Supplementary information on Material and Methods

Cell culture: Primary human hepatocytes were cultured in HCM medium as described before (Kleine et al., 2014) (Lonza; Cologne, Germany; HBM with single aliquots of ascorbic acid, hydrocortisone, transferrin, insulin, gentamycin, BSA and rhEGF). Isolation of human hepatocytes was accomplished by a modified 2-step collagenase perfusion technique. Briefly, liver specimens were obtained after partial hepatectomy, immediately cannulated under sterile conditions and flushed once with 500 mL pre-warmed (37ºC) washing buffer containing 2.5 mM EGTA. Subsequently, perfusion was performed with 100 mL of a pre-warmed (37ºC) digestion buffer containing 0.05% collagenase (Roche; Mannheim, Germany) allowing for recirculation of the perfusate. Upon sufficient digestion, the tissue was mechanically disrupted and the emerging cell suspension poured through a gauze-lined funnel followed by centrifugation (50 g, 5 min, 4ºC). The resulting cell pellet was washed twice using ice-cold PBS (50 g, 5 min, 4ºC) and resuspended in William´s medium E (all Biochrom AG; Berlin, Germany) supplemented with 1 µM insulin, 1 µM dexamethason/fortecortin, 100 U/mL penicillin, 100 µg/mL streptomycin, 1 mM sodium pyruvate, 15 mM HEPES buffer, 4 mM L-glutamine and 5% FCS. Cell number and viability were determined by the Trypan blue exclusion test. Hepatocytes were seeded in collagen pre-coated 6-well plates at a concentration of 1.5 x 10^6 viable cells per well. Sixteen to eighteen hours after plating, culture medium was changed to remove dead and non-adherent cells.

Virus titration by immunohistochemical staining and luciferase assays: Briefly, Huh-7.5 cells were seeded into 96-well plates at a concentration of 1 x 10^5 cells/mL. One day later, these cells were infected with serial dilutions of filtered virus stocks. Three days thereafter, cells were washed with PBS and fixed for 20 minutes with ice-cold methanol at -20°C. The viral protein NS5A was detected with a monoclonal antibody (9E10, 1:1000) diluted in PBS for 1 hour at room temperature. For immunohistochemical staining and detection of bound 9E10, cells were washed and incubated for 1 hour at room temperature with a peroxidase-

conjugated mouse antibody IgG (Sigma-Aldrich; Munich, Germany) diluted at 1:200 in PBS. Peroxidase activity was detected by incubating cells with 0.32% (w/v) of 3-amino-9-ethylcarbazole (Sigma-Aldrich, Munich, Germany) in N,N-dimethylformamide diluted at a ratio of 1:3.3 with 15 mM acetic acid, 35 mM sodium acetate, pH 8.0, and 0.4% H_2O_2. Following incubation for 10 to 30 minutes, the carbazole substrate was removed and water was added to stop the reaction. The 50% tissue culture dose was calculated by the method of Spearman and Kärber. To measure luciferase activity from cells infected with renilla reporter viruses, cells were lysed in passive lysis buffer (Promega, Mannheim, Germany) diluted 1:5 in water. Renilla luciferase activity was measured by adding 100 µL of renilla luciferase substrate in PBS (1 µM of coelenterazin; P.J.K.) to 20 µL cell lysates and measured in a plate luminometer (Lumat LB9507; Berthold; Bad Wildbad, Germany).

2.6 Density Gradient Centrifugation: A iodixanol gradient centrifugation was performed as described recently (Bankwitz et al., 2010; Haid et al., 2009). Briefly, viruses were separated according to their buoyant density by centrifugation through a 0% to 30% iodixanol gradient at 154,000 × g in a TH641 swing-out rotor at 4°C using a Sorvall WX80 centrifuge. After 16 to 18 hours, fractions of 1 mL were collected from the bottom and analyzed for virus infectivity and the density of the respective fraction.

Binding and fusion of R18 labeled viruses: HCV JFH-1 virions were labeled with 0.5 µM R18 as recently described (Colpitts and Schang, 2014; Colpitts et al., 2013). R18-labeled HCV JFH-1 virions were exposed to PGG or vehicle for 10 minutes at 37°C before cooling on ice at 4°C for 10 minutes. Virions were adsorbed onto Huh-7.5 cell monolayers in 24-well plates for 1 hour at 4°C. Cells were washed with ice-cold phosphate-buffered saline. The cells and attached virions were then lysed with 0.1% Triton-X 100. R18 fluorescence was excited at 560 nm and the emitted fluorescence was detected at 590 nm. In order to assess fusion, R18-labeled HCV was exposed to PGG or vehicle for 10 minutes at 37°C, and then incubated on ice for 3 minutes. The exposed virions were then mixed with pre-chilled Huh-7.5 cells in

180 mM 25 Na_2HPO_4, 10 mM citric acid (pH 7.4) fusion buffer and incubated on ice for 1 hour to allow binding but not fusion.

MTT-assay: The MTT-assay was performed as described before (Riss et al., 2004). In brief, medium of the assayed cells (96-well plate) was removed and 50µl of the MTT-substrate was added (1:10 diluted in PBS ; stock solution 5mg/ml). This was incubated at 37°C for 1 hour. The reaction was terminated by addition of 50µl DMSO. The OD 570/630 was measured in a BioTek Synergy 2 ELISA plate reader.

RNA protection assay: Jc1 viruses were incubated with PGG (20 µM) or vehicle control for 4 hours at 37ºC. Impact on RNA was assessed by detection of qRT-PCR following RNA extraction using the kit High Pure Viral RNA Kit (Roche, Mannheim, Germany) and infectivity was assessed by limiting dilution assay. The quality of the extracted RNA was assessed by transfecting it to naïve Huh-7.5 cells by electroporation and measuring RNA levels at 4 and 72 hours post-electroporation. Infectivity levels of the 72 hours post-electroporation samples were determined by limiting dilution assay of supernatants. As a positive control we used heat-inactivated virus.

Time of addition assay: Huh7-Lunet-hCD81 cells were seeded at a concentration of 5.3 x 10^4 cells/well 24 hours before infection with firefly reporter Jc1 at 4ºC for 1 hour. Cells were washed and DMEM was added. Cells were transferred to 37ºC and compounds were added at different time points during infection. PGG (6 µM), heparin (50 µg/mL) or Concanamycin A (ConcaA) (5 nM) were added during binding (4ºC) or after transfer to 37ºC (20, 40, 60, 80, 100, 120, 180 minutes post-transfe). Following 4 hours incubation with each compound, media was changed. After 48 hours, luciferase activity was measured in lysed cells.

Fusion at the plasma membrane assay: Huh-7.5 cells were seeded at a concentration of 3 x 10^5 cells/mL in a 6-well plate. After 24 hours, cell monolayers were treated with ConcaA (5 nM) for 1 hour at 37ºC, before infection with renilla reporter Jc1 in presence of ConcaA. Cell monolayers were washed twice with PBS and incubated with medium containing ConcaA for

1 hour at 37ºC. Subsequently, the monolayers were incubated for 5 minutes at 37ºC with pH 7 or pH 5 citric acid buffer (McIlvaine buffer system). The pH buffers were removed and new media were added to the cells in continuous presence of ConcaA. After 3 hours, medium was changed and infectivity was measured by assessment of reporter activity at 48 hours post infection. In order to assess at which time point PGG acts on, this compound was added throughout infection for 4 hours, during the binding stage for 2 hours, only during the receptor interaction stage for 1 hour at 37ºC, only during the pH shift for 5 minutes or immediately after the pH shift for 3 hours.

Proteinase K (PK) protection assay: Jc1 viruses were incubated with PGG (10 µM) or vehicle control for 4 hours at 37ºC. The virus/compound mixture was divided in three parts. One fraction was treated with PBS, one with 50 µg/mL PK for 1 hour on ice and one was lysed with 2% (v/v) triton X-100 prior to PK treatment. Protease digestion was terminated by addition of 5 mM PMSF (phenylmethylsulfonyl fluoride; AppliChem; Darmstadt, Germany). The amount of core protein was measured by ELISA (Architect Anti-HCV, Abbott; Hannover, Germany).

Inhibition of ZIKV: The Puerto Rico strain of ZIKV was used at an MOI of 1 to infect Vero B4 cells with different concentrations of PGG or solvent control. After 4 hours of infection, cells were washed and DMEM + compound were added to the cells. After 48 hours, supernatant was harvested for detection of viral RNA.

Compounds: ZBDHW was purchased from Lanzhou traditional herbs (Temple city, CA, USA). For extraction, 3 pills were scrambled, resuspended in 1ml DMEM and left at 37°C for 1 hour. The supernatant was filtered through a 0.45µm filter and stored at -20°C. The constituents of ZBDHW (Rhemannia, Phellodendron, Poria, CM, Fr. Corni, Dioscorea, Alismatis orientalis and Anemorrhena) were purchased from a local pharmacy. Extraction of each root/bark was performed accordingly to extraction of the pills (Rhemannia 990 mg,

Phellodendron 240 mg, Poria 495 mg, CM 375 mg, Fr. Corni 375 mg, Dioscorea 495 mg, Alismatis orientalis 375 mg, and Anemorrhena 240 mg).

The CM ingredients bencoic acid, gallic acid, methyl gallate, paeoniflorin, paenol and PGG were purchased from Santa Cruz Biotechnologies. Daclatasvir was purchased from Selleckchen.com (BMS-790052).

References

Bankwitz, D., Steinmann, E., Bitzegeio, J., Ciesek, S., Friesland, M., Herrmann, E., Zeisel, M.B., Baumert, T.F., Keck, Z.Y., Foung, S.K., Pecheur, E.I., Pietschmann, T., 2010. Hepatitis C virus hypervariable region 1 modulates receptor interactions, conceals the CD81 binding site, and protects conserved neutralizing epitopes. Journal of virology 84, 5751-5763.

Colpitts, C.C., Schang, L.M., 2014. A small molecule inhibits virion attachment to heparan sulfate- or sialic acid-containing glycans. J Virol 88, 7806-7817.

Colpitts, C.C., Ustinov, A.V., Epand, R.F., Epand, R.M., Korshun, V.A., Schang, L.M., 2013. 5-(Perylen-3-yl)ethynyl-arabino-uridine (aUY11), an arabino-based rigid amphipathic fusion inhibitor, targets virion envelope lipids to inhibit fusion of influenza virus, hepatitis C virus, and other enveloped viruses. J Virol 87, 3640-3654.

Haid, S., Pietschmann, T., Pecheur, E.I., 2009. Low pH-dependent hepatitis C virus membrane fusion depends on E2 integrity, target lipid composition, and density of virus particles. The Journal of biological chemistry 284, 17657-17667.

Kleine, M., Riemer, M., Krech, T., DeTemple, D., Jager, M.D., Lehner, F., Manns, M.P., Klempnauer, J., Borlak, J., Bektas, H., Vondran, F.W., 2014. Explanted diseased livers - a possible source of metabolic competent primary human hepatocytes. PloS one 9, e101386.

Riss, T.L., Moravec, R.A., Niles, A.L., Duellman, S., Benink, H.A., Worzella, T.J., Minor, L., 2004. Cell Viability Assays, in: Sittampalam, G.S., Coussens, N.P., Brimacombe, K.,

Grossman, A., Arkin, M., Auld, D., Austin, C., Baell, J., Bejcek, B., Chung, T.D.Y., Dahlin, J.L., Devanaryan, V., Foley, T.L., Glicksman, M., Hall, M.D., Hass, J.V., Inglese, J., Iversen, P.W., Kahl, S.D., Kales, S.C., Lal-Nag, M., Li, Z., McGee, J., McManus, O., Riss, T., Trask, O.J., Jr., Weidner, J.R., Xia, M., Xu, X. (Eds.), Assay Guidance Manual, Bethesda (MD).