utilized in order to reduce the numbers of false positive hits. Both targeted and general small molecule libraries were screened for inhibitors of SARS-CoV entry, and a number of compounds were identified that inhibit SARS-CoV entry and replication. In particular, we took advantage of our previous findings that cathepsin L in target cells is required for activation of SARS-CoV Spike, in order to focus on libraries of cysteine protease inhibitors. 16 positive "hits" with 95% inhibition or higher in the primary screen were further studied for drug dose–response, cell toxicity, and the ability to inhibit coronavirus 229E, Ebola and live SARS-CoV. Three related compounds, exhibiting potent antiviral activity ($IC_{50} < 10^{-4}$ μM) were selected for small animal studies.

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Escaping Development of Drug-Resistant Mutants: Basis for Effective Chemotherapy of Enterovirus Infections

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Enteroviruses are causative agents of more than 50 various diseases, including meningitis, encephalitis, pleurodinia, myocarditis, pericarditis, insulin-dependent diabetes mellitus, etc. Most of enteroviruses induce several clinical syndromes, a phenomenon unique in the infectious pathology. The great majority of these infections are unapparent ones or have a subclinical course. Prevailing role in the strategy of counteraction of enteroviral infections is the use of anti-enteroviral chemotherapeutic agents administered during disease latency period—urgent prophylaxis. It might reduce to a minimum the risk of enterovirus induced myocarditis, acquired diabetes in infant age and other enterovirus infections with severe course. The main obstacle of the development of effective anti-enteroviral chemotherapy is the development of drug-resistance, phenomenon based on the unusually high level of mutation rate (10^{-3}). We carried out systematic study of the drug-resistance on the model of coxsackievirus B1 neuroinfection in mice treated with disoxaril, WIN compounds, binding to the hydrophobic pocket of enteroviral VP1 protein. In parallel, disoxaril-resistant and disoxaril-dependent Cox B1 mutants have been developed in vitro, in FL cells. Phenotypic characteristics, VP1 genome sequencing and VP1 protein sequence deduction of disoxaril mutants have been determined. Sequence changes gave satisfactory explanation for mutant resistance and on the unusual effect of inhibitor-dependence. Combination effects of anti-enteroviral agents with different modes of action have been carried out in cell culture experiments and a series of synergistic combinations have been selected. Administration of antivirals in synergistic combinations could be considered as a prospective approach to decrease the level of drug-resistance and to improve the chemotherapy efficacy. A new scheme of application of the partners in the synergistic combinations was developed on the model of experimental coxsackievirus B1 infection in newborn mice. The maximum protective effect was reached with the combination disoxaril/guanidine.HCl/oxoglaucine.

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Novel Small Molecule Inhibitors of Dengue Virus Replication

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There is an urgent need for new antivirals for both treatment and control of dengue virus, given that over 50 million people are infected worldwide every year and there are no approved vaccines or antiviral drugs available. An antiviral drug that inhibits viral replication without increasing the risk for antibody-dependent enhancement (ADE) of infection would be extremely valuable for public health by providing a means to control outbreaks, as well as to travelers to endemic regions. The goal of the SIGA dengue program is to develop a small molecule therapeutic for the treatment and/or prevention of disease caused by dengue virus, with a final drug product that will be a safe, effective, and orally administered antiviral compound. A sensitive and specific high throughput screening (HTS) assay has been developed to evaluate compounds from the SIGA chemical library for inhibitory activity against dengue-2 (DEN-2) virus replication. Hits have been identified that are potent (EC50 < 5 μM) and selective (CC50 > 50 μM), with initial structure activity relationship in several series of related compounds. Early hits have structures that are chemically tractable, in that they possess chemically stable functionalities and have potential drug-like qualities. Lead series have been identified with activity against all four serotypes of dengue virus which are being defined by spectrum of activity, mechanism of action, preliminary absorption, distribution, metabolism, and excretion (ADME) profiles, and pharmacokinetic (PK) evaluations. One of these series has shown proof-of-concept efficacy in a murine model of disease. The identification and characterization of early stage dengue virus inhibitors with activity in a murine model of dengue virus infection represents a compelling start toward our goal.

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Escaping Development of Drug-Resistant Mutants: Basis for Effective Chemotherapy of Enterovirus Infections

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Enteroviruses are causative agents of more than 50 various diseases, including meningitis, encephalitis, pleurodinia, myocarditis, pericarditis, insulin-dependent diabetes mellitus, etc. Most enteroviruses induce several clinical syndromes, a phenomenon unique in the infectious pathology. The great majority of these infections are unapparent ones or have a subclinical course. Prevailing role in the strategy of counteraction of enteroviral infections is the use of anti-enteroviral chemotherapeutic agents administered during disease latency period—urgent prophylaxis. It might reduce to a minimum the risk of enterovirus induced myocarditis, acquired diabetes in infant age and other enterovirus infections with severe course. The main obstacle for the development of effective anti-enteroviral chemotherapy is the development of drug-resistance, phenomenon based on the unusually high level of mutation rate (10^{-3}). We carried out a systematic study of the drug-resistance on the model of coxsackievirus B1 neuroinfection in mice treated with disoxaril, WIN compounds, binding to the hydrophobic pocket of enteroviral VP1 protein. In parallel, disoxaril-resistant