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Human T-cell lymphotropic virus type 1 (HTLV-1) is a retrovirus that can cause aggressive T cell leukemia or neurological disorders. Up to 10 million people are infected with HTLV-1 worldwide, but no antivirals are available yet. This project aims to discover novel inhibitors able to inhibit the transmission and/or proliferation of HTLV-1-infected cells in vitro. First, a cell-to-cell infection assay was set up. To quantify de novo HTLV-1 infection, uninfected HEK293T cells were modified by CRISPR/Cas9 to contain an enhanced green fluorescent protein (eGFP) reporter under control of the HTLV-1 5'LTR promoter. Upon infection by HTLV-1-infected C91/PL cells, the viral Tax protein will be expressed and induce eGFP expression, which is quantified by flow cytometry. The optimal ratio of the number of HEK293T reporter cells versus infected C91/PL cells showed to be 5:1, resulting in 7.38% eGFP positive cells. The assay was validated using compounds known to affect 5'LTR activity, such as rapamycin and A485, which both caused a dose-dependent reduction of eGFP expression. To facilitate screening, imaging techniques (e.g. Incucyte) are currently being implemented to assess eGFP expression. The antiviral activity will be confirmed in susceptible human dendritic cells. Infection with cell-free HTLV-1 will be evaluated by detection of integrated viral DNA using qPCR, or viral proteins using multi-parameter flow cytometry. In parallel, compounds will be evaluated for their inhibitory effect on the viability of HTLV-1-infected and uninfected cell lines. To conclude, the assays set up in this project will play a pivotal role in our aim to discover new and selective HTLV-1 antivirals.

707V. Discovery, Mechanistic Investigations and Crystallographic Study of Benzopyrimidinone-bearing Phenylalanine Derivatives as Novel HIV-1 Capsid Modulators

Shujing Xu, Ph.D., *Shandong University, Jinan, Shandong Province, China*
Lin Sun, *China*
Dang Ding, *China*
Xiaoyu Shao, *China*
Alexej Dick, *China*
Christophe Pannecouque, *China*
Xinyong Liu, Ph.D., *Shandong University, Jinan, Shandong, China*
Peng Zhan, Ph.D., *Shandong University, Jinan, Shandong, China*

The multifunctional HIV-1 capsid protein (CA) represents a highly appealing target in HIV-1 medication research. With PF74 as the lead compound, our previous efforts involved replacing the indole moiety with benzenesulfonamide piperazinone and obtained a novel HIV-1 CA modulator 11L. However, its antiviral activity and metabolic stability still need to be further improved. Herein, we obtained a series of phenylalanine derivatives containing benzopyrimidinone with improved antiviral activity and metabolic stability by cyclizing the amide bond with metabolic liabilities in 11L while retaining the privileged structure of benzenesulfonamide piperazinone, represented by compound RB-8f. Mechanism of action studies showed that RB-8f could effectively bind to the CA hexamer and interfere with the binding of host factor CPSF6 to CA, and it exhibited excellent antiviral effect with dual-stage inhibitory profile. To investigate the interaction mode between these benzopyrimidinone-bearing phenylalanine derivatives and CA hexamer, we analyzed the co-crystal structure of RB-8f. The phenylalanine core skeleton maintained the original binding mode of PF74. The carbonyl group on piperazinone formed a key hydrogen bond with Lys70. Additionally, the benzenesulfonamide moiety extended into the NTD-CTD interface, forming multiple hydrogen bonds with surrounding amino acids such as Lys70, Arg173 and Lys182. The newly introduced benzopyrimidinone group displayed a cation-π interaction with Asn53, and a hydrogen bond was built between C=O and the backbone NH of the crucial residue Thr107. Overall, these studies proved that RB-8f is a promising lead compound for further modification.

800. Strigolactones as Broad-spectrum Antivirals Against β-coronaviruses Through Targeting the Main Protease Mpro

Greta Bajetto, M.S., *University of Piemonte Orientale, Center for Translational Research on Autoimmune and Allergic Disease-CAAD,, Novara, Italy*
Matteo Biolatti, *Department of Public Health and Pediatric Sciences, University of Turin, Italy*
Marco Blangetti, *Department of Chemistry, University of Turin, Turin, Italy*
Melissa Baggieri, *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
Davide Arnodo, *Department of Chemistry, University of Turin, Turin, Italy*
Raoul Fioravanti, *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
Marco De Andrea, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*
Francesca Gugliesi, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*

Camilla Albano, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*
Selina Pasquero, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*
Ivan Visentin, *Department of Agricultural, Forestry, and Food Sciences, University of Turin, Turin, Italy*
Francesca Esposito, *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*
Paolo Malune, *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*
Matteo Bersani, *Department of Drug Science and Technology, University of Turin, Turin, Italy*
Enzo Tramontano, Ph.D., *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*
Cristina Prandi, *Department of Chemistry, University of Turin, Turin, Italy*
Francesca Spyrakis, *Department of Drug Science and Technology, University of Turin, Turin, Italy*
Fabio Magurano, *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*

The current SARS-CoV-2 pandemic and the likelihood that new coronavirus strains will emerge in the immediate future point out the urgent need to identify new pan-coronavirus inhibitors. Strigolactones (SLs) are a class of plant hormones with multifaceted activities whose role in plant-related fields has been extensively explored. Recently, we proved that SLs also exert an antiviral activity toward herpesviruses, such as human cytomegalovirus (HCMV). Here we show that the synthetic SLs TH-EGO and EDOT-EGO impair β-coronavirus replication, including SARS-CoV-2 and the common cold human coronavirus HCoV-OC43. Interestingly, in-silico simulations suggest the binding of SLs in the SARS-CoV-2 main protease (Mpro) active site, and this was further confirmed by an in-vitro activity assay. Overall, our results highlight the potential efficacy of SLs as broad-spectrum antivirals against β-coronaviruses, which may provide the rationale for repurposing this class of hormones for the treatment of COVID-19 patients.

801. Novel Benzodiazepine Antivirals Selectively Active against Yellow Fever Virus with a GABAA Receptor Refractory Property

Yanming Du, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Nicky Hwang, B.S., *Baruch S. Blumberg Institute, PA, United States*
Anilkumar Karampoori, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Fang Guo, M.D., Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Lin Zhang, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Xuexiang Zhang, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Zhengyuan Jiang, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
William Kinney, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Ju-Tao Guo, M.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Jinhong Chang, M.D., Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

Yellow fever is an acute viral hemorrhagic disease that is endemic in tropical areas of Africa, Central and South America, causing an estimated 1.7 million infections and 29,000–60,000 deaths per year. An effective live attenuated yellow fever vaccine has been available since 1938, but the vaccine coverage has been insufficient and has failed to prevent outbreaks in at-risk regions. In addition, there is currently no approved treatment for yellow fever. BDAA is a benzodiazepine (BD) compound that we discovered through a high throughput screening to inhibit yellow fever virus (YFV) in cultured cells and in infected hamsters. Benzodiazepines are considered to have a privileged core structure that has been found to be effective in tranquilizer drugs by binding to the α- and γ-subunits of α- and γ-subunit containing GABAA receptors; thus, lack of binding activities to the GABAA receptors should be preferred for antivirals based on BD core. Here, we report our effort to develop benzodiazepine antivirals meeting this criterion. We analyzed BDAA and its analogs; and identified a structural variation that enables BDAA and its derivatives devoid of interactions with central and peripheral nervous system benzodiazepine receptors at 10 μM. Further structure-activity relationship studies at this position led to the discovery of novel BDAA analogs with sub-micromolar EC50s and favorable pharmacokinetic profiles.

802. Evaluation of in Vitro Selected Nirmatrelvir Resistant SARS-COV-2 in A549-ACE2 Cells

Hussin Rothan, Ph.D., *Pfizer INC, NY, United States Minor Outlying Islands*
Irina Yurgelonis, M.S., *Pfizer, New York, United States*
Patricia McMonagle, M.S., *Pfizer, NY, New York, United States*
Devendra Rai, Ph.D., *Pfizer, NY, New York, United States*
Zhenghui Li, Ph.D., *Pfizer, NY, New York, United States*