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Inhibition of hepatitis C virus infection by polyoxometalates

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ABSTRACT

Hepatitis C virus (HCV) infects about 2% of the world population. The standard treatment of chronic HCV infection is still discontented because of the low sustained virological response rate. The development of new HCV antivirals is a healthcare imperative. We explored the potentials of polyoxometalates to inhibit HCV infection using newly developed HCVcc cell culture system. We found one polyoxometalate compound (named POM-12) can inhibit HCV infection at the nanomolar range while displayed little cytotoxicity. We showed that POM-12 inhibited pseudotyped HCV infection but had no effect on HCV RNA replication. Furthermore, we showed that POM-12 was virucidal and can disrupt HCV particles. Finally we demonstrated that POM-12 had no effect on the vesicular stomatitis virus infection while had weak inhibitory activity against the influenza virus infection. In conclusion, we identified a potent anti-HCV compound which may provide an attractive drug candidate to cure HCV infection.

1.Introduction

Hepatitis C virus (HCV) is an enveloped positive-strand RNA virus belonging to the Flaviviridae family. At least 70% of HCV infection results in persistent infection (Afdhal et al., 2004; Poynard et al., 2003) and leads to progressive liver diseases and other diseases related to the HCV infection (Alter and Seeff, 2000; Fartoux et al., 2005; Noto and Raskin, 2006; Romero-Gomez, 2006), such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, which can necessitate liver transplantation if the infection is not successfully treated. The current standard treatment of chronic HCV infection is combination of pegylated interferon-alpha (IFN-α) and ribavirin (Glue et al., 2000). The sustained virological response (SVR) of this treatment is only achieved in about 80% of individuals infected with HCV genotype 2 or 3 and 40–50% of individuals infected with genotype 1 or 4 (Ahmed and Keeffe, 1999). Small-molecule inhibitors of NS3 protease, Boceprevir (Sarrazin et al., 2007) and Telaprevir (Hezode et al., 2009) were recently approved, and more antivirals targeting NS5B polymerase, NS5A and other viral or host encoded proteins are under development.

Polyoxometalates (POMs) are negatively charged clusters of inorganic substances that consist of three or more transition metal oxyanions linked together by shared oxygen atoms. These inorganic compounds have potential multiple applications such as catalysis (El Moll et al., 2011), functional materials and medicine (Shigeta, 1999). The medicinal properties of POMs have been a subject of interest for their low cytotoxicity and lower cost than the majority of organic pharmaceuticals (Judd et al., 2001; Shigeta et al., 2003; Witvrouw et al., 2000). Anti-virus, anti-bacteria and anti-tumor activities of POMs have been reported (Compain et al., 2010; Guo et al., 2011; Inoue et al., 2006a,b). Particularly, POMs substituted with titanium or vanadium atoms have been demonstrated to inhibit several RNA viruses, including Flaviviridae (Dengue virus), Orthomyxoviridae (influenza virus type A), Paramyxoviridae (respiratory syncytial virus), and Lentiviridae (human immunodeficiency virus type 1) (Shigeta et al., 2003). Other POMs were also reported to inhibit the replication of the human immunodeficiency virus, herpes simplex virus, influenza virus and respiratory syncytial virus (Dan et al., 2002; Flutsch et al., 2011). In this study, we identified a POM compound that possesses a potent inhibitory activity against HCV.

2. Materials and methods

2.1. Synthesis and characterization of the POM compound and X-ray crystallography

POM-12 ($Cs_2K_4Na[SiW_9Nb_3O_{40}]\cdot H_2O$) was prepared as described below. First, a hexaniobate solution was made by dissolving 6.5 g of $K_7H[Nb_6O_{19}]$ in 400 mL of deionized H_2O. 11.6 M hydrogen peroxide was added with gentle stirring. The reaction

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mixture was then acidified by the addition of 3 M HCl (20 mL), and 25.1 g A-α-$Na_9H[SiW_9O_{34}]$ was mixed in until fully dissolved. The solution was diluted with H_2O to a final volume of 700 mL. 10 g cesium chloride was added and the solution was evaporated at room temperature. Approximately 350 mL of a yellow precipitate resulted. The yellow precipitate was added to 300 mL distilled water and heated to 45 °C in a water bath. The solution was then filtered through 0.2 μm mesh and allowed to evaporate at room temperature. After a few minutes, yellow-colored crystals of $Cs_2K_4Na[SiW_9Nb_3O_{40}]\cdot H_2O$ were produced. Elemental analysis was carried out on the crystalline, and the weight percents were: Cs, 8.67%/8.97%; K, 5.09%/4.93%; Nb, 9.09%/9.19%; W, 54.02%/54.15%. Infrared spectra (IR) measured by the KBr pellet method (cm^{-1}) were: 3429 (strong), 2357 (strong), 1631 (strong), 990 (middle), 939 (middle), 864 (strong), 808 (strong), 745 (strong), 506 (weak), 488 (strong). The measurement for POM-12 was collected on a Rigaku R-AXIS RAPID IP diffractometer with Mo-Kα monochromated radiation (λ = 0.71073o) at 150 K. Empirical absorption correction was applied. The structure was solved by the direct method and refined by the Full-matrix least-squares on F^2 using the SHELXL-97 software. All of the non-hydrogen atoms except the disordered atoms were refined anisotropically.

Synthesis of POM-4 (A-β-$Na_9H[SiW_9O_{34}]$) was previously described (Téazéa et al., 2007). 3.42 g $NaSiO_3$ was dissolved in 50 ml of distilled water, and then 30.04 g $NaWO_4\cdot 2H_2O$ was added slowly. The pH value of the solution was adjusted to 8.0 with 6 M hydrochloric acid. After filtering to remove the precipitate, the solution was kept at room temperature for several days. The formed white crystals were flushed with cold water for several times. Elemental analysis was carried out on the crystalline A-β-$Na_9H[SiW_9O_{34}]$. Weight percents were (expected/actual): Na, 8.50%/8.48%; Si, 1.15%/1.17%; W, 67.96%/68.72%. Infrared spectra (IR) measured by the KBr pellet method (cm^{-1}) were: 3428 (s), 2360 (s), 1630 (s), 990 (m), 934 (m), 864 (s), 808 (s), 745 (s), 668 (s), 546 (w).

Synthesis of POM-6 ($K_6[SiNiW_{11}O_{40}H_2O]\cdot 15H_2O$) was previously described (Inouye et al., 1992). 3.42 g Ni_2SO_4 dissolved in H_2O was dropwisely added to a solution of 30.04 g, 10 mmol $H_4SiW_{12}O_{40}\cdot 7H_2O$ in 100 ml H_2O with stirring, then, 31.40 g, 320 mmol CH_3COOK in H_2O was added. The solution was acidified to pH 7.0 by addition of CH_3COOH. After standing for 1 min at 95 °C, the undissolved residue was filtered off. The resulting green needle precipitate was recrystallized at room temperature overnight. Elemental analysis was carried out on the crystalline $K_6[SiNiW_{11}O_{40}H_2O]\cdot 15H_2O$. Weight percents were (expected/actual): W, 61.81%/61.88%; Ni, 1.79%/1.68%. Infrared spectra (IR) measured by the KBr pellet method (cm^{-1}) were: 3430 (s), 2360 (s), 1626 (s), 996 (m), 958 (m), 906 (s), 813 (s), 787 (s), 697 (s), 524 (w).

2.2. Cell culture and virus preparation

Huh7.5.1, HEK293T and MDCK cells were maintained in complete Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 10 mM HEPES buffer, 100 U/ml penicillin and 100 mg/ml streptomycin (Zhong et al., 2005).

The generation of HCVcc (JFH1 strain and chimera) (Lu et al., 2013; Zhong et al., 2005), vesicular stomatitis virus Indiana 1 serotype (VSV-IN1) (Liu et al., 2010; Rodriguez et al., 2002) and Influenza A/WSN/1933 (WSN) (Liu et al., 2010; Rodriguez et al., 2002) were as previously described.

HCV and VSV pseudotyped retroviral particles (HCVpp or VSVpp) were generated as previously described (Hsu et al., 2003). Briefly HEK293T cells were cotransfected with the envelope-deficient HCV genome PNL4-3.Luc.R-.E- and a plasmid expressing glycoproteins of HCV and VSV, respectively. Viral supernatants were harvested at 72 h posttransfection and filtered.

2.3. HCV focus reduction assay

POM compounds dissolved in growth medium were pre-incubated with 50 focus-forming units (ffu) of JFH1-HCVcc for 1 h at room temperature. The virus–drug mixture was then transferred to 8000 Huh7.5.1 cells in a 96-well plate. The inocula were removed at 4 h post-inoculation, and the cells were supplemented with 100 μl of fresh growth medium without compounds, and incubated at 37 °C for 3 days. The cells were fixed with paraformaldehyde and immunostained with a human monoclonal antibody (C1) against HCV envelope protein E2 (Zhong et al., 2005). Bound primary antibodies were detected by using Alexa 555-conjugated secondary antibodies (Molecular probes, Eugene, OR, USA). Nuclei were stained with Hoechst dye. The number of HCV-positive foci was counted by fluorescence microscopy.

2.4. HCV RNA quantification

Total RNA was extracted from viral culture supernatants or HCV infected Huh7.5.1 cell lysates using the previously described guanidine thiocyanate method (Zhong et al., 2005). HCV RNA levels were determined by quantitative reverse transcription-PCR (RT-qPCR) as described previously (Zhong et al., 2005).

2.5. Pseudotyped virus infection

Pseudotyped viruses containing HCV (H77 strain) or VSV glycoproteins were normalized using their luciferase reading values upon infection, and the same amount of viruses were inoculated to 1×10^4 Huh7.5.1 cells for 72 h. The infected cells were lysed with 20 μl of cell culture lysis reagent (Promega, Madison, WI, USA), and infection was measured by quantifying the expression of the luciferase reporter using 50 μl of luciferase substrate on a GloMax 96 microplate luminometer (Promega).

2.6. Cytotoxicity assay

Cytotoxicity of the POM compound was examined by determining cell viability after treating Huh7.5.1 cells for 3 days using the cell proliferation reagent WST-1 (Roche, Basel, Switzerland) following the protocol provided.

2.7. HCV replicon assay

HCV replicon cells were incubated with culture medium containing POM-12 for 3 days. HCV RNA was quantified as described above with cellular GAPDH used as an internal control.

2.8. Time of addition assay

Eight thousand of Huh7.5.1 cells in a 96-well plate were infected with 50 ffu HCV. POM-12 was added at a final concentration of 20 μM under the following three conditions: (i) Preinoculation: POM-12 was added to cells for 4 h at 37 °C followed by washing four times with growth medium before virus infection. (ii) Coinoculation: the mixture of POM-12 and virus was added to cells for 4 h at 37 °C followed by washing four times with growth medium. (iii) Postinoculation: cells were first infected for 4 h at 37 °C followed by washing four times with growth medium. POM-12 was added and incubated with the cells for the duration of the experiment. At 72 h postinfection, cells were analyzed by HCV E2 immunostaining as described above.

2.9. HCV particle disruption assay

Two hundred thousand ffu of JFH1-HCVcc or 100 μl of HCV patient sera were incubated with various concentrations of POM-12 and RNase A (40 μg/ml) at 37 °C for 1 h before RNA extraction. HCV RNA was quantified by RT-qPCR as described above.

2.10. Flu and VSV plaque assay

MDCK and Huh7.5.1 cells were used for the plaque assay for the influenza virus (WSN) and VSV infection, respectively. The 2% soft agar solution was cooled down to 45 °C, mixed with the pre-warmed 2× MEM containing 4% FBS and 2× NEAA and poured to the infected cells. The cells were maintained in 37 °C for 48 h for the plaque detection.

2.11. Co-treatment of POM-12 and other anti-HCV agents

One thousand ffu of JFH1-HCVcc was pretreated with 5 μM POM-12 and 100 IU/mL IFN-α or 150 nM HCV-796, an NS5B inhibitor (Howe et al., 2008) at room temperature for 1 h, and then inoculated to 1×10^4 Huh7.5.1 cells at 37 °C for 3 days. HCV RNA was quantified by RT-qPCR as described above.

3. Results

3.1. Anti-HCV activity of POMs

We synthesized three POM compounds (POM-4, POM-6 and POM-12). The structure of these compounds is shown in Fig. 1A. First we evaluated the ability of these POM compounds to inhibit HCV infection using a previously established HCV focus reduction assay (Cheng et al., 2008). About 50 focus-forming units (ffu) of HCVcc (JFH1) were incubated with 2, 20 or 50 μM of the POM compounds for 1 h before inoculated to Huh7.5.1 cells, and the foci of infection were counted at day 3 post-infection. As shown in Fig. 1B, all three POM compounds could inhibit HCV infection in a dose-dependent manner, with POM-12 the most potent one and POM-4 the least potent one. At 2 μM concentration, POM-4, POM-6 and POM-12 resulted in about 90%, 70% and 20% foci of infection, respectively compared to the mock treatment. Notably, 20 and 50 μM of POM-12 completely abolished HCV infection, clearly indicating that this compound possesses the most potent anti-HCV activity. Therefore we chose POM-12 for further studies.

3.2. Anti-HCV efficacy and cytotoxicity of POM-12

Next, using the HCV focus reduction assay we determined that the 50% maximal effective concentration (EC_{50}) of POM-12 on HCV infection was 0.8 μM (Table 1 and Fig. 2A). To assess cytotoxicity of POM-12, we first examined the viability of Huh7.5.1 cells treated with POM-12 at the concentrations from 0.5 to 50 μM used to determine EC_{50}. No cytotoxicity was observed for POM-12 at the concentrations up to 20 μM. About 85% cell viability remained at 50 μM of POM-12, while HCV infectivity was entirely abolished at this concentration (Fig. 2A), suggesting that POM-12 inhibits HCV infection without apparently impairing cell viability. To calculate the 50% toxic concentration (TC_{50}) of POM-12, we treated Huh7.5.1 cells with POM-12 at a higher dose range (from 20 to 250 μM). As presented in Fig. 2B and Table 1, the TC_{50} of POM-12 was 119 μM. The drug selection index of POM-12, defined as the ratio of TC_{50} over EC_{50}, was about 150. Furthermore, we showed that POM-12 inhibited HCV at different multiplicity of infection (MOI) (Fig. 2C), and it appeared that the inhibitory efficiency of POM-12 decreased with the increase of viral inocula.

Table 1
Antiviral efficacy of POM-12.

EC_{50} for HCVcc infection (μM)	0.8
EC_{50} for Flu V infection (μM)	16.07
EC_{50} for HCVpp infection (μM)	1.19
TC_{50} for Huh7.5 cell (μM)	119

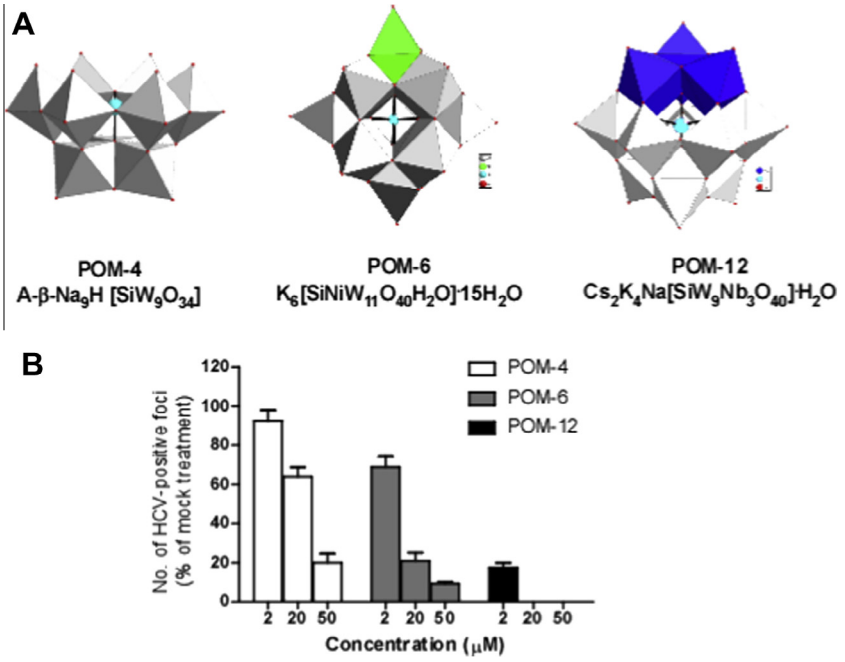

Fig. 1. POM compounds inhibit HCV infection. (A) The structure of three POM compounds. (B) HCV focus reduction assay was performed to screen the three POM compounds (POM-4, POM-6 and POM-12) for their anti-HCV activities. JFH1-HCVcc were pre-incubated with 2, 20 and 50 μM of the POM compounds for 1 h, and then inoculated to Huh7.5.1 cells for 4 h. The infection was analyzed by the HCV E2 immunofluorescence. The results were presented as the percentage of the HCV-positive foci numbers compared to the mock treatment. The error bars represented three independent experiments.

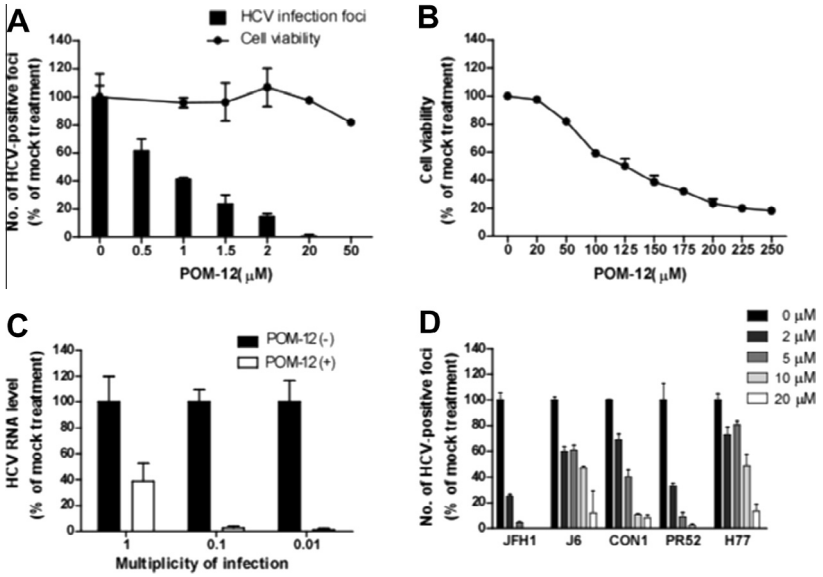

Fig. 2. Anti-HCV efficacy and cytotoxicity of POM-12. (A) Measurement of EC_{50} of POM-12 on HCV infection. Fifty ffu of JFH1-HCV were pre-incubated with various concentrations of POM-12 for 1 h, and then inoculated to Huh7.5.1 cells for 4 h. The inocula were removed, and the infection was allowed for 3 days prior to immunofluorescence analysis to quantify HCV infection. The cell proliferation reagent WST-1 was used to measure the viability of the cells treated with POM-12 for 3 days. The results were presented as the percentage of the HCV-positive foci numbers (bars) or viability OD readings (line) compared to the mock treatment. The error bars represent three independent experiments. (B) Measurement of TC_{50} of POM-12. Huh7.5.1 cells were treated with indicated concentrations of POM-12 for 3 days, and the cell viability was measured using the WST-1 assay, and presented as the percentage of the mock treatment. The error bars represented three independent experiments. (C) Effect of POM-12 on HCV infection at different MOIs. Different amount of JFH1-HCVcc were treated with 5 μM of POM-12 for 1 h, then inoculated to Huh7.5.1 cells at an MOI of 1, 0.1 or 0.01 for 4 h. The infection was measured by RT-qPCR analysis of intracellular HCV RNA levels at day 3 postinfection. The results were normalized against the intracellular GAPDH levels, and presented as values relative to the mock treatment for each infection. (D) Antiviral effect of POM-12 on HCV of different genotypes. Fifty ffu of HCVcc of indicated genotypes were pretreated with various concentrations of POM-12 for 1 h, and then inoculated to Huh7.5.1 cells for 4 h. The inocula were removed, and the infection was allowed for 3 days prior to immunofluorescence analysis to quantify HCV infection. The results were presented as the percentage of the HCV-positive foci numbers compared to the mock treatment.

To determine the effect of POM-12 on other HCV genotypes, we performed the focus reduction assay using the chimeric HCVcc expressing envelope glycoproteins of other HCV strains, including H77 (genotype 1a), Con1 (genotype 1b), PR52 (genotype 1b) (Lu et al., 2013), J6 (genotype 2a). As shown in Fig. 2D, our results demonstrated that POM-12 inhibited the infection of all these HCVcc albeit with various efficiencies, suggesting POM-12 is a promising anti-HCV lead compound.

3.3. POM-12 inhibits the infection of pseudotyped HCV particles

To investigate the mechanism of action of POM-12 on HCV infection, we first examined the effect of POM-12 on the infection of pseudotyped HCV particles (HCVpp). Various concentrations of POM-12 compounds were used to treated HCVpp and a control virus pseudotyped with VSV glycoproteins (VSVpp). As shown in Fig. 3, POM-12 inhibited HCVpp infection in a dose-dependent manner while had no effect on VSVpp infection. The EC_{50} of POM-12 inhibition on HCVpp infection was calculated as 1.19 μM (Table 1), slightly higher than the EC_{50} (0.8 μM) on HCVcc infection. These data suggested that POM-12 inhibits HCV infection at an early stage of HCV life cycle, and this inhibitory effect displays significant specificity possibly due to HCV envelope proteins.

3.4. POM-12 has no apparent effect on HCV RNA replication

To examine whether POM-12 affects HCV RNA replication, we treated Huh7 cells expressing an HCV subgenomic replicon with a serial concentrations of POM-12 for 3 days, and HCV RNA levels were determined by RT-qPCR. BMS-790052, a potent NS5A inhibitor (Gao et al., 2010), was included as the control. As shown in Fig. 4, POM-12 had no apparent effect on HCV RNA replication even at the dose of 20 μM, while 3.5 nM of BMS-790052 almost completely abolished HCV RNA replication.

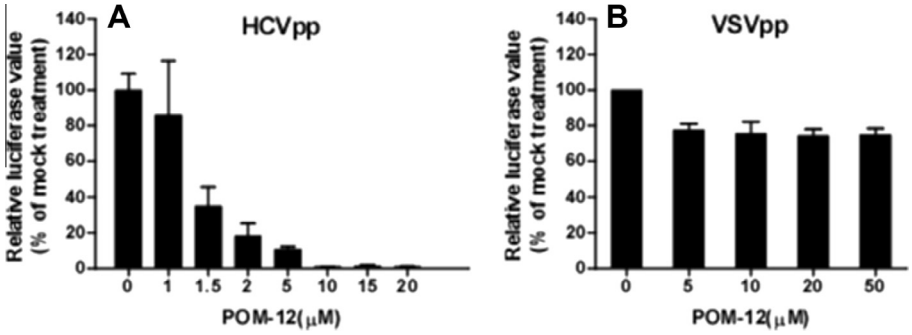

Fig. 3. POM-12 inhibits HCVpp infection. Pseudotyped viruses containing HCV envelope proteins (A) or VSV glycoproteins (B) were incubated with POM-12 for 1 h before the infection. Infection was measured by the luciferase assay. Data shown are mean value ± SD of at least three independent experiments.

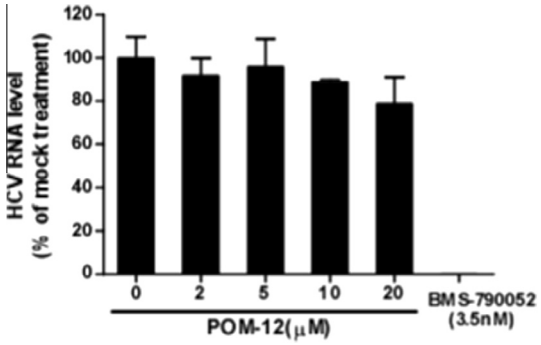

Fig. 4. POM-12 has no apparent effect on HCV RNA replication. HCV subgenomic replicon cells were incubated with POM-12 for 72 h. HCV RNA was quantified by RT-qPCR. NS5A inhibitor BMS-790052 was used as a positive control. Data shown are mean value ± SD of at least three independent experiments.

3.5. POM-12 can disrupt HCV particles

To investigate at which step POM-12 inhibits HCV infection, we performed a time of addition experiment. POM-12 was incubated with the Huh7.5.1 cells before, simultaneously or after HCV inoculation. As shown in Fig. 5A, addition of POM-12 before or after the infection only reduced the infection to 45% and 60%, respectively, whereas addition of the compound and HCV at the same time to the cells completely abolished the infection, suggesting POM-12 likely acts directly against viral particles.

To further define its mechanism of action, 1×10^5 ffu of JFH1-HCVcc were pre-incubated with 10 μM POM-12 at room temperature for 1 h, and then diluted for 1000 folds with fresh medium to reach the final concentration of 100 ffu JFH1-HCVcc and 10 nM POM-12. As the control, 100 ffu JFH1-HCVcc was incubated with 10 nM POM-12 at room temperature for 1 h prior to inoculation. As shown in Fig. 5B, although 10 nM POM-12 had weak inhibitory effect on HCV infection, preincubation of HCVcc with 10 μM POM-12 followed by dilution almost completely abolished the infection, suggesting that POM-12 has inactivated HCVcc before the step of dilution.

Next we examined the effect of POM-12 on the integrity of HCV particles. The HCV supernatants were incubated with various concentrations of POM-12 followed by the RNaseA treatment to degrade non-protected free HCV genomic RNA. The remaining HCV RNA was quantified by RT-QPCR. As a control, 1% of Triton-100, a detergent known to disrupt HCV envelope leading to exposure of viral RNA genome. As shown in Fig. 5C, the POM-12 treatment could promote the degradation of HCV genomic RNA in HCV virions in a dose-dependent manner measured by RT-qPCR, while it had no interfering effect on amplification efficiency of RT-qPCR (data not shown).

Furthermore, we tested whether POM-12 can disrupt the HCV virions in patient serum. Two genotype 1b and two genotype 2a HCV patient sera were treated with POM-12 at 37 °C for 1 h. As shown in Fig. 5D, POM-1 reduced HCV RNA levels significantly, suggesting that the compound is stable and retains the anti-HCV activity in human sera.

Taken together, our results above demonstrated that POM-12 can break down HCV envelope and disrupt the integrity of viral particles.

3.6. Specificity of antiviral activities of POM-12

To determine the specificity of POM-12 on the virus infection, we examined the effects of this compound on other two enveloped viruses, vesicular stomatitis virus (VSV) and influenza virus. As shown in Fig. 6, POM-12 had no significant effect on VSV infection as 50 μM POM-12 only reduced infection for about 10%, similar to the effect on the pseudotyped VSV (VSVpp) infection (Fig. 3). In

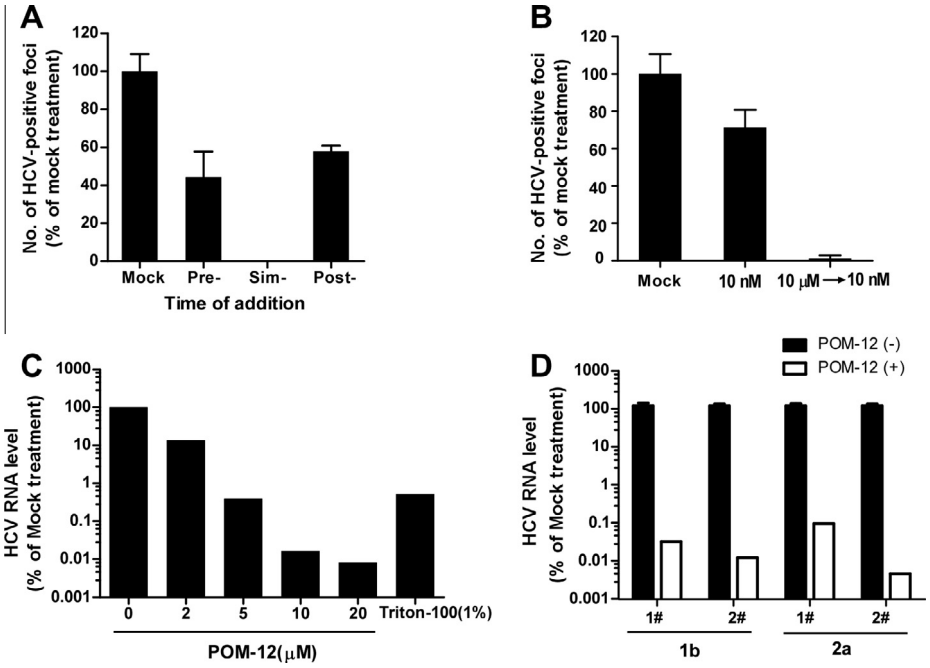

Fig. 5. POM-12 can directly disrupt HCV virion. (A) Time of addition experiment of POM-12. Huh7.5.1 cells were incubated with 20 μM of POM-12 for 4 h before infection, simultaneously or at 4 h postinfection, respectively. The infection was measured by the HCV E2 immunofluorescence. (B) 1×10^5 ffu of JFH1-HCVcc were pre-incubated with 10 μM POM-12 at room temperature for 1 h, followed by 1000-fold dilution to reach the final concentration of 100 ffu HCVcc and 10 nM POM-12. As the control, 100 ffu JFH1-HCVcc was incubated with 10 nM POM-12 at room temperature for 1 h. The both samples were used to infect Huh7.5.1 cells, and the infection was analyzed by the HCV E2 immunofluorescence at day 3 postinfection. (C) JFH1 virions were incubated with POM-12 and RNase A at 37 °C for 1 h. The HCV RNA was quantified by RT-qPCR. D. Patient sera containing HCV virions of indicated genotypes were incubated with 10 μM of POM-12 and RNase A at 37 °C for 1 h. The HCV RNA was quantified by RT-qPCR.

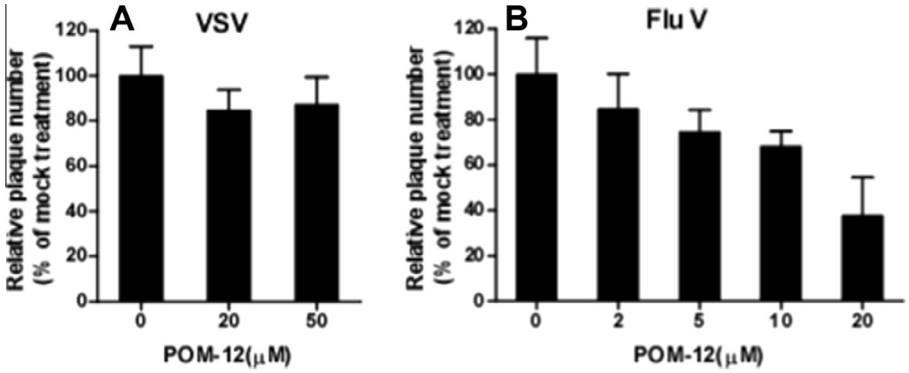

Fig. 6. The inhibitory effect of POM-12 on VSV and influenza virus. VSV (A) and influenza virus (B) were incubated with POM-12 at room temperature for 1 h prior to infection of Huh7.5.1 cells and MDCK cells, respectively. The infection was quantified by plaque assay. Data shown are mean value ± SD of at least three independent experiments.

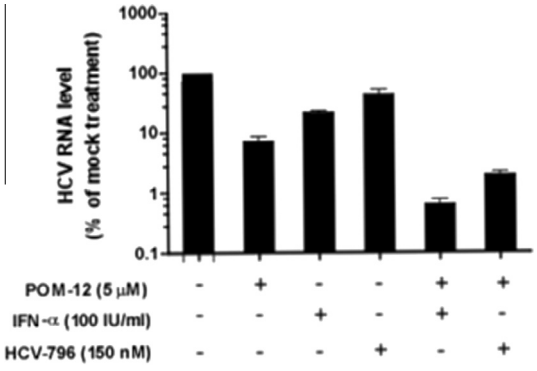

Fig. 7. Synergistic effect of POM-12 with other anti-HCV agents. HCVcc were pretreated with POM-12 and the known anti-HCV agents for 1 h, then inoculated into Huh7.5.1 for 3 days. The HCV RNA was quantified by RT-qPCR.

contrast, POM-12 displayed a weak inhibitory effect on the influenza virus infection (Fig. 6). The EC_{50} of POM-12 on the influenza virus infection was calculated as 16.07 μM, 20 times higher than that on HCV infection (0.8 μM) (Table 1). These results demonstrated that the inhibitory effect of POM-12 is specific to HCV infection.

3.7. Synergistic effect of POM-12 with other anti-HCV agents

Finally we determined the anti-HCV effects of POM-12 in combination with known anti-HCV agent, including IFN-α and HCV-796, an NS5B inhibitor (Howe et al., 2008). As shown in Fig. 7, treatment with either 5 μM POM-12, 100 IU/ml IFN-α or 150 nM HCV-796 reduced HCV RNA levels for 90%, 80% or 50%, respectively. Remarkably, combination of POM-12 with IFN-α and HCV-796 reduced 99% HCV infection, suggesting that combination of POM-12 with IFN-α or HCV-796 could synergistically inhibit HCV infection.

4. Discussion and conclusion

The Keggin-type structures polyoxotungstates have displayed antiviral activity against a variety of enveloped RNA viruses (Shigeta et al., 2003). In addition, there was reported that silicon-containing POM had greater antiviral activity than the other model compound. Herein, we synthesized three kinds of silicon-containing POMs. They all belong to the Keggin-type structures polyoxotungstates and show anti-HCV activity in vitro. We found a polyoxometalate compound POM-12 inhibits HCV infection efficiently in vitro. The EC_{50} of this compound was 0.8 μM on HCVcc infection, while the TC_{50} was 119 μM, indicating that it is a good lead compound for the future development. POM-12 inhibits the pseudotyped HCV infection, and shows the highest inhibitory effect only when the compound and HCV are administrated to the cells at the same time. In consistent with this finding, we found that POM-12 does not reduce the number of HCV-infected cell with an infection focus if the compound is added after the virus inoculation (data not shown), suggesting POM-12 may not inhibit the cell to cell transmission of HCV. Furthermore, the POM-12 treatment makes RNA genome of HCV viral particles either derived from cell culture or patient serum vulnerable for RNase degradation. All these results suggest that POM-12 is a virucidal agent and can directly disrupt virus particles.

Several other HCV entry inhibitors have been identified recently, such as RAFIs (St. Vincent et al., 2010), ladanein (Haid et al., 2012) and EGCG (Calland et al., 2012; Ciesek et al., 2011). These inhibitors either prevent attachment of virus to cells or block virus infection at a post-attachment stage. Different from these entry inhibitors, POM-12 acts directly on viral particles and destabilize the integrity virion structure. Therefore, POM-12 could provide a new alternative for development of therapeutic options.

Interestingly, POM-12 is not merely a detergent to break up the lipid layer of viral envelope as the compound does not inhibit pseudotyped VSVpp, and authentic VSV infection, and has a much weaker inhibitory effect on influenza virus infection, strongly suggesting that POM-12 has certain specificity on HCV infection. Given that POM-12 can inhibit the infection of HCVcc and HCVpp that both consist of HCV envelope proteins on the surface, it is possible that this specificity might be conferred by HCV E1 and E2 glycoproteins. Further investigations are needed to address this interesting question.

In conclusion, we discovered a polyoxometalate compound that shows potent and specific anti-HCV activity but little cytotoxicity. Our data suggest that this lead compound warrants further evaluation as potential agent for use against HCV infections in combination with or as alternatives to ribavirin, or the protection of individuals from the exposure of HCV.

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