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Identification of structurally re-engineered rocaglates as inhibitors against hepatitis E virus replication

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ABSTRACT

Hepatitis E virus (HEV) infections are a leading cause of acute viral hepatitis in humans and pose a considerable threat to public health. Current standard of care treatment is limited to the off-label use of nucleoside-analog ribavirin (RBV) and PEGylated interferon-α, both of which are associated with significant side effects and provide limited efficacy.

In the past few years, a promising natural product compound class of eukaryotic initiation factor 4A (eIF4A) inhibitors (translation initiation inhibitors), called rocaglates, were identified as antiviral agents against RNA virus infections. In the present study, we evaluated a total of 205 synthetic rocaglate derivatives from the BU-CMD compound library for their antiviral properties against HEV. At least eleven compounds showed inhibitory activities against the HEV genotype 3 (HEV-3) subgenomic replicon below 30 nM (EC_{50} value) as determined by *Gaussia* luciferase assay. Three amidino-rocaglates (ADRs) (CMLD012073, CMLD012118, and CMLD012612) possessed antiviral activity against HEV with EC_{50} values between 1 and 9 nM. In addition, these three selected compounds inhibited subgenomic replicons of different genotypes (HEV-1 [Sar55], wild boar HEV-3 [83-2] and human HEV-3 [p6]) in a dose-dependent manner and at low nanomolar concentrations. Furthermore, tested ADRs tend to be better tolerated in primary hepatocytes than hepatoma cancer cell lines and combination treatment of CMLD012118 with RBV and interferon-α (IFN-α) showed that CMLD012118 acts additive to RBV and IFN-α treatment. In conclusion, our results indicate that ADRs, especially CMLD012073, CMLD012118, and CMLD012612 may prove to be potential therapeutic candidates for the treatment of HEV infections and may contribute to the discovery of pan-genotypic inhibitors in the future.

1. Introduction

Hepatitis E virus infections have been recognized as a global health problem in both developing and industrialized regions over the past decade (Nimgaonkar et al., 2018). Every year, an estimated 20 million people become infected with HEV, resulting in 3.3 million acute cases

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and 70,000 deaths annually, making HEV the leading cause for acute viral hepatitis worldwide (World Health Organization (WHO), 2021). HEV is a member of the *Hepeviridae* family and is a quasi-enveloped virus that inherits a 7.2-kilobase (kb) single-stranded, positive-sense RNA genome. To date, eight different genotypes have been identified, including five human-pathogenic genotypes (HEV-1-4, 7). Genotypes 1 (HEV-1) and 2 (HEV-2) solely infect humans and cause water-borne outbreaks in developing countries with poor sanitary and hygiene conditions. Infections with HEV-1 and HEV-2 are generally self-limiting and are not associated with chronic disease. In contrast, HEV-3, HEV-4, and HEV-7 can cause chronic infections in immunocompromised individuals (Nimgaonkar et al., 2018; Wißing et al., 2021). Currently, the European Association of the Study of the Liver (EASL) recommends treatment with off-label drugs such as the nucleoside-analog ribavirin (RBV) and PEGylated interferon-α (IFN-α) in immunocompromised patients in instances when a reduction in administered immunosuppressants fails to clear infection (Dalton et al., 2018). However, these off-label drugs can have severe side effects and are contraindicated in selected patients (i.e. pregnant women) (Kamar et al., 2014; Todt et al., 2018a). Moreover, several reports have recently emerged, indicating treatment failure due to the emergence of single-nucleotide variants (Debing et al., 2014, 2016a, Todt et al., 2016a,b). Evidently, the development of effective and safer drugs for treatment of HEV infections, especially for individuals suffering from chronic infection and pregnant women, is becoming increasingly urgent.

Translation initiation is a key process in viral proliferation. Because RNA viruses do not encode their own translational machinery, they rely on host protein synthesis. In the past, targeting the translation machinery of the host has been extensively studied and proposed as a therapeutic strategy for the treatment of viral infections (Harford, 1995; Montero et al., 2019). One of the most promising targets for inhibition of viral protein synthesis is the eukaryotic initiation factor (eIF) 4F complex (comprised of eIF4A, 4E, and 4G) (Gale et al., 2000; Jan et al., 2016; Taroncher-Oldenburg et al., 2021). Due to a highly structured viral 5′-untranslated region (5′UTR), a large number of RNA viruses require the DEAD-box RNA helicase activity of eIF4A to unwind the viral genome and to allow for the recruitment and scanning of the 43S-preinitiation complexes (43S-PIC) during translation initiation (Toribio et al., 2016). Intriguingly, several studies have reported that inhibition of the eIF4A complex by a class of natural products and their synthetic derivatives termed rocaglates prevents replication of RNA viruses including HEV *in vitro* and *in vivo* (Müller et al., 2018; Nebigil et al., 2020; Obermann et al., 2022; Schulz et al., 2021; Taroncher-Oldenburg et al., 2021; Todt et al., 2018b). The rocaglate chemotype is defined by the cyclopenta[*b*]benzofuran structure and is thought to repress translation initiation mostly by blocking 43S-PIC scanning through the stabilization of eIF4A to 5′ leader mRNA in a ternary complex (Chu et al., 2020; Iwasaki et al., 2016, 2016, 2016; Pelletier and Sonenberg, 2019).

Herein, we screened 205 unique rocaglate derivatives from the BU-CMD collection (Roche et al., 2010; Rodrigo et al., 2012; Wang et al., 2016; Zhang et al., 2019a, 2019b) and characterized their antiviral activity against HEV. We identified three candidates that exhibit pan-genotypic inhibitory properties against HEV replication of HEV-1 and HEV-3: CMLD012073, CMLD012118, and CMLD012612. Treatment of tested ADRs also resulted in improved tolerability in primary porcine hepatocytes compared to hepatoma cells. To assess putative antagonistic or synergistic effects of CMLD012118 treatment with RBV and IFN-α we performed combination treatment experiments and found that CMLD012118 acts additive during combination treatment with RBV and IFN-α. Overall, our results suggest that amidino-rocaglates (ADRs) are pan-genotypic inhibitors of HEV replication and may be considered potential alternatives to current standard of care treatments.

2. Materials and methods

2.1. Compounds and reagents

A total of 205 rocaglate derivatives were provided by the Boston University Center for Molecular Discovery (BU-CMD) as racemic mixtures (Roche et al., 2010; Rodrigo et al., 2012; Wang et al., 2016; Zhang et al., 2019a, 2019b). In the initial screen we also included 63 rocaglates as enantioenriched samples (see also Supplementary Table S1). Notably, the BU-CMD collection also contains multiple copies of selected compounds. Top performing compounds identified were all ADRs which are synthesized using an intercepted retro-Nazarov reaction according to a literature procedure (Zhang et al., 2019a). Compounds were dissolved in neat dimethyl sulfoxide (DMSO) to 2 mM concentration and stored at −80 °C prior to use. Silvestrol was purchased from MedChem Express (Monmouth Junction, NJ, USA) and type I IFN-α was purchased from R&D systems. All compounds were stored and diluted according to manufacturer's recommendations.

2.2. Cell culture

Human liver hepatoma cells HepG2 were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Karlsruhe, Germany) supplemented with 10% FCS (GE Healthcare), 100 μg/mL of streptomycin, 100 IU/mL of penicillin (Invitrogen), 2 mM L-glutamine and 1% nonessential amino acids (Invitrogen) (DMEM complete) at 37 °C in a 5% (vol/vol) CO_2 incubator. Primary porcine hepatocytes (PPHs) were isolated from liver specimens by perfusion technique as previously described (Fráguas-Eggenschwiler et al., 2021). Hepatocytes were plated at a density of 3×10^5 on collagen-precoated 24-well plates, and kept in hepatocyte culture medium (Williams medium E; Invitrogen) supplemented with 5% FCS (GE Healthcare), 100 μg/mL of streptomycin, 100 IU/mL of penicillin (Invitrogen), 1% GlutaMAX (Invitrogen), 2% nonessential amino acids (Invitrogen), 10 mM HEPES buffer (Invitrogen), 2% Dimethylsulfoxid HybriMax (Merck), 5 μg/mL insulin (Sigma-Aldrich), 5.41 μM hydrocortisone, 5.5 ng/mL epidermal growth factor (EGF) (Med Chem Express).

2.3. Plasmids and in vitro transcription

Three assembly-deficient subgenomic HEV replicons which encode a *Gaussia* luciferase reporter gene were used to generate T7 polymerase-based HEV *in vitro* transcripts as previously described (Drave et al., 2016; Todt et al., 2020). Subgenomic *Gaussia* luciferase-encoding plasmid constructs of HEV-3 Kernow-C1 p6 strain was kindly provided by Shukla et al. (Shukla et al., 2011, 2012) and HEV-3 replicon derived from the G3-HEV83-2-27 virus (HEV-3; GenBank accession no. AB740232) was a kind gift from the laboratory of Takaji Wakita. In addition, a Sar55/S17 (HEV-1, based on clone pSK-E2, GenBank accession no. AF444002) *Gaussia* luciferase-coupled construct was used to test for pan-genotypic inhibition of HEV (Nguyen et al., 2014).

2.4. Screening of rocaglate derivatives and dose-response assays

To perform the rocaglate derivative screening and dose-response assay, capped RNA was delivered to HepG2 cells by electroporation according to Koutsoudakis et al. (2006). In brief, 5×10^6 cells were resuspended in 400 μL Cytomix containing 2 mM ATP and 5 mM glutathione and mixed with 5 μg of the respective *in vitro* transcribed HEV RNA. Cells were electroporated with the Gene Pulser Xcell™ apparatus (Bio-Rad) and seeded at a density of 20,000 cells/well in a 96-well format. To screen rocaglate derivatives, cells were incubated in the presence of 25 nM compound while cells used for dose-response assay were treated with triplicate 2-fold serial dilutions of drugs at concentrations ranging from 0.39 to 100 nM. Supernatants were sampled 24 h and 48 h post-treatment and stored at 4 °C until

luminometer reading.

2.5. Gaussia luciferase assay

Gaussia luciferase activity was measured by adding 20 μL of harvested cell culture supernatant per well on a 96-well LUMITRAC 600 plate, followed by the addition of Coelenterazine substrate and the detection of luminescence using a Centro XS^3 LB 960 luminometer (Berthold Technologies). The microplate reader was set to dispense 50 μL of substrate, followed by shaking for 2 s and reading for 5 s. Samples were assayed in triplicate and read sequentially.

2.6. MTT assay

Cell viability was determined by adding 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) substrate to cells and subsequent incubation at 37 °C and 5% CO_2 for 1–2 h. Medium was removed and DMSO was added to each well. The absorbance of each well was read on a microplate absorbance reader (Tecan) at 570 nm. Cells treated with 70% ethanol for 10 min served as background control.

2.7. Toxicity assay in primary and hepatoma cells

PPHs and HepG2 cells were treated with duplicate 5-fold serial dilutions of CMLD012118, CMLD012073 and CMLD012612 at concentrations ranging from 0.032 to 100 nM. Cell viability was determined by MTT assay 24 h, 48 h and 72 h post treatment (see 2.6).

2.8. Statistical analysis

Dose-dependent inhibition of replication was plotted and adjusted to a non-linear regression model using GraphPad Prism v9.3.1 for Windows (La Jolla, California, USA, www.graphpad.com). EC_{50}, and CC_{50} were calculated using the four-parameter log-logistic model.

3. Results

3.1. Screening a rocaglate derivative library for antiviral activity against HEV

Recently, we identified one of the most well-characterized, naturally-occurring rocaglates, silvestrol, as a pan-genotypic inhibitor of HEV replication *in vitro* and *in vivo* (Todt et al., 2018b). However, silvestrol treatment is hampered by its limited production in nature, challenging synthetic production, poor oral bioavailability, and toxicity in larger animals (Agarwal et al., 2021). To identify rocaglates with improved properties for patient treatment, we screened a compound library consisting of 205 synthetic rocaglates for antiviral activity against HEV. We first transfected HepG2 cells with HEV-3 replicon p6-Gluc (ORF2 region is partially replaced by a *Gaussian* luciferase) (Shukla et al., 2012) (Fig. 1A). Subsequently, each rocaglate derivative was added at a final concentration of 25 nM. At 24 h and 48 h post-treatment, HEV replication and cell viability were assessed in both luciferase and MTT assays, respectively. Screening results are shown in Fig. 1B as scatter plots after data normalization to DMSO (vehicle control). Using the BU-CMD library, we identified 50 compounds that inhibited HEV-3 replication by at least 50% 24 h post-treatment (below the horizontal dashed black line in the left panel, Fig. 1B; Table S1). From these 50 compounds, 36 derivatives reduced cell viability less than 70%. At 48 h post-treatment, a total of 42 compounds reduced HEV-3 replication below 30% (below the horizontal dashed black line in the right panel, Figure 1B), 18 of which reduced cell viability by as much as 50%. As expected, silvestrol (orange dots), an established, naturally-occurring rocaglate and known inhibitor of HEV replication, inhibited HEV replication at 25 nM (RLU 19.0 ± 3.3% [24 h], RLU 11.4 ± 2.5 [48 h]). For further analysis we selected 7 compounds (purple dots) that fulfilled selection criteria set for both 24 h and 48 h (Fig. 1C).

3.2. Dose-response profiles of selected rocaglate derivatives on HEVp6-Gluc replicon

Since several compounds strongly reduced replication of HEV-3 at 25 nM, the efficacies of 7 selected derivatives (purple dots; Fig. 1C) were further assessed by determining the half-maximum inhibitory concentrations (EC_{50}), half-maximum cytotoxic concentration (CC_{50}) and selectivity indices (SI) (Fig. 2, Table S3).

In addition, given the predominance of racemic ADR esters among the 7 selected derivatives, we also opted to include several additional ADR esters and hydroxamates as racemic mixtures for dose-response studies. ADR hydroxamates such as CMLD012612 were recently found to be among the most potent rocaglate translation inhibitors (Chu et al., 2019) characterized to-date. In addition to CMLD012612, ADRs CMLD012073, and CMLD012072 were also included. Lastly, we included the racemic rocaglate hydroxamate CMLD011880 (aka SDS-1-021, also a highly potent translation inhibitor) into our analysis. Compound structures and identifiers are summarized in Supplementary Tables S1 and S2. In the dose-response assay, the EC_{50} of these rocaglate/ADR derivatives were found to range between 1.1 nM and 28.4 nM (Fig. 2A), whereas CC_{50} values ranged between 18 and > 100 nM, resulting in SI values of 3.8 to >100 at 24 h post treatment. EC_{50}, CC_{50} and SI values determined at 48 h post-treatment were overall reduced and found to be between 1 and 24.8 nM, 3.7 and 77.9 nM and 1.9–14.1, respectively (Fig. 2B), suggesting suppression of HEVp6 replication in a dose-dependent manner with low EC_{50} values. Notably, longer incubation times with all rocaglate derivatives reduced cell viability.

Furthermore, we found robust inhibition of HEV-3 replication by two racemic ADRs at both 24 h and 48 h after treatment (CMLD012612 [EC_{50} = 1.0 nM at 24 h, = 1.0 nM at 48 h] and CMLD012073 [EC_{50} = 2.0 nM at 24 h, ~1.5 nM at 48 h]). While cytotoxicity was comparably low at 24 h (CMLD012612 [CC_{50} = 97.0 nM], CMLD012073 [CC_{50} = 97.1 nM]) cell viability significantly decreased at 48 h post treatment (CMLD012612 [CC_{50} = 11.7 nM], CMLD012073 [CC_{50}, = 19.7 nM]). Interestingly, treatment with CMLD012118, a racemic guanidinyl (N-substituted) ADR resulted in modest antiviral activity HEV-3 (with SI = 12) at 24 h, which decreased marginally 48 h after treatment (SI = 8.9). Based on the promising SI, we selected CMLD012612, CMLD012073, and CMLD012118 for further analysis (Fig. 2; black arrows).

3.3. Pan-genotypic inhibition of HEV by CMLD012073, CMLD012118, and CMLD012612

Given the fact that HEV is a highly heterogenous virus with a broad host range, the development of pan-genotypic antiviral therapeutics targeting HEV is beneficial to contain the spread of future emerging and re-emerging HEV outbreaks. To assess the antiviral potency of CMLD012073 (Fig. 3A), CMLD012118 (Fig. 3B) and CMLD012612 (Fig. 3C) on different HEV genotypes, we treated HepG2 cells transfected with replicons representing HEV-3-based wild boar isolate 83-2-27 (Shiota et al., 2013), human derived HEV-3 p6 and the HEV-1 strain Sar55 (harboring an S17 insertion in the ORF1 like the p6 strain) (Emerson et al., 2004; Shukla et al., 2012) with increasing concentrations of ADRs for 24 h and 48 h. The three ADRs were effective against all tested replicons at low nanomolar concentrations (Fig. 3, Table 1), albeit HEVSar55/S17 (orange curve) replication was inhibited even more efficiently compared to HEV-3 replication (EC_{50} = 0.40–4.57 nM for HEVSar55/S17 vs. EC_{50} = 1.14–14.80 nM for HEV-3 replicons). Apart from this, inhibition of HEV-3 HEVp6 (dark purple) was similar to that of HEV-3 HEV83-2 (light purple) for tested ADRs, indicated by almost identical sigmoidal dose-response curve profiles and similar EC_{50} ranges (1.14 nM–8.79 nM for HEVp6 and 1.20 nM–14.8 nM for HEV83-2). Furthermore, for compounds CMLD012073 and

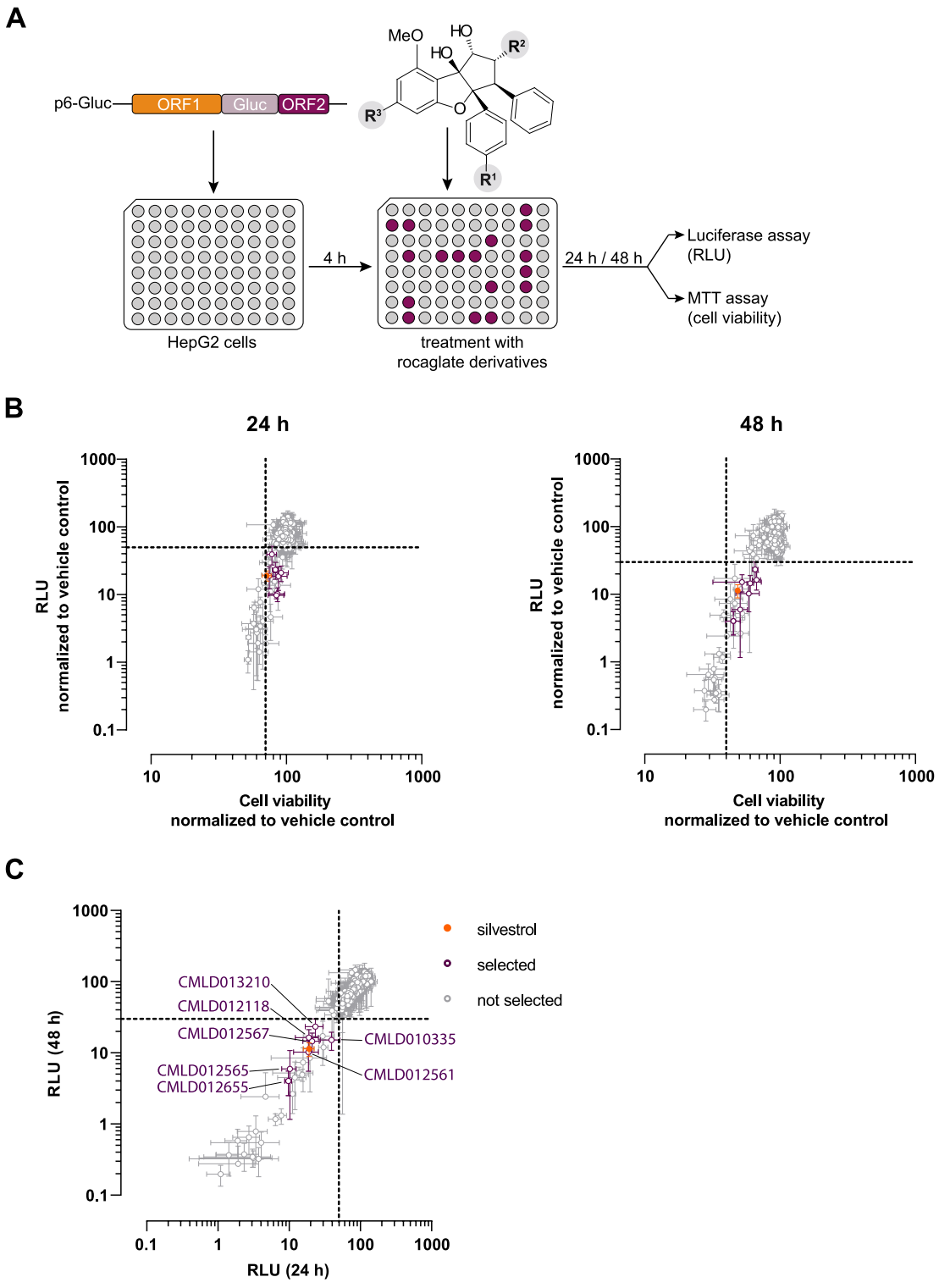

Fig. 1. Screening of rocaglate derivatives using HEV subgenomic replicon. (**A**) Schematic representation of assay setup: HEVp6-Gluc was delivered to HepG2 cells via electroporation. Four hours after the transfection, cells were treated with 25 nM of each rocaglate derivative. (**B**) Supernatants were sampled after 24 h and 48 h HEVp6 replication was measured via reporter luciferase read-out (relative light units [RLU]) and normalized to the respective vehicle treated control (DMSO) while cell viability was monitored via MTT assay. Silvestrol (orange dots) was included as a reference. Candidates selected for downstream analysis are highlighted as purple dots. (**C**) The screening provided a total of 7 candidates (purple dots) with antiviral properties against HEV and were selected based on fulfillment of both selection criteria at 24 h and 48 h for further analysis. Derivatives excluded from further analysis are represented as grey dots. Error bars indicate standard error of the mean, n = 3.

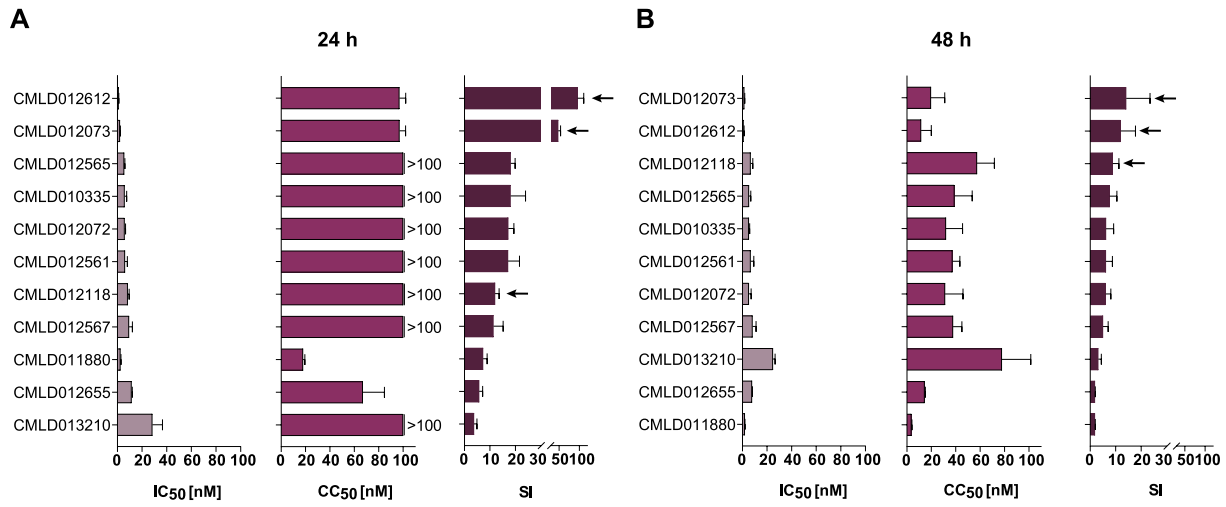

Fig. 2. Comparison of EC_{50}, CC_{50} and SI of eleven racemic rocaglates against HEVp6 replication. HepG2 cells were transfected with HEVp6-Gluc replicon and treated with drugs at concentrations ranging from 0.39 nM to 100 nM for (**A**) 24 h and (**B**) 48 h. Dose-response curves of eleven derivatives were adjusted to a non-linear fit regression model and calculated with a four-parameter logistic curve from three experiments with three replicates. For each derivative EC_{50}, CC_{50} and SI values are plotted and ordered according to SI values. Based on selective indices at 48 h, 3 derivatives (black arrows) were selected to test for pan-genotypic inhibition.

CMLD012612, we observed a shift of the CC_{50} value from 65.76 nM to 10.46 nM (CMLD012073) and 43.37 nM–5.43 nM (CMLD012612) from 24 h to 48 h (Fig. 3A and C black curves; Table 1), thus indicating decreased cell viability at 48 h post treatment. Interestingly, CC_{50} values determined for treatment with CMLD012118 did marginally decrease at 48h from >100 nM at 24 h to CC_{50} levels observed for CMLD012612 at 24 h (45.57 nM for CMLD012118 vs. 43.37 nM for CMLD012612), suggesting an improved safety profile for CMLD012118. Taken together, all three ADRs suppressed HEV replication of HEV strains of genotype 1 and 3 *in vitro*, while treatment with CMLD012118 maintained cell viability most efficiently.

3.4. Determining cell toxicity in primary porcine hepatocytes and hepatoma cells

To dissect whether the decrease of cell viability observed in HepG2 cells treated with CMLD012118, CMLD012073 and CMLD012612 is limited to cancer cells, we evaluated cytotoxicity in natural target cells for HEV-3. To this end, we treated primary porcine hepatocytes (PPHs) with up to 100 nM of ADRs for 24 h, 48 h and 72 h and determined cell viability via MTT assay (Fig. 4). Overall, cell toxicity increased over time in CMLD012073 (Fig. 4A), CMLD012118 (Fig. 4B) and CMLD012612 (Fig. 4C) treated hepatoma cells (straight lines) and PPHs (dotted lines). Nonetheless, compared to HepG2 cells, cell viability was higher in PPHs in the presence of ADRs, indicating improved tolerability of ADRs in primary liver cells.

3.5. Combination treatment of CMLD012118 with RBV and silvestrol

To test whether combination treatment with RBV (current treatment of choice for HEV infections) or IFN-α offers increased antiviral efficacy or even antagonized the antiviral effects of ADRs, we subjected CMLD012118 to combination treatment with RBV and IFN-α based on its superior safety profile. Depicted in Fig. 5 is a three-dimensional surface plot constructed according to the Bliss independence model (Prichard and Shipman, 1990), representing deviations from expected interactions for combination treatment with RBV and IFN-α.

Herein, we observed no decrease or increase below/above the plane for treatment with RBV or IFN-α for both 24 h and 48 h, suggesting an additive effect. Similarly, we noticed an additive effect on the inhibition of HEVp6-Gluc replication by co-treatment of CMLD012118 and silvestrol (shares the cyclopenta[*b*]benzofuran core structure with rocaglates) for 48 h (Fig. S1).

4. Discussion

Although RBV and PEGylated interferon-α are currently approved for the management of HEV-related viral hepatitis, HEV infections remain to be associated with morbidity and persistent chronicity. Notably, side effects and resistance to current medications have been reported, warranting future development of novel therapeutics (Debing et al., 2016b; Todt et al., 2016a, 2018a). HEV, like several other RNA viruses contains a highly structured 5′-UTR and relies on the interaction with the eukaryotic translation machinery for viral protein synthesis. Indeed, a recent investigation on the involvement of eukaryotic translation initiation factor (DEAD-box helicase) 4A (eIF4A), 4G (eIF4G) and 4E (eIF4E) in HEV replication, found that efficient replication of HEV requires the cap-dependent translation machinery of host cells (Zhou et al., 2015), suggesting eIF4A as a putative target for antiviral therapy. So far, numerous *in vitro* and *in vivo* studies have investigated the antiviral activity of natural and synthetic rocaglates against a broad range of RNA viruses including Ebola, Chikungunya, several coronaviruses (i.e. MERS-CoV, SARS-CoV, SARS-CoV-2, HCoV-229E), Zika, Lassa virus, hepatitis C virus and HEV (Biedenkopf et al., 2017; Elgner et al., 2018; Glitscher et al., 2018; Henss et al., 2018; Liu et al., 2015; Müller et al., 2018, 2020, 2021; Obermann et al., 2022; Todt et al., 2018b; Zhang et al., 2019b).

To identify novel synthetic rocaglates as potential antiviral agents against HEV, we assessed the antiviral activity of 205 racemic rocaglate-derivatives (+63 enantioenriched samples) from the BU-CMD collection (Chu et al., 2019; Zhang et al., 2019a). Inhibition of HEV replication by rocaglates was evaluated in a primary screen using state-of-the-art replicon systems, followed by confirmation of 'hit' compounds in dose–response assays. The screening identified at least 7 rocaglate derivatives that reduced the expression of *Gaussia* luciferase from a HEV-3 HEVp6 subgenomic replicon by at least 50%. Performing dose-response assays of selected compounds, we have identified three ADRs, CMLD012073, CMLD012612, and CMLD012118 as inhibitors of HEVp6 HEV-3 and wild-boar derived HEV-3 HEV83-2 replication. Strikingly, these ADRs were most effective against HEV-1 HEVSar55/S17. However, it should be noted, that HEVSar55/S17 HEV-1 replicon generally tends to replicate less efficient than HEV-3 replicons in cell culture.

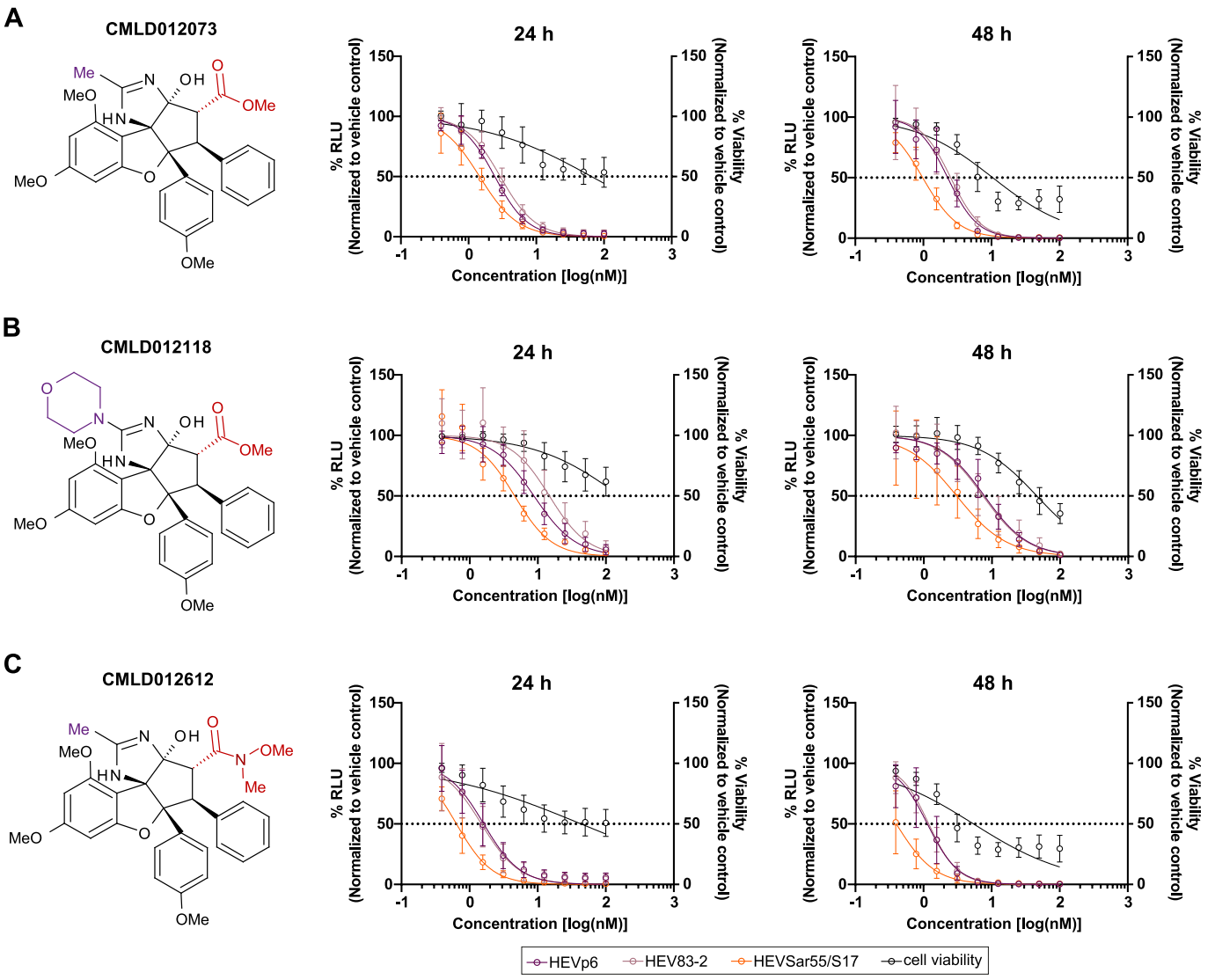

Fig. 3. Pan-genotypic inhibition of HEV replication by CMLD012073, CMLD012118, and CMLD012612. HEV subgenomic replicons HEVp6-Gluc, HEV83-2-Gluc, HEVSar55/S17-Fluc were electroporated into HepG2 cells. Four hours post transfection, cells were treated with (**A**) CMLD012073, (**B**) CMLD012118 and (**C**) CMLD012612 at concentrations ranging from 0.39 nM to 100 nM for 24 h and 48 h. Depicted are non-linear fit response curves representative of three experiments with three replicates for HEVp6 (dark purple lines), HEV83-2 (bright purple lines) and Sar55/S17 (orange lines). Cell viability was monitored by MTT assay (black lines). EC_{50} and CC_{50} were calculated using GraphPad Prism 8 software. Error bars indicate standard error of the mean, n = 3.

Table 1
EC_{50} and CC_{50} values of CMLD012073, CMLD012118 and CMLD012612 against different HEV genotype replicons.

	CMLD012073 (nM)		CMLD012118 (nM)		CMLD012612 (nM)	
	EC_{50}	CC_{50}	EC_{50}	CC_{50}	EC_{50}	CC_{50}
	24 h	65.76		>100		43.37
HEVp6	2.45		8.79		1.62	
HEV83-2	2.95		14.80		1.49	
HEVSar55/S17	1.46		4.57		0.64	
	48 h	10.46		45.57		5.43
HEVp6	2.28		7.76		1.14	
HEV83-2	2.52		7.41		1.20	
HEVSar55/S17	0.98		3.05		0.40	

Nevertheless, we did not observe increased silvestrol sensitivity for HEVSar55/S17 in our previous study (Todt et al., 2018b), thus determining genotype-dependent ADRs sensitivity remains to be investigated.

CMLD012073, CMLD012612, and CMLD012118 have recently been synthesized by intercepted retro-Nazarov reactions and have shown improved eIF4A-dependent translation inhibition compared to previous described rocaglates (i.e. CR-1-31-B (−)) in an *in vitro* bicistronic reporter assay (Zhang et al., 2019a). We found CMLD012612 to be most effective against HEV replication, followed closely by CMLD012073. However, treatment with CMLD012612 and CMLD012073 also resulted in considerable cytotoxicity 48 h post treatment. These observations are most probably owed to the fact that the hydroxamate moiety of CMLD012612 (Fig. 3, red) is a more optimal hydrogen-bond acceptor to Gln195 of eIF4A than the corresponding methyl ester substitutions of CMLD012073 and CMLD012118 (Zhang et al., 2019a). In addition, structure-activity relationship analysis revealed that cytotoxicity increased in NIH/3T3 cells with decreasing size and rigidity of the imidazoline substituent (Fig. 3; purple), (Chu et al., 2019). Hence, ADRs such as CMLD012073 and CMLD012612 that harbor a methyl group at the imidazoline moiety might also display increased cytotoxicity in HepG2 cells compared to CMLD012118 bearing a large and rigid morpholine functional group.

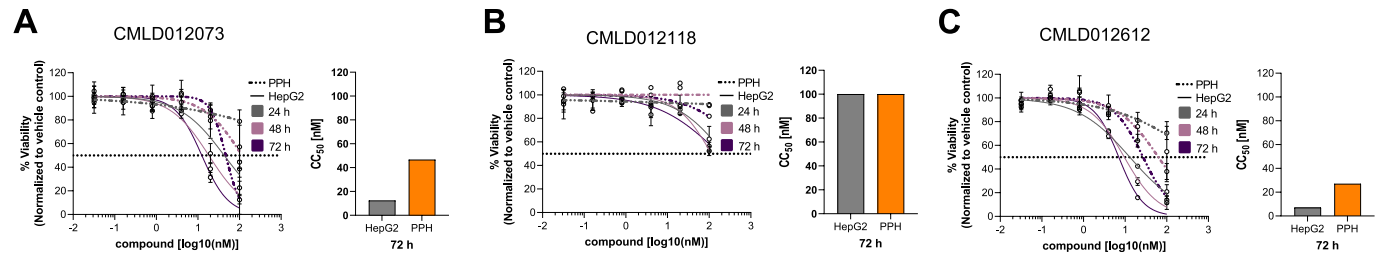

Fig. 4. Cell viability of amidino-rocaglate treated primary and hepatoma cells. PPHs and HepG2 cells were treated with (**A**) CMLD012073, (**B**) CMLD012118 and (**C**) CMLD012612 at concentrations ranging from 0.032 nM to 100 nM for 24 h (grey lines), 48 h (bright purple lines) and 72 h (dark purple lines). Depicted are non-linear fit response curves representative of two experiments with two replicates. Cell viability was monitored by MTT assay. CC_{50} values were calculated using GraphPad Prism 8 software and 72 h values were plotted. n = 2 (except CMLD012118 treatment; n = 1).

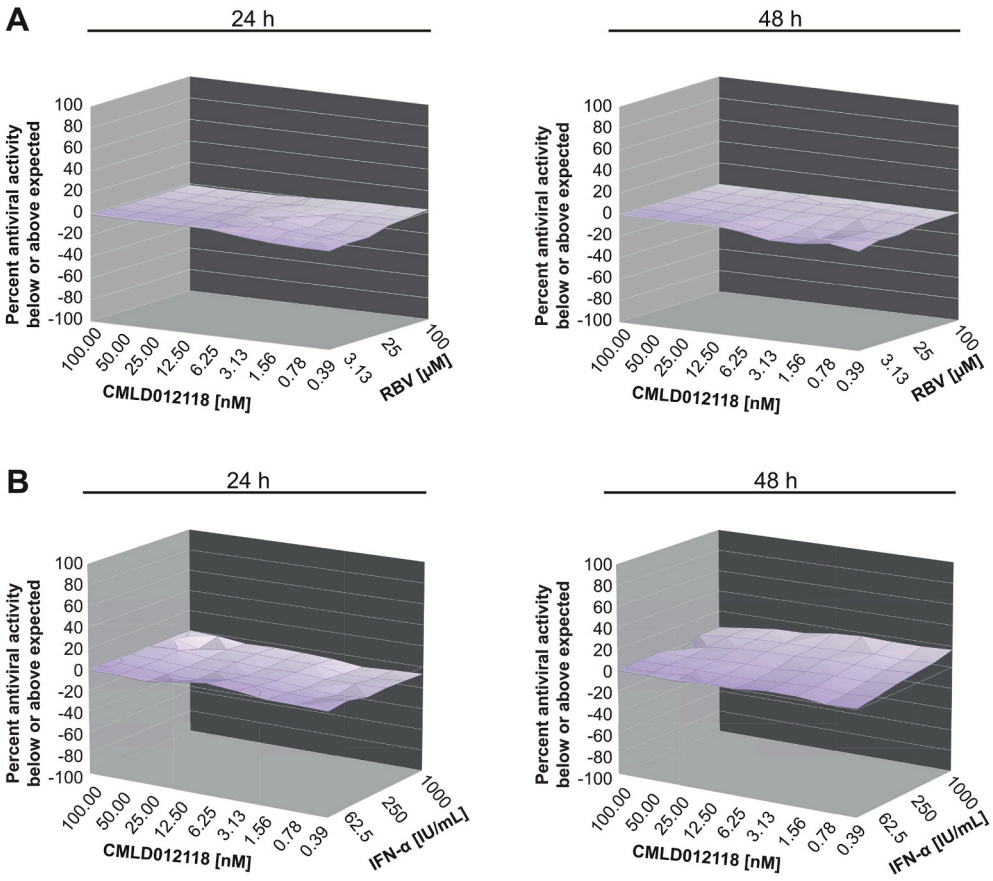

Fig. 5. Combination treatment of CMLD012118 and RBV on HEV replication. Representation the dose-dependent inhibition of transfected HEV replicons after RBV/IFN-α combination treatment with CMLD012118 for 24h and 48 h. Compounds were mixed at different ratios (CMLD012118: 0.39–100 nM, RBV: 3.13–100 µM; IFN: 31.25–1000 IU/mL). HEV replication was measured via reporter luciferase and normalized to DMSO treated control. Data were analyzed according to the Bliss independence model (Prichard and Shipman, 1990) and plotted as three-dimensional differential surface plots based on three technical replicates. Note that synergistic drug interactions appear as a peak above the plane. Conversely, antagonistic interactions appear as a pit in the plane with a negative value.

As rocaglates are known for their cancer-inhibiting properties, we hypothesized that the cytotoxic effects observed in human hepatoma cells might be restricted to cancer cells and may not be found in primary liver cells. To test this, we treated PPHs with increasing concentrations of ADRs and observed that cell toxicity was reduced in primary porcine hepatocytes compared to hepatoma cancer cells treated with ADRs, suggesting improved tolerability in primary cells. In accordance with these results, previous studies found rocaglates to selectively target cancer cells without being toxic to primary cells or animals (Wolfe et al., 2014) and that inhibition by rocaglates is generally limited to proto-oncogenic mRNAs (Chan et al., 2019; Rubio et al., 2014). At the same time, studies by Wolfe et al. and Chan et al. also suggest minimal or no toxicities for long-term dosing regimens in mice intraperitoneally injected with (−)-CR-1-31-B (CMLD010513) (Chan et al., 2019; Wolfe et al., 2014), emphasizing the fact that cytotoxic effects observed in cancer cells might not occur in primary cells or animals. Furthermore, the fact that zotatifin, a synthetic rocaglate (eFFECTOR Therapeutics) is currently in phase 1/2 clinical trial against advanced solid tumors and has recently entered a phase 1 clinical study to evaluate its safety and efficacy for adults with mild or moderate COVID-19 infection (ClinicalTrials.gov, 2021), is highly encouraging for a potential antiviral application of rocaglates in humans. Moreover, additional derivatization of the guanidine and carboxylate moieties in CMLD012118 may further reduce putative toxic side effects and improve safety profiles. Nevertheless, whether CMLD012073, CMLD012612 and especially CMLD012118 are safe for the treatment of HEV *in vivo* has yet to be established.

While the ADRs identified here represent potential therapeutic leads, their chemical synthesis remains challenging due to their complex structures (Zhang et al., 2019a). At the same time, supply of natural rocaglates is often limited by extracting from leaves, twigs and roots of the *Aglaia* tree. Therefore, advances in large-scale chemical synthesis or extraction of rocaglates need to be pursued further to make rocaglates suitable for clinical applications.

Finally, we investigated whether combination treatment of CMLD012118 with RBV results in combinatory effects. Collectively, our data suggests an additive effect for RBV and IFN-α when combined with CMLD012118. These results are consistent with previous combination treatment experiments with silvestrol and RBV which also indicated additive effects of RBV/silvestrol co-treatment (Todt et al., 2018b).

5. Conclusions

This study demonstrates for the first time that synthetic ADRs are putative candidates for the treatment of HEV infections. Host-targeting antiviral (HTAs) drugs that target the host cell machinery have great potential as broad-spectrum pan-antivirals and reduce the likelihood for resistance development, making rocaglates putative candidates for HEV treatment. Despite the observation that ADRs inhibit HEV replication *in vitro* at low nanomolar concentration, determination of potential toxicity *in vivo* as well as the development of large-scale synthesis procedures are still necessary to establish ADRs as a potential antiviral HEV treatment option.

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Data availability statement

The data presented in this study are available upon request from the corresponding author.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2022.105359.

References

Agarwal, G., Chang, L.-S., Soejarto, D.D., Kinghorn, A.D., 2021. Update on phytochemical and biological studies on rocaglate derivatives from Aglaia species. Planta Med. 87, 937–948. https://doi.org/10.1055/a-1401-9562.

Biedenkopf, N., Lange-Grünweller, K., Schulte, F.W., Weißer, A., Müller, C., Becker, D., Becker, S., Hartmann, R.K., Grünweller, A., 2017. The natural compound silvestrol is a potent inhibitor of Ebola virus replication. Antivir. Res. 137, 76–81. https://doi.org/10.1016/j.antiviral.2016.11.011.

Chan, K., Robert, F., Oertlin, C., Kapeller-Libermann, D., Avizonis, D., Gutierrez, J., Handly-Santana, A., Doubrovin, M., Park, J., Schoepfer, C., Da Silva, B., Yao, M., Gorton, F., Shi, J., Thomas, C.J., Brown, L.E., Porco, J.A., Pollak, M., Larsson, O., Pelletier, J., Chio, I.I.C., 2019. eIF4A supports an oncogenic translation program in pancreatic ductal adenocarcinoma. Nat. Commun. 10, 5151. https://doi.org/10.1038/s41467-019-13086-5.

Chu, J., Zhang, W., Cencic, R., Devine, W.G., Beglov, D., Henkel, T., Brown, L.E., Vajda, S., Porco, J.A., Pelletier, J., 2019. Amidino-rocaglates: a potent class of eIF4A inhibitors. Cell Chemical Biology 26, 1586–1593. https://doi.org/10.1016/j.chembiol.2019.08.008 e3.

Chu, J., Zhang, W., Cencic, R., O'Connor, P.B.F., Robert, F., Devine, W.G., Selznick, A., Henkel, T., Merrick, W.C., Brown, L.E., Baranov, P.V., Porco, J.A., Pelletier, J., 2020. Rocaglates induce gain-of-function alterations to eIF4A and eIF4F. Cell Rep. 30, 2481–2488. https://doi.org/10.1016/j.celrep.2020.02.002 e5.

ClinicalTrials.gov, 2021. NTC04632381.

Dalton, H.R., Kamar, N., Baylis, S.A., Moradpour, D., Wedemeyer, H., Negro, F., 2018. EASL clinical practice guidelines on hepatitis E virus infection. J. Hepatol. 68, 1256–1271. https://doi.org/10.1016/j.jhep.2018.03.005.

Debing, Y., Gisa, A., Dallmeier, K., Pischke, S., Bremer, B., Manns, M., Wedemeyer, H., Suneetha, P.V., Neyts, J., 2014. A mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. Gastroenterology 147, 1008–1011. https://doi.org/10.1053/j.gastro.2014.08.040 e7.

Debing, Y., Moradpour, D., Neyts, J., Gouttenoire, J., 2016a. Update on hepatitis E virology: implications for clinical practice. J. Hepatol. 65, 200–212. https://doi.org/10.1016/j.jhep.2016.02.045.

Debing, Y., Ramière, C., Dallmeier, K., Piorkowski, G., Trabaud, M.-A., Lebossé, F., Scholtès, C., Roche, M., Legras-Lachuer, C., de Lamballerie, X., André, P., Neyts, J., 2016b. Hepatitis E virus mutations associated with ribavirin treatment failure result in altered viral fitness and ribavirin sensitivity. J. Hepatol. 65, 499–508. https://doi.org/10.1016/j.jhep.2016.05.002.

Drave, S.A., Debing, Y., Walter, S., Todt, D., Engelmann, M., Friesland, M., Wedemeyer, H., Neyts, J., Behrendt, P., Steinmann, E., 2016. Extra-hepatic replication and infection of hepatitis E virus in neuronal-derived cells. J. Viral Hepat. 23, 512–521. https://doi.org/10.1111/jvh.12515.

Elgner, F., Sabino, C., Basic, M., Ploen, D., Grünweller, A., Hildt, E., 2018. Inhibition of Zika virus replication by silvestrol. Viruses 10, 149. https://doi.org/10.3390/v10040149.

Emerson, S.U., Nguyen, H., Graff, J., Stephany, D.A., Brockington, A., Purcell, R.H., 2004. In vitro replication of hepatitis E virus (HEV) genomes and of an HEV replicon expressing green fluorescent protein. J. Virol. 78, 4838–4846. https://doi.org/10.1128/jvi.78.9.4838-4846.2004.

Fráguas-Eggenschwiler, M., Eggenschwiler, R., Söllner, J.-H., Cortnumme, L., Vondran, F.W.R., Cantz, T., Ott, M., Niemann, H., 2021. Direct conversion of porcine primary fibroblasts into hepatocyte-like cells. Sci. Rep. 11, 9334. https://doi.org/10.1038/s41598-021-88727-1.

Gale, M., Tan, S.-L., Katze, M.G., 2000. Translational control of viral gene expression in eukaryotes. Microbiol. Mol. Biol. Rev. 64, 239–280. https://doi.org/10.1128/MMBR.64.2.239-280.2000.

Glitscher, M., Himmelsbach, K., Woytinek, K., Johne, R., Reuter, A., Spiric, J., Schwaben, L., Grünweller, A., Hildt, E., 2018. Inhibition of hepatitis E virus spread by the natural compound silvestrol. Viruses 10, 301. https://doi.org/10.3390/v10060301.

Harford, J.B., 1995. Translation-targeted Therapeutics for Viral Diseases.

Henss, L., Scholz, T., Grünweller, A., Schnierle, B., 2018. Silvestrol inhibits Chikungunya virus replication. Viruses 10, 592. https://doi.org/10.3390/v10110592.

Iwasaki, S., Floor, S.N., Ingolia, N.T., 2016. Rocaglates convert DEAD-box protein eIF4A into a sequence-selective translational repressor. Nature 534, 558–561. https://doi.org/10.1038/nature17978.

Jan, E., Mohr, I., Walsh, D., 2016. A cap-to-tail guide to mRNA translation strategies in virus-infected cells. Annu. Rev. Virol. 3, 283–307. https://doi.org/10.1146/annurev-virology-100114-055014.

Kamar, N., Izopet, J., Tripon, S., Bismuth, M., Hillaire, S., Dumortier, J., Radenne, S., Coilly, A., Garrigue, V., D'Alteroche, L., Buchler, M., Couzi, L., Lebray, P., Dharancy, S., Minello, A., Hourmant, M., Roque-Afonso, A.-M., Abravanel, F., Pol, S., Rostaing, L., Mallet, V., 2014. Ribavirin for chronic hepatitis E virus infection in transplant recipients. N. Engl. J. Med. 370, 1111–1120. https://doi.org/10.1056/NEJMoa1215246.

Koutsoudakis, G., Kaul, A., Steinmann, E., Kallis, S., Lohmann, V., Pietschmann, T., Bartenschlager, R., 2006. Characterization of the early steps of hepatitis C virus infection by using luciferase reporter viruses. J. Virol. 80, 5308–5320. https://doi.org/10.1128/JVI.02460-05.

Liu, S., Wang, W., Brown, L.E., Qiu, C., Lajkiewicz, N., Zhao, T., Zhou, J., Porco, J.A., Wang, T.T., 2015. A novel class of small molecule compounds that inhibit hepatitis C virus infection by targeting the prohibitin-CRaf pathway. EBioMedicine 2, 1600–1606. https://doi.org/10.1016/j.ebiom.2015.09.018.

Montero, H., Pérez-Gil, G., Sampieri, C.L., 2019. Eukaryotic initiation factor 4A (eIF4A) during viral infections. Virus Gene. 55, 267–273. https://doi.org/10.1007/s11262-019-01641-7.

Müller, C., Obermann, W., Karl, N., Wendel, H.-G., Taroncher-Oldenburg, G., Pleschka, S., Hartmann, R.K., Grünweller, A., Ziebuhr, J., 2021. The rocaglate CR-31-B (−) inhibits SARS-CoV-2 replication at non-cytotoxic, low nanomolar concentrations in vitro and ex vivo. Antivir. Res. 186, 105012 https://doi.org/10.1016/j.antiviral.2021.105012.

Müller, C., Obermann, W., Schulte, F.W., Lange-Grünweller, K., Oestereich, L., Elgner, F., Glitscher, M., Hildt, E., Singh, K., Wendel, H.-G., Hartmann, R.K., Ziebuhr, J., Grünweller, A., 2020. Comparison of broad-spectrum antiviral activities of the synthetic rocaglate CR-31-B (−) and the eIF4A-inhibitor Silvestrol. Antivir. Res. 175, 104706 https://doi.org/10.1016/j.antiviral.2020.104706.

Müller, C., Schulte, F.W., Lange-Grünweller, K., Obermann, W., Madhugiri, R., Pleschka, S., Ziebuhr, J., Hartmann, R.K., Grünweller, A., 2018. Broad-spectrum

antiviral activity of the eIF4A inhibitor silvestrol against corona- and picornaviruses. Antivir. Res. 150, 123–129. https://doi.org/10.1016/j.antiviral.2017.12.010.
Nebigil, C.G., Moog, C., Vagner, S., Benkirane-Jessel, N., Smith, D.R., Désaubry, L., 2020. Flavaglines as natural products targeting eIF4A and prohibitins: from traditional Chinese medicine to antiviral activity against coronaviruses. Eur. J. Med. Chem. 203, 112653 https://doi.org/10.1016/j.ejmech.2020.112653.
Nguyen, H.T., Shukla, P., Torian, U., Faulk, K., Emerson, S.U., 2014. Hepatitis E virus genotype 1 infection of swine kidney cells in vitro is inhibited at multiple levels. J. Virol. 88, 868–877. https://doi.org/10.1128/JVI.02205-13.
Nimgaonkar, I., Ding, Q., Schwartz, R.E., Ploss, A., 2018. Hepatitis E virus: advances and challenges. Nat. Rev. Gastroenterol. Hepatol. 15, 96–110. https://doi.org/10.1038/nrgastro.2017.150.
Obermann, W., Friedrich, A., Madhugiri, R., Klemm, P., Mengel, J.P., Hain, T., Pleschka, S., Wendel, H.-G., Hartmann, R.K., Schiffmann, S., Ziebuhr, J., Müller, C., Grünweller, A., 2022. Rocaglates as antivirals: comparing the effects on viral resistance, anti-coronaviral activity, RNA-clamping on eIF4A and immune cell toxicity. Viruses 14, 519. https://doi.org/10.3390/v14030519.
Pelletier, J., Sonenberg, N., 2019. The organizing principles of eukaryotic ribosome recruitment. Annu. Rev. Biochem. 88, 307–335. https://doi.org/10.1146/annurev-biochem-013118-111042.
Prichard, M.N., Shipman, C., 1990. A three-dimensional model to analyze drug-drug interactions. Antivir. Res. 14, 181–205. https://doi.org/10.1016/0166-3542(90)90001-N.
Roche, S.P., Cencic, R., Pelletier, J., Porco, J.A., 2010. Biomimetic photocycloaddition of 3-hydroxyflavones: synthesis and evaluation of rocaglate derivatives as inhibitors of eukaryotic translation. Angew. Chem. Int. Ed. 49, 6533–6538. https://doi.org/10.1002/anie.201003212.
Rodrigo, C.M., Cencic, R., Roche, S.P., Pelletier, J., Porco, J.A., 2012. Synthesis of rocaglamide hydroxamates and related compounds as eukaryotic translation inhibitors: synthetic and biological studies. J. Med. Chem. 55, 558–562. https://doi.org/10.1021/jm201263k.
Rubio, C.A., Weisburd, B., Holderfield, M., Arias, C., Fang, E., DeRisi, J.L., Fanidi, A., 2014. Transcriptome-wide characterization of the eIF4A signature highlights plasticity in translation regulation. Genome Biol. 15, 476. https://doi.org/10.1186/s13059-014-0476-1.
Schulz, G., Victoria, C., Kirschning, A., Steinmann, E., 2021. Rocaglamide and silvestrol: a long story from anti-tumor to anti-coronavirus compounds. Nat. Prod. Rep. 38, 18–23. https://doi.org/10.1039/D0NP00024H.
Shiota, T., Li, T.-C., Yoshizaki, S., Kato, T., Wakita, T., Ishii, K., 2013. The hepatitis E virus capsid C-terminal region is essential for the viral life cycle: implication for viral genome encapsidation and particle stabilization. J. Virol. 87, 6031–6036. https://doi.org/10.1128/JVI.00444-13.
Shukla, P., Nguyen, H.T., Faulk, K., Mather, K., Torian, U., Engle, R.E., Emerson, S.U., 2012. Adaptation of a genotype 3 hepatitis E virus to efficient growth in cell culture depends on an inserted human gene segment acquired by recombination. J. Virol. 86, 5697–5707. https://doi.org/10.1128/JVI.00146-12.
Shukla, P., Nguyen, H.T., Torian, U., Engle, R.E., Faulk, K., Dalton, H.R., Bendall, R.P., Keane, F.E., Purcell, R.H., Emerson, S.U., 2011. Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus-host recombinant. Proc. Natl. Acad. Sci. USA 108, 2438–2443. https://doi.org/10.1073/pnas.1018878108.
Taroncher-Oldenburg, G., Müller, C., Obermann, W., Ziebuhr, J., Hartmann, R.K., Grünweller, A., 2021. Targeting the DEAD-box RNA helicase eIF4A with rocaglates—a pan-antiviral strategy for minimizing the impact of future RNA virus pandemics. Microorganisms 9, 540. https://doi.org/10.3390/microorganisms9030540.
Todt, D., Friesland, M., Moeller, N., Praditya, D., Kinast, V., Brüggemann, Y., Knegendorf, L., Burkard, T., Steinmann, J., Burm, R., Verhoye, L., Wahid, A., Meister, T.L., Engelmann, M., Pfankuche, V.M., Puff, C., Vondran, F.W.R., Baumgärtner, W., Meuleman, P., Behrendt, P., Steinmann, E., 2020. Robust hepatitis E virus infection and transcriptional response in human hepatocytes. Proc. Natl. Acad. Sci. U.S.A. 117, 1731–1741. https://doi.org/10.1073/pnas.1912307117.
Todt, D., Gisa, A., Radonic, A., Nitsche, A., Behrendt, P., Suneetha, P.V., Pischke, S., Bremer, B., Brown, R.J.P., Manns, M.P., Cornberg, M., Bock, C.T., Steinmann, E., Wedemeyer, H., 2016a. In vivo evidence for ribavirin-induced mutagenesis of the hepatitis E virus genome. Gut 65, 1733–1743. https://doi.org/10.1136/gutjnl-2015-311000.
Todt, D., Meister, T.L., Steinmann, E., 2018a. Hepatitis E virus treatment and ribavirin therapy: viral mechanisms of nonresponse. Curr. Opin. Virol. 32, 80–87. https://doi.org/10.1016/j.coviro.2018.10.001.
Todt, D., Moeller, N., Praditya, D., Kinast, V., Friesland, M., Engelmann, M., Verhoye, L., Sayed, I.M., Behrendt, P., Dao Thi, V.L., Meuleman, P., Steinmann, E., 2018b. The natural compound silvestrol inhibits hepatitis E virus (HEV) replication in vitro and in vivo. Antivir. Res. 157, 151–158. https://doi.org/10.1016/j.antiviral.2018.07.010.
Todt, D., Walter, S., Brown, R., Steinmann, E., 2016b. Mutagenic effects of ribavirin on hepatitis E virus—viral extinction versus selection of fitness-enhancing mutations. Viruses 8, 283. https://doi.org/10.3390/v8100283.
Toribio, R., Díaz-López, I., Ventoso, I., 2016. New insights into the topology of the scanning ribosome during translation initiation: lessons from viruses. RNA Biol. 13, 1223–1227. https://doi.org/10.1080/15476286.2016.1247146.
Wang, W., Cencic, R., Whitesell, L., Pelletier, J., Porco, J.A., 2016. Synthesis of *aza*-rocaglates via ESIPT-mediated (3+2) photocycloaddition. Chem. Eur J. 22, 12006–12010. https://doi.org/10.1002/chem.201602953.
Wißing, M.H., Brüggemann, Y., Steinmann, E., Todt, D., 2021. Virus–host cell interplay during hepatitis E virus infection. Trends Microbiol. 29, 309–319. https://doi.org/10.1016/j.tim.2020.07.002.
Wolfe, A.L., Singh, K., Zhong, Y., Drewe, P., Rajasekhar, V.K., Sanghvi, V.R., Mavrakis, K.J., Jiang, M., Roderick, J.E., Van der Meulen, J., Schatz, J.H., Rodrigo, C.M., Zhao, C., Rondou, P., de Stanchina, E., Teruya-Feldstein, J., Kelliher, M.A., Speleman, F., Porco, J.A., Pelletier, J., Rätsch, G., Wendel, H.-G., 2014. RNA G-quadruplexes cause eIF4A-dependent oncogene translation in cancer. Nature 513, 65–70. https://doi.org/10.1038/nature13485.
World Health Organization (WHO), 2021. (Hepatitis E).
Zhang, W., Chu, J., Cyr, A.M., Yueh, H., Brown, L.E., Wang, T.T., Pelletier, J., Porco, J.A., 2019a. Intercepted retro-nazarov reaction: syntheses of amidino-rocaglate derivatives and their biological evaluation as eIF4A inhibitors. J. Am. Chem. Soc. 141, 12891–12900. https://doi.org/10.1021/jacs.9b06446.
Zhang, W., Liu, S., Maiga, R.I., Pelletier, J., Brown, L.E., Wang, T.T., Porco, J.A., 2019b. Chemical synthesis enables structural reengineering of aglaroxin C leading to inhibition bias for hepatitis C viral infection. J. Am. Chem. Soc. 141, 1312–1323. https://doi.org/10.1021/jacs.8b11477.
Zhou, X., Xu, L., Wang, Y., Wang, W., Sprengers, D., Metselaar, H.J., Peppelenbosch, M.P., Pan, Q., 2015. Requirement of the eukaryotic translation initiation factor 4F complex in hepatitis E virus replication. Antivir. Res. 124, 11–19. https://doi.org/10.1016/j.antiviral.2015.10.016.

Further reading

Meister, T.L., Bruening, J., Todt, D., Steinmann, E., 2019. Cell culture systems for the study of hepatitis E virus. Antivir. Res. 163, 34–49. https://doi.org/10.1016/j.antiviral.2019.01.007.