant problem. Moreover, these compounds are expensive due to their complex structure and the many steps required for their total synthesis. To overcome the drug-resistant virus and achieve a long-term antiviral effect, multidrug treatment is essential. Thus, the development of drugs targeting a different step in the HCV life cycle and presumably requiring low cost is urgently needed.

HCV propagates in hepatocytes through its viral life cycle including: attachment and entry (defined as the early step in this study); translation, polyprotein processing, and RNA replication (the middle step); and assembly, trafficking, budding, and release (the late step) (Supplementary Fig. S1). The middle step has been extensively analysed, especially after the establishment of the HCV replicon system [4]. The early step can be analysed with HCV pseudoparticle (HCVpp) [5,6], which is a murine leukemia virus- or human immunodeficiency virus-based pseudovirus carrying HCV E1 and E2 as envelope proteins. The HCV-producing cell culture system (HCVcc) is used for analyzing the whole life cycle [7–9]. In addition, the HCV trans-complemented particle (HCVtcp) system carrying an HCV subgenomic replicon RNA packaged in HCV E1 and E2-containing particles can evaluate the life cycle from the early to the middle step [10]. The majority of anti-HCV agents currently under clinical development, such as inhibitors of protease, polymerase, NS5A, and cellular cyclophilin, inhibit polyprotein processing and/or RNA replication. A desirable approach

Abbreviations: HCV, hepatitis C virus; IFN, interferon; HCVpp, HCV pseudoparticle; HCVcc, HCV derived from cell culture; HCVtcp, HCV trans-complemented particle; MOI, multiplicity of infection; HBs, HBV envelope protein; CsA, cyclosporin A; VSV, vesicular stomatitis virus.

* Corresponding author. Adress: Department of Virology II, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan. Fax: +81 3 5285 1161.

E-mail address: kwatashi@nih.go.jp (K. Watashi).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.100

to achieving efficient multidrug therapy is to identify new antiviral drugs targeting different steps in the viral life cycle. A combination of drugs with different targets can greatly decrease the emergence of drug-resistant virus.

Natural products generally contain more characteristics of high chemical diversity than combinatorial chemical collections, and therefore have a wider range of physiological activities [11,12]. They offer major opportunities for finding novel lead structures that are active in a biological assay. Moreover, biologically active natural products are generally small molecules with drug-like properties, and thus development costs of producing orally active agents tend to be lower than that derived from combinatorial chemistry [13]. In addition, there is a wide variety of natural compounds reported to possess antiviral activity [14,15]. In the present study, we have taken advantage of the potential of natural products by screening a natural product library derived from fungal extracts with a cell-based assay that supports the whole life cycle of HCV.

2. Materials and methods

2.1. Cell culture

Huh-7.5.1 [8] and HepaRG cells [16] were cultured as described previously.

2.2. Natural product library and reagents

Natural products were extracted essentially as previously described [17]. Culture broths of fungal strains isolated from seaweeds, mosses, and other plants were extracted with CH_2Cl_2. The crude extracts were separated by silica gel column chromatography to purify compounds. The chemical structure of each compound was determined by NMR and mass spectrometry analyses. Thus, we prepared an in-house natural product library consisting of approximately 300 isolated compounds.

Cyclosporin A was purchased from Sigma. Bafilomycin A1 and chlorpromazine were purchased from Wako. Heparin was obtained from Mochida Pharmaceutical. IFNα was purchased from Schering-Plough.

2.3. Compound screening

Huh-7.5.1 cells were treated with HCV J6/JFH1 at a multiplicity of infection (MOI) of 0.15 for 4 h. The cells were washed and then cultured with growth medium treated with 10 μM of each compound for 72 h. The infectivity of HCV in the medium was quantified. Cell viability at 72 h post-treatment was simultaneously measured. Compounds that decreased the cell viability to less than 50% of that without treatment were eliminated for further evaluations. Normalised infectivity was calculated as HCV infectivity divided by cell viability. Compounds reducing the normalised infectivity to less than 40% were selected as initial hits. The initial hits were further evaluated for data reproduction and dose-dependency.

2.4. HCVcc assay

HCVcc was recovered from the medium of Huh-7.5.1 cells transfected with HCV J6/JFH-1 RNA as described [7]. HCVcc was infected into Huh-7.5.1 cells at 0.15 MOI for 4 h. After washing out the inoculated virus, the cells were cultured with normal growth medium in the presence or absence of compounds for 72 h. The infectivity of HCV and the amount of HCV core protein in the medium were quantified by infectious focus formation assay and chemiluminescent enzyme immunoassay (Lumipulse II HCV core assay, ortho clinical diagnostics), respectively [7,18].

2.5. Immunoblot analysis

Immunoblot analysis was performed as described previously [19]. The anti-HCV core antibody (2H9) was used as a primary antibody with 1:1000 dilution [7].

2.6. MTT assay

The viability of cells was quantified by using a Cell Proliferation Kit II XTT (Roche Diagnostics) as described previously [20].

2.7. HCV replicon assay

Huh-7.5.1 cells were transfected with an HCV subgenome replicon RNA (SGR–JFH1/Luc) for 4 h and then incubated with or without compounds for 48 h [21]. The cells were lysed with 1xPLB (Promega), and the luciferase activity was determined with a luciferase assay system (Promega) according to the manufacturer's protocol [22].

2.8. HCVpp assay

HCVpp was recovered from the medium of 293T cells transfected with expression plasmids for HCV JFH-1 E1E2, MLV Gag-Pol, and luciferase, which were kindly provided from Dr. Francois-Loic Cosset at Universite de Lyon [5]. Vesicular stomatitis virus pseudoparticles (VSVpp) was similarly recovered with transfection by replacing HCV E1E2 with VSV G.

Huh-7.5.1 cells were preincubated with compounds for 3 h and were then infected with HCVpp in the presence of compounds for 4 h. After washing out virus and compounds, cells were incubated for an additional 72 h before recovering the cell lysates and quantifying the luciferase activity.

2.9. HCVtcp assay

The HCVtcp assay was essentially performed as described [10]. Briefly, Huh-7 cells were transfected with expression plasmids for the HCV subgenomic replicon carrying the luciferase gene and for HCV core-NS2 based on genotype 1a (RMT) (kindly provided by Dr. Michinori Kohara at Tokyo Metropolitan Institute of Medical Science), 1b (Con1), and 2a (JFH-1) [4,10,23] to recover HCVtcp. HCVtcp can reproduce RNA replication as well as HCV-mediated entry into the cells [10].

2.10. Synergy analysis

To determine whether the effect of the drug combination was synergistic, additive, or antagonistic, MacSynergy (kindly provided by Mark Prichard), a mathematical model based on the Bliss independence theory, was used to analyse the experimental data shown in Fig. 3A. In this model, a theoretical additive effect with any given concentrations can be calculated by $Z = X + Y(1-X)$, where X and Y represent the inhibition produced by each drug alone, and Z represents the effect produced by the combination of two compounds if they were additive. The theoretical additive effects were compared to the actual experimental effects at various concentrations of the two compounds and were plotted as a three-dimensional differential surface that would appear as a horizontal plane at 0 if the combination were additive. Any peak above this plane (positive values) indicates synergy, whereas any depression below the plane (negative values) indicates antagonism. The 95% confidence interval of the experimental dose–response was considered to reveal only effects that were statistically significant.

3. Results

3.1. Screening of natural products possessing anti-HCV activity

We extracted culture broths of fungal strains isolated from seaweeds, mosses, and other plants and purified compounds as described in the Section 2 [17]. The chemical structure of each compound was determined by NMR and mass spectrometry analyses. Thus, we prepared an in-house natural product library consisting of approximately 300 isolated compounds. As shown in the Section 2, compounds reducing the normalised HCV infectivity to less than 40% as compared with DMSO were selected as primary hits. The primary hits were then validated by examining the reproducibility, dose-dependency, and cell viability in the HCVcc system. Sulochrin [methyl 2-(2,6-dihydroxy-4-methylbenzoyl)-5-hydroxy-3-methoxybenzoate] (Fig. 1A) was one of the compounds showing the highest anti-HCV activity, and the following analyses focus mainly on this compound.

3.2. Sulochrin decreased HCV infectivity in HCV cell culture assay

To characterise the anti-HCV activity of the compounds, Huh-7.5.1 cells were infected with HCV J6/JFH1 at an MOI of 0.15 and then cultured for 72 h in the presence or absence of compounds. In this system, infectious HCV is secreted into the medium and then re-infects into uninfected cells to support the spread of HCV during a 72 h period (Section 2). Cell cultures were treated with sulochrin or cyclosporin A (CsA) as a positive control in this mul-

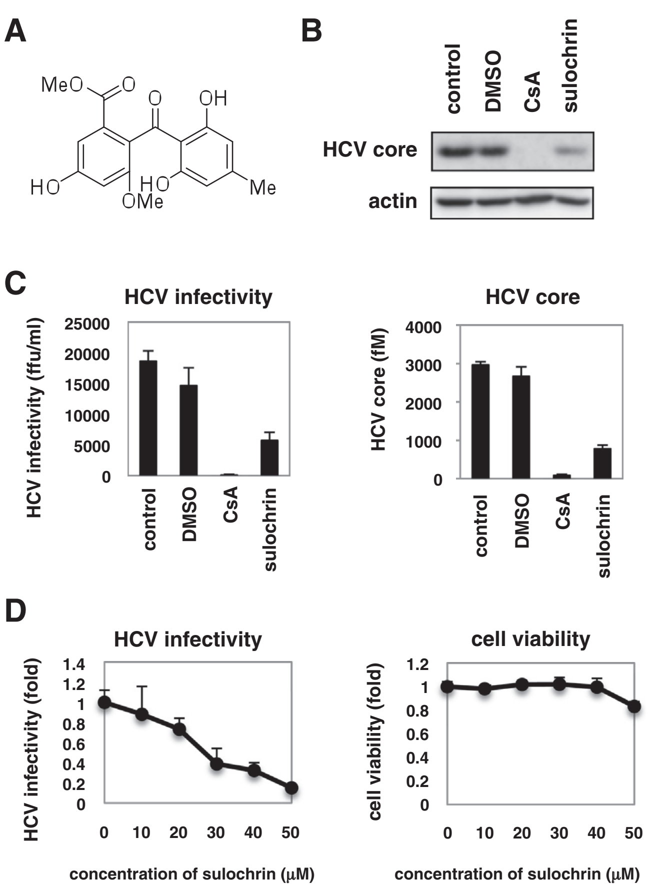


Fig. 1. Sulochrin decreased HCV production in a multi-round HCV infection assay. (A) Chemical structure of sulochrin. (B) Huh-7.5.1 cells were infected with HCV J6/JFH-1 at an MOI of 0.15 for 4 h and then incubated with or without 0.3% DMSO, 2 μM cyclosporin A (CsA), or 30 μM sulochrin for 72 h. The resultant medium was inoculated into naïve Huh-7.5.1 cells to detect intracellular HCV core and actin protein at 48 h postinoculation by immunoblot. (C) HCV infectivity (left) and HCV core protein (right) in the medium as prepared in (B) were quantified as shown in the Section 2. (D) HCV infectivity (left) determined as shown in (C) with varying concentrations (0–50 μM) of sulochrin. Cell viability was examined by MTT assay (right).

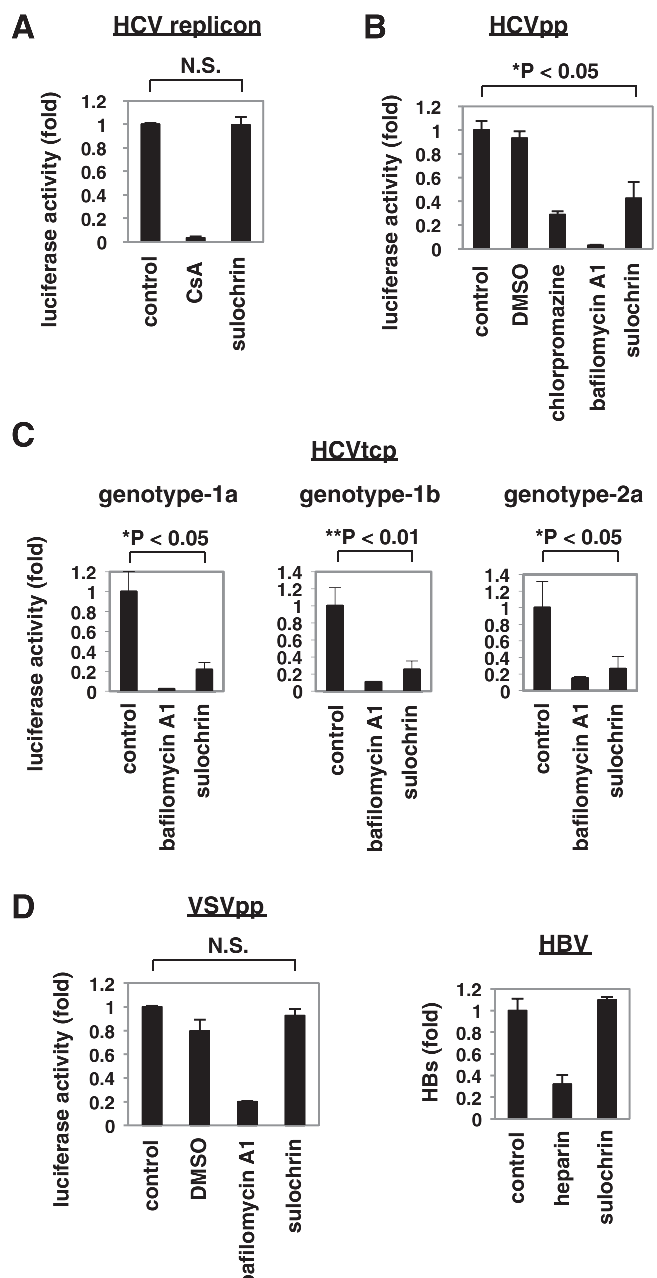


Fig. 2. Sulochrin blocked HCV entry. (A) Replicon assay. Huh-7.5.1 cells were transfected with an HCV subgenomic replicon RNA for 4 h followed by treatment with or without the indicated compounds for 48 h. Luciferase activity driven by the replication of the subgenomic replicon was quantified. (B and C) HCV pseudoparticle (HCVpp) and trans-complemented particle (HCVtcp) assay. Huh-7.5.1 cells were pretreated with the indicated compounds for 3 h and then infected with HCVpp (B) or HCVtcp (C) for 4 h. After washing out virus and compounds, cells were further incubated for 72 h and harvested for measuring luciferase activity driven by the infection of HCVpp or HCVtcp. HCVtcp assay was performed with HCV E1 and E2 derived from genotypes 1a (RMT), 1b (Con1), and 2a (JFH1). (D) Left, the pseudoparticle assay was performed as shown in (B) with VSV G instead of HCV E1 and E2. Right, HBV infection assay. HepaRG cells were pretreated with the indicated compounds for 3 h and then infected with HBV for 16 h. After washing out virus and compounds, cells were incubated for an additional 12 days. HBV infection was evaluated by measuring HBs secretion from the infected cells. Heparin was used as a positive control that inhibits HBV entry.

ti-round infection system. To examine the level of infectious HCV particles produced from the cells, the resultant medium was inoculated into naive Huh-7.5.1 cells to detect HCV core protein in the cells. As shown in Fig. 1B, intracellular production of HCV core but not that of actin was reduced in the cells inoculated with sulochrin- and CsA-treated medium (Fig. 1B). Quantitative analysis showed that sulochrin decreased HCV infectivity and HCV core protein in the medium to 1/3–1/4 of the untreated levels (Fig. 1C). Reduction of HCV infectivity by sulochrin was dose-dependent without serious cytotoxicity up to 50 μM (Fig. 1D).

3.3. Sulochrin blocked HCV entry

We investigated the step in the HCV life cycle that was inhibited by sulochrin. The middle step of the life cycle including translation and RNA replication was evaluated with the transient replication assay by using the HCV subgenomic replicon. Sulochrin had little effect on the replicon activity at doses up to 50 μM (Fig. 2A). In the HCVpp system, which reproduced the early step of HCV infection including entry, sulochrin significantly inhibited HCVpp infection (Fig. 2B). Sulochrin also inhibited the infection of HCVtcp, which reproduced both the viral entry and RNA replication, further supporting that this compound targeted the entry step (Fig. 2C). In contrast, VSV G-mediated viral entry efficiency was not altered by sulochrin treatment (Fig. 2D). Additionally, HBV entry was not inhibited by the presence of sulochrin (Fig. 2D). These data suggest that the inhibitory activity of sulochrin on viral entry is specific to HCV. The anti-HCV entry activity of sulochrin was conserved among different HCV genotypes, 1a (RMT), 1b (Con1), and 2a (JFH-1) [4,10,23] (Fig. 2C).

3.4. Synergistic effect of cotreatment of sulochrin with IFNα or telaprevir

We examined the anti-HCV activity of sulochrin co-administered with clinically available anti-HCV agents, IFNα and a prote-

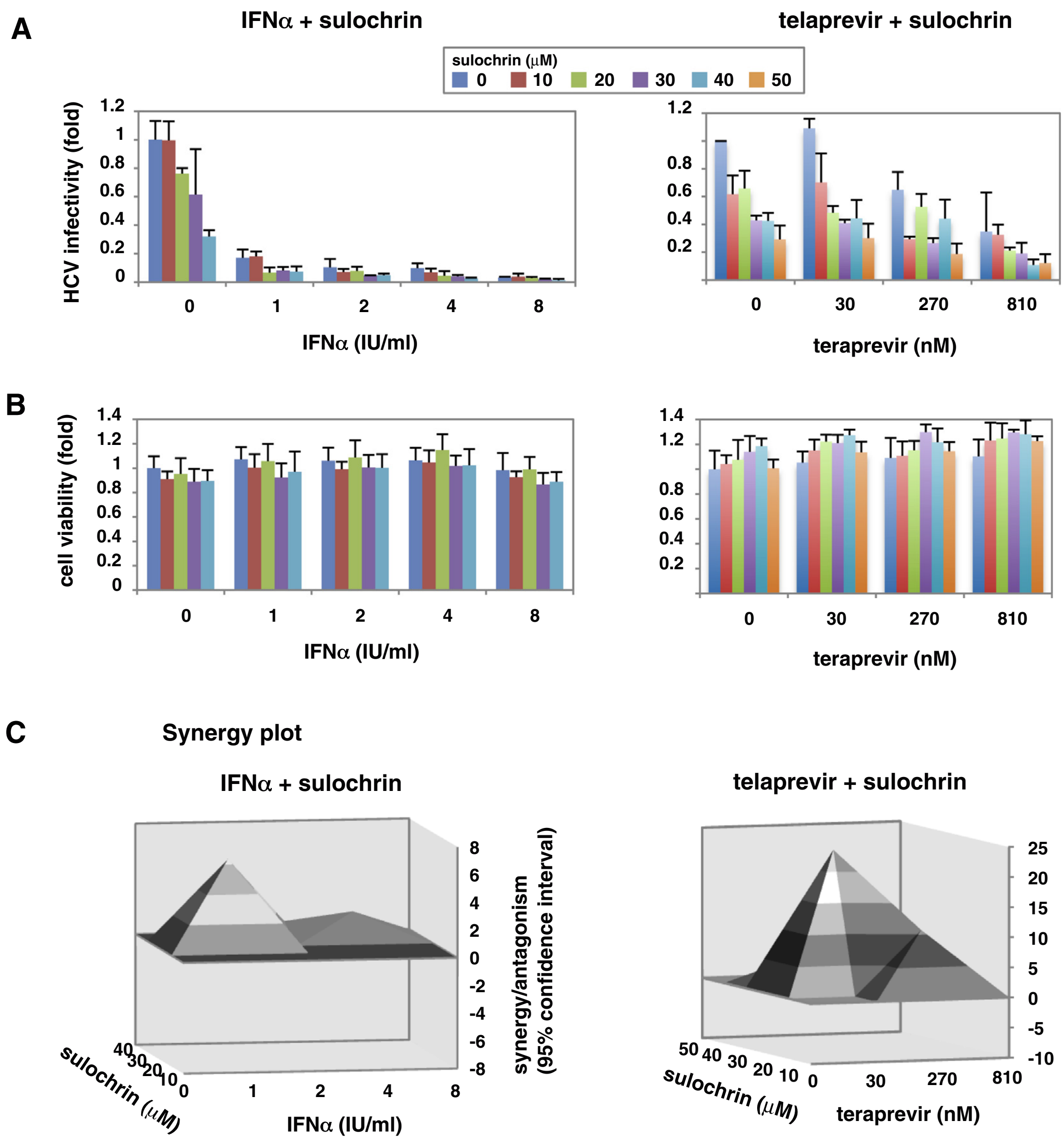


Fig. 3. Cotreatment of sulochrin with IFNα or telaprevir. (A, B) Huh-7.5.1 cells infected with HCV were treated with the indicated concentrations of sulochrin with IFNα (left) or telaprevir (right) to determine HCV infectivity in the medium (A) as shown in Fig. 1C. Cell viability was also quantified (B). (C) Synergy analysis. The results of the combinations shown in (A) were analysed with a mathematical model, MacSynergy, as described in the Section 2. The three-dimensional surface plot represents the difference between actual experimental effects and theoretical additive effects of the combination treatment (95% confidence interval). The theoretical additive effects are shown as the zero plane (dark gray) across the z-axis. A positive value in the z-axis as a peak above the plane indicates synergy, and a negative value with a valley below the plane indicates antagonism. Sulochrin in combination with IFNα (left) or telaprevir (right) produced synergistic antiviral effects that were greater than the theoretical additive effects.

ase inhibitor telaprevir. As shown in Fig. 3, addition of sulochrin with IFNα or telaprevir led to a further decrease in HCV infectivity (Fig. 3A) without significantly enhancing cytotoxicity (Fig. 3B) at any given concentrations. Thus, the combination of sulochrin and IFNα or telaprevir always resulted in a greater reduction in HCV infectivity as compared with that achieved by either agent alone. Synergy/antagonism analysis with the Bliss independence model showed that the experimental anti-HCV activity in combination with sulochrin and IFNα or telaprevir showed a peak above the zero plane in the *z*-axis, which shows the calculated theoretical additive effect (Fig. 3C). Any peak above the zero plane indicates more than an additive effect, namely, synergy (Section 2). The data clearly indicate that sulochrin had a synergistic anti-HCV effect with both IFNα and telaprevir.

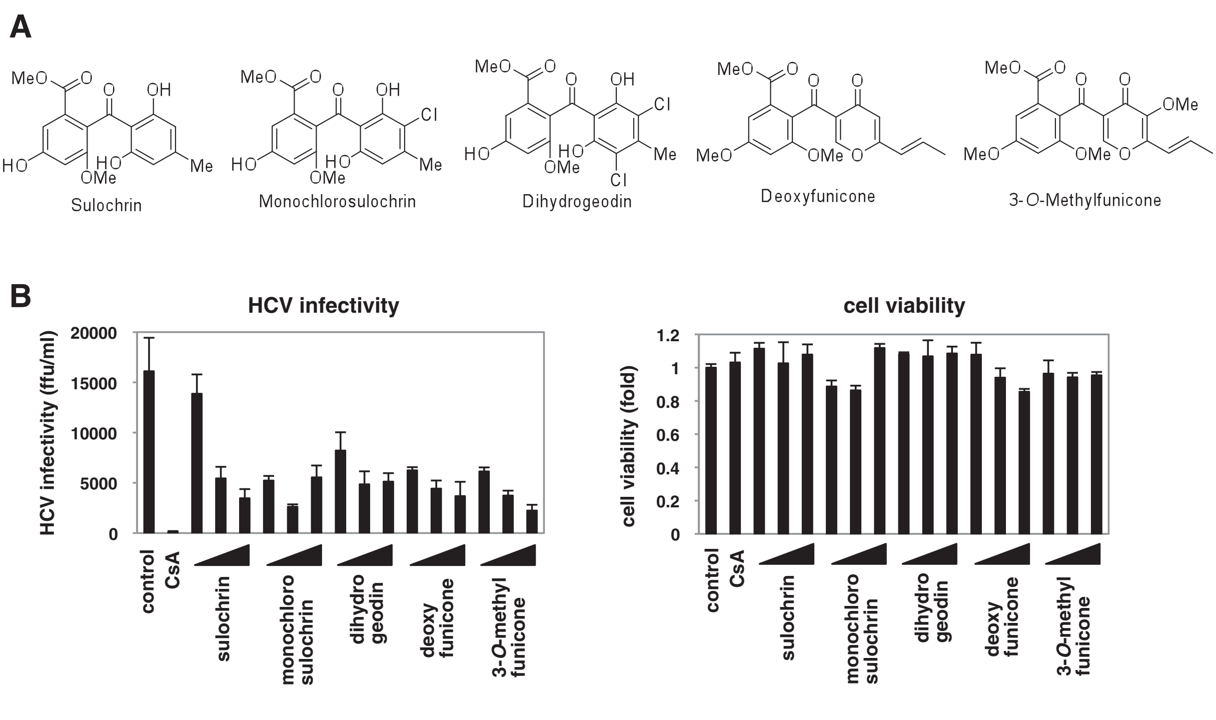
3.5. Derivative analysis of sulochrin

We examined the anti-HCV activity of a series of sulochrin derivatives (Fig. 4A) in the HCVcc system. Monochlorosulochrin and dihydrogeodin, mono- or dichloro-substituted derivatives of sulochrin, possessed even higher anti-HCV activity than sulochrin (Fig. 4B and C). Deoxyfunicone, of which one aromatic ring was replaced by a 4-pyrone ring, had approximately 5-fold greater HCV inhibitory activity as compared with sulochrin (Fig. 4B and C). An additional compound, 3-*O*-methylfunicone, also possessed anti-HCV activity (Fig. 4B and C). These data suggest that the 1,3-dihydroxy-5-methylbenzene moiety of sulochrin is important for anti-HCV activity. Furthermore, funicone derivatives as well as sulochrin derivatives are likely to be lead compounds for a new class of anti-HCV agents.

4. Discussion

In the present study, we prepared a natural product library consisting of approximately 300 isolated compounds derived from fungi extract [17]. Among these compounds, we focused on sulochrin, which reduced HCV infectivity in the HCVcc system. Sulochrin suppressed the viral entry efficiencies both in the HCVpp and the HCVtcp systems, suggesting that this compound blocked HCV envelope-mediated entry. HCV was reported to enter host cells through clathrin-dependent endocytosis after engagement to host receptors [24–27]. Sulochrin is not likely to be a general inhibitor of clathrin-dependent endocytosis, but rather is specific for HCV entry, as it did not affect the entry of other viruses such as VSVpp and HBV, which were reported to enter by clathrin-dependent manners [28,29].

Sulochrin inhibits eosinophil degranulation, activation, and chemotaxis [30,31]. It also inhibits VEGF-induced tube formation of human umbilical vein endothelial cells [32]. In addition, 3-*O*-methylfunicone, a sulochrin derivative possessing anti-HCV activity, has an anti-tumor activity [33]. It is unknown if these activities of the compounds are related to their anti-HCV activity. The establishment of drug-resistant virus and the identification of the target molecule are in progress to reveal the mechanism of action of sulochrin and its derivatives. However, the present study is the



C IC$_{50}$s and CC$_{50}$s

compound	IC$_{50}$ (μM)	CC$_{50}$ (μM)
sulochrin	24.4 ± 2.0	> 50
monochlorosulochrin	4.3 ± 1.1	> 50
dihydrogeodin	10.3 ± 0.3	> 50
deoxyfunicone	4.6 ± 1.1	> 50
3-*O*-methylfunicone	6.2 ± 2.3	> 50

Fig. 4. Derivative analysis of sulochrin. (A) Chemical structures of sulochrin derivatives examined in this study, monochlorosulochrin, dihydrogeodin, deoxyfunicone, 3-*O*-methyfunicone, as well as sulochrin. (B) Anti-HCV effects of the sulochrin derivatives (10, 30, and 50 μM) were investigated as shown in Fig. 1C. (C) The IC$_{50}$ and CC$_{50}$ values of the sulochrin derivatives are shown.

first report to demonstrate the antiviral activity of these compounds. It is important to note that sulochrin inhibited the entry of HCV genotype 1a and b, which are the dominant genotypes in North America, Europe, and East Asia, indicating that this compound has potential clinical applications. Promising applications of entry inhibitors include the prevention of HCV recurrence in patients after liver transplantation. In patients with HCV-related end-stage liver diseases undergoing liver transplantation, re-infection of the graft is universal and characterised by accelerated progression of liver diseases. Entry inhibitors may be effective especially in these conditions under robust re-infection of HCV into hepatocytes. In the present study, we showed that co-treatment of sulochrin with IFNα and a protease inhibitor, teleprevir, synergistically augmented the anti-HCV effects of these approved drugs. These results suggest the possibility that co-treatment with sulochrin and probably its effective derivatives helps to inhibit the spread of HCV infection. We also identified the chemical structure and the derivatives of sulochrin as lead compounds for anti-HCV agents. Further derivatives analysis may identify more preferable anti-HCV agents.

In conclusion, our results demonstrate that sulochrin and its derivatives are potent and selective inhibitors of HCV infection in cell culture. Although further studies including an analysis of mode of action and pharmacological properties *in vivo* are required, this class of compounds should be pursued for its clinical potential in the treatment of HCV infection.

Acknowledgments

Huh-7.5.1 cells were kindly provided by Dr. Francis Chisari at The Scripps Research Institute. The expression plasmids for producing HCVpp were a generous gift from Dr. Francois-Loic Cosset at Universite de Lyon. The expression plasmid for HCV E1E2 of genotype 1a (RMT) was kindly provided by Dr. Michinori Kohara at Tokyo Metropolitan Institute of Medical Science. We thank all of the members of the Department of Virology II, National Institute of Infectious Diseases, for their helpful discussions. This study was supported by grants-in-aid from the Ministry of Health, Labour, and Welfare, Japan, from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and from the Japan Society for the Promotion of Science.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.09.100.

References

[1] T.J. Liang, V. Rustgi, E. Galun, H.E. Blum, HCV RNA in patients with chronic hepatitis C treated with interferon-alpha, J. Med. Virol. 40 (1993) 69–75.
[2] J.G. McHutchison, S.C. Gordon, E.R. Schiff, M.L. Shiffman, W.M. Lee, V.K. Rustgi, Z.D. Goodman, M.H. Ling, S. Cort, J.K. Albrecht, Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis interventional therapy group, N. Engl. J. Med. 339 (1998) 1485–1492.
[3] A.W. Tai, R.T. Chung, Treatment failure in hepatitis C: mechanisms of non-response, J. Hepatol. 50 (2009) 412–420.
[4] V. Lohmann, F. Korner, J. Koch, U. Herian, L. Theilmann, R. Bartenschlager, Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line, Science 285 (1999) 110–113.
[5] B. Bartosch, J. Dubuisson, F.L. Cosset, Infectious hepatitis C virus pseudo-particles containing functional E1–E2 envelope protein complexes, J. Exp. Med. 197 (2003) 633–642.
[6] M. Hsu, J. Zhang, M. Flint, C. Logvinoff, C. Cheng-Mayer, C.M. Rice, J.A. McKeating, Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles, Proc. Natl. Acad. Sci. USA 100 (2003) 7271–7276.
[7] T. Wakita, T. Pietschmann, T. Kato, T. Date, M. Miyamoto, Z. Zhao, K. Murthy, A. Habermann, H.G. Krausslich, M. Mizokami, R. Bartenschlager, T.J. Liang, Production of infectious hepatitis C virus in tissue culture from a cloned viral genome, Nat. Med. 11 (2005) 791–796.
[8] J. Zhong, P. Gastaminza, G. Cheng, S. Kapadia, T. Kato, D.R. Burton, S.F. Wieland, S.L. Uprichard, T. Wakita, F.V. Chisari, Robust hepatitis C virus infection *in vitro*, Proc. Natl. Acad. Sci. USA 102 (2005) 9294–9299.
[9] B.D. Lindenbach, M.J. Evans, A.J. Syder, B. Wolk, T.L. Tellinghuisen, C.C. Liu, T. Maruyama, R.O. Hynes, D.R. Burton, J.A. McKeating, C.M. Rice, Complete replication of hepatitis C virus in cell culture, Science 309 (2005) 623–626.
[10] R. Suzuki, K. Saito, T. Kato, M. Shirakura, D. Akazawa, K. Ishii, H. Aizaki, Y. Kanegae, Y. Matsuura, I. Saito, T. Wakita, T. Suzuki, Trans-complemented hepatitis C virus particles as a versatile tool for study of virus assembly and infection, Virology 432 (2012) 29–38.
[11] G.M. Cragg, D.J. Newman, Natural products: a continuing source of novel drug leads, Biochim. Biophys. Acta 2013 (1830) 3670–3695.
[12] D.J. Newman, G.M. Cragg, Natural products as sources of new drugs over the 30 years from 1981 to 2010, J. Nat. Prod. 75 (2012) 311–335.
[13] A.L. Harvey, Natural products as a screening resource, Curr. Opin. Chem. Biol. 11 (2007) 480–484.
[14] S.S. Yang, G.M. Cragg, D.J. Newman, J.P. Bader, Natural product-based anti-HIV drug discovery and development facilitated by the NCI developmental therapeutics program, J. Nat. Prod. 64 (2001) 265–277.
[15] K. Kitazato, Y. Wang, N. Kobayashi, Viral infectious disease and natural products with antiviral activity, Drug Discovery Ther. 1 (2007) 14–22.
[16] K. Watashi, G. Liang, M. Iwamoto, H. Marusawa, N. Uchida, T. Daito, K. Kitamura, M. Muramatsu, H. Ohashi, T. Kiyohara, R. Suzuki, J. Li, S. Tong, Y. Tanaka, K. Murata, H. Aizaki, T. Wakita, Interleukin-1 and tumor necrosis factor-alpha trigger restriction of hepatitis B virus infection via a cytidine deaminase AID, J. Biol Chem. PMID: 24025329.
[17] Y. Myobatake, T. Takeuchi, K. Kuramochi, I. Kuriyama, T. Ishido, K. Hirano, F. Sugawara, H. Yoshida, Y. Mizushina, Pinophilins A and B, inhibitors of mammalian A-, B-, and Y-family DNA polymerases and human cancer cell proliferation, J. Nat. Prod. 75 (2012) 135–141.
[18] A. Murayama, N. Sugiyama, K. Watashi, T. Masaki, R. Suzuki, H. Aizaki, T. Mizuochi, T. Wakita, T. Kato, Japanese reference panel of blood specimens for evaluation of hepatitis C virus RNA and core antigen quantitative assays, J. Clin. Microbiol. 50 (2012) 1943–1949.
[19] K. Watashi, M. Khan, V.R. Yedavalli, M.L. Yeung, K. Strebel, K.T. Jeang, Human immunodeficiency virus type 1 replication and regulation of APOBEC3G by peptidyl prolyl isomerase Pin1, J. Virol. 82 (2008) 9928–9936.
[20] K. Watashi, M.L. Yeung, M.F. Starost, R.S. Hosmane, K.T. Jeang, Identification of small molecules that suppress microRNA function and reverse tumorigenesis, J. Biol. Chem. 285 (2010) 24707–24716.
[21] T. Kato, T. Date, M. Miyamoto, M. Sugiyama, Y. Tanaka, E. Orito, T. Ohno, K. Sugihara, I. Hasegawa, K. Fujiwara, K. Ito, A. Ozasa, M. Mizokami, T. Wakita, Detection of anti-hepatitis C virus effects of interferon and ribavirin by a sensitive replicon system, J. Clin. Microbiol. 43 (2005) 5679–5684.
[22] H. Marusawa, M. Hijikata, K. Watashi, T. Chiba, K. Shimotohno, Regulation of Fas-mediated apoptosis by NF-kappa B activity in human hepatocyte derived cell lines, Microbiol. Immunol. 45 (2001) 483–489.
[23] F. Yasui, M. Sudoh, M. Arai, M. Kohara, Synthetic lipophilic antioxidant BO-653 suppresses HCV replication, J. Med. Virol. 85 (2013) 241–249.
[24] M.B. Zeisel, I. Fofana, S. Fafi-Kremer, T.F. Baumert, Hepatitis C virus entry into hepatocytes: molecular mechanisms and targets for antiviral therapies, J. Hepatol. 54 (2011) 566–576.
[25] E. Blanchard, S. Belouzard, L. Goueslain, T. Wakita, J. Dubuisson, C. Wychowski, Y. Rouille, Hepatitis C virus entry depends on clathrin-mediated endocytosis, J. Virol. 80 (2006) 6964–6972.
[26] A. Codran, C. Royer, D. Jaeck, M. Bastien-Valle, T.F. Baumert, M.P. Kieny, C.A. Pereira, J.P. Martin, Entry of hepatitis C virus pseudotypes into primary human hepatocytes by clathrin-dependent endocytosis, J. Gen. Virol. 87 (2006) 2583–2593.
[27] L. Meertens, C. Bertaux, T. Dragic, Hepatitis C virus entry requires a critical postinternalisation step and delivery to early endosomes via clathrin-coated vesicles, J. Virol. 80 (2006) 11571–11578.
[28] D.K. Cureton, R.H. Massol, S.P. Whelan, T. Kirchhausen, The length of vesicular stomatitis virus particles dictates a need for actin assembly during clathrin-dependent endocytosis, PLoS Pathog. 6 (2010) e1001127.
[29] H.C. Huang, C.C. Chen, W.C. Chang, M.H. Tao, C. Huang, Entry of hepatitis B virus into immortalised human primary hepatocytes by clathrin-dependent endocytosis, J. Virol. 86 (2012) 9443–9453.
[30] H. Ohashi, M. Ishikawa, J. Ito, A. Ueno, G.J. Gleich, H. Kita, H. Kawai, H. Fukamachi, Sulochrin inhibits eosinophil degranulation, J. Antibiotechnol. (Tokyo) 50 (1997) 972–974.
[31] H. Ohashi, Y. Motegi, H. Kita, G.J. Gleich, T. Miura, M. Ishikawa, H. Kawai, H. Fukamachi, Sulochrin inhibits eosinophil activation and chemotaxis, Inflamm. Res. 47 (1998) 409–415.
[32] H.J. Lee, J.H. Lee, B.Y. Hwang, H.S. Kim, J.J. Lee, Fungal metabolites, asterric acid derivatives inhibit vascular endothelial growth factor (VEGF)-induced tube formation of HUVECs, J. Antibiotechnol. (Tokyo) 55 (2002) 552–556.
[33] R. Nicoletti, E. Manzo, M.L. Ciavatta, Occurence and bioactivities of funicone-related compounds, Int. J. Mol. Sci. 10 (2009) 1430–1444.