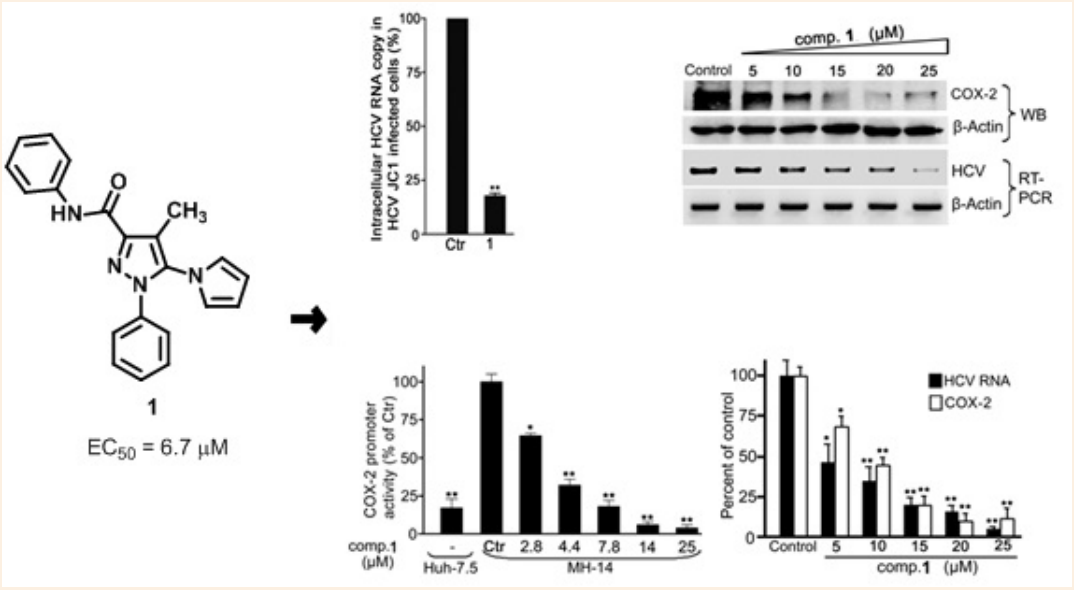
84 Design and Synthesis of New 1-Phenyl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-carboxamides as anti-HCV agents targeting Cyclooxygenase-2

Sveva Pelliccia[1], Giuseppe La Regina[1], Dinesh Manvar[2], Neerja Kaushik-Basu[2], Johan Neyts[3], Romano Silvestri[1]
[1]Sapienza University, Rome, Italy, [2]New Jersey Medical School, Newark, USA, [3]Katholieke Universiteit Leuven, Leuven, Belgium

Hepatitis C virus (HCV) is single-stranded (ss) RNA Hepacivirus in the Flaviviridae family. The current HCV therapy suffers from inadequate sustained viral response rate, rapid emergence of drug resistance, in particular for patients infected with genotype 1 HCV. (1,2) We synthesized new pyrazolecarboxamide derivatives (3) as anti-Hepatitis C virus agents, and investigated the mechanism of inhibition (Chart 1). The most active compounds inhibited the subgenomic HCV replicon 1b in Huh 5-2 cells with EC50 of 6.7 μM and selectivity index of 23 against HCV 1b. Hit compound 1 did not target HCV NS5B or HCV IRES mediated translation; evaluation of the mechanism of anti-HCV activity of 1 revealed that it suppressed HCV-induced COX-2 mRNA and protein expression, exhibiting an IC50 of 3.2 μM in COX-2 promoter-linked luciferase reporter assay. Our data suggest that the pyrazolecarboxamide derivatives behave as anti-HCV agents by targeting COX-2 at both the transcriptional and translational levels. These results provide a strong basis for hit optimization of this new chemical class of HCV inhibitors. (1) Tan, S. L., Pause, A. et al. Nat. Rev. Drug. Disc. 2002, 1, 867-881. (2) Barreca, M. L., Manfroni, G. et al. J. Med. Chem. 2013, 56, 2270-2282. (3) Manvar, D., Pelliccia, S. et al. Eur. J. Med. Chem. 2015, 90, 497-506



85 Novel potent antiviral derivatives against poliovirus

Luca Pescatori[1], Roberta Costi[1], Lucia Fiore[2], Eric Rhoden[3], M.Steven Oberste[3], Roberto Di Santo[1]
[1]Sapienza, University of Roma, Rome, Italy, [2]Istituto Superiore di Sanità, CRIVIB, Rome, Italy, [3]Division of Viral Diseases,Centers for Disease Control and Prevention, Atlanta, USA

The Global Polio Eradication Initiative, established in 1988, has made substantial progress toward its target, with only 3 countries that have not yet eliminated wild poliovirus (PV).[1] More problematic for the eradication is the persistent infections that Sabin strains can establish in immune-deficient individuals (iVDPV), with evolution rates similar to those for wild virus and circulating vaccine-derived polioviruses (cVDPVs). Thus, iVDPVs must be considered a potential source for outbreaks and for reemergence of polio after eradication. Antiviral drugs offer a promising complementary or alternative approach to the use of vaccines to control poliovirus infections, particularly for chronic persistent infections in immunodeficient individuals during the final stages of eradication and for post-eradication reemergence.[2] WHO has recently stressed the importance of research on antivirals and recommended the development of antiviral drugs against PV as a tool to maintain polio-free status after global eradication.

We screened the in vitro antiviral activity of our in-house chemical library against PV Sabin strains and we found two hits, RC 304 and RC 305, active at submicromolar concentration against Sabin 2 and 3 strains. To increase the potency of these antiviral agents, we designed derivatives of these hits and identified antivirals that were also active against Sabin 1. These newly synthesized

compounds were highly active against the three PV Sabin reference strains and a large panel of wild and vaccine-derived PVs isolated from patients with acute flaccid paralysis or immune-deficient subjects. In particular, compounds RC 402 and RC 444 were active at nanomolar concentration against PV Sabin 1, 2, 3 serotypes. Time of addition experiments suggested that these compounds are active in an early stage of viral replication, possibly the uncoating.[3]

REFERENCES:
[1] www.polioeradication.org/ [MSO1]
[2] Tijsma et al. *Antiviral Research*, 2014, (110), 1–9.
[3] Shulman et al., *PLoS One*, 2011, *6(5)*, e18360.

86 Design, Synthesis and Anti-corona Virus Activity of a Series of Acyclic Fleximer Analogues

Hannah L. Peters[1], Dirk Jochmans[2], Adriaan H. de Wilde[3], Clara C. Posthuma[3], Eric J. Snijder[3], Johan Neyts[2], Katherine L. Seley-Radtke[1]

[1]University of Maryland, Baltimore County (UMBC), Baltimore, USA, [2]KULeuven, Leuven, Belgium, [3]Leiden University Medical Center, Leiden, Netherlands

Replication is intrinsically involved in the lifecycle of all viruses, thus their survival relies on DNA or RNA polymerases. One of the most effective strategies in targeting this enzyme is through the use of nucleos(t)ide analogues. An area in which these analogues have been barely explored is the coronaviruses (CoVs), especially Severe Acute Respiratory Syndrome-CoV and Middle East Respiratory Syndrome-CoV. Previously it was found that a “split base” guanosine analogue (Flex-GTP) developed in our laboratory not only served as a better substrate for, but also retained full potency against, binding site mutations in guanosine fucose pyrophosphorylase due to interactions with secondary amino acid residues. Flex-GTP also served as an inhibitor of S-adenosylhomocysteine hydrolase, an adenosine-metabolizing enzyme. These observations strongly indicate that flexibility in the nucleobase scaffold can be a powerful tool for developing drugs that can bind to atypical enzymes in biologically significant conformations. Based on this information we designed and synthesized a series of novel nucleoside analogues by combining our “fleximer” base modification with various modified sugars of FDA-approved antiviral nucleoside drugs, including Acyclovir. This led to potent biological activity against a number of targets, including CoVs, with EC_{50} values ranging from 9-51 μM. This is one of the best activity profiles for a nucleoside against coronaviruses observed to date, and has inspired a second generation of analogues, with biological testing currently underway. The results will be presented herein.

87 Synthesis and in vitro Assay of Deoxyhypusine Synthase Inhibitors as Potential Anti-HIV Agents

Katharina Pfaff[1], Jan Chemnitz[2], Joachim Hauber[2], Chris Meier[1]

[1]University of Hamburg, Hamburg, Germany, [2]Heinrich Pette Institute – Leibniz Institute for Experimental Virology, Hamburg, Germany, [3]Heinrich Pette Institute – Leibniz Institute for Experimental Virology, Hamburg, Germany, [4]University of Hamburg, Hamburg, Germany

HIV chemotherapy mainly focuses on parallel inhibition of several viral enzymes (cART). However, in order to reduce long-term toxicities and the development of (multi-)drug resistance, it is mandatory to identify new potential targets. One option are cellular cofactors that are essential for viral replication like the eukaryotic initiation factor 5A (eIF-5A). This protein acts as a cellular cofactor of the HIV Rev protein in the process of nucleocytoplasmic transport of incompletely-spliced and unspliced viral transcripts. Activation of eIF-5A involves a unique post-translational modification which is catalyzed by two enzymes, the deoxyhypusine synthase (DHS) and the deoxyhypusine hydroxylase (DOHH). Targeting the DHS efficiently suppresses the activation of eIF-5A leading to an inhibition of HIV replication, which has been shown by active compounds like the guanylhydrazone CNI-1493 and analogues of the natural substrate, e.g. GC7.

Recently, we reported an *in silico* designed structurally different inhibitor containing an indole core fragment and amine/guanidino moieties that showed dose-dependent activity against DHS and HIV-1 *in vitro* without causing cytotoxic effects. This hit compound has been employed as a lead structure for further optimization of binding affinity and development of new potential drugs. Here we present the synthesis and biological evaluation of a second substance identified in the initial virtual screening and several new compounds with modifications regarding the substitution pattern, alkyl chain lengths and the aromatic scaffold. The synthesis is based on *in situ* C-C cross coupling and indole cyclisation or click chemistry as the key step to obtain 2,5- and 3,5-substituted indole derivatives as well as one 1,4-substituted triazole compound.