was evaluated against influenza A (H5N1) virus. Results of biological tests show that most of synthesized compounds reveal antiviral activity to a greater or lesser extent. Also adamantane containing hydrazide has marked antiviral potency against influenza A virus (H5N1), it inhibits their reproduction at 0.5 mM concentration. Amino derivative, containing adamantylidene unit, suppresses replication of H5N1 virus at 0.7 mM concentration. The presence of great number of high active compounds indicates some common principles of antiviral action of compounds, containing saturated cage moiety. It determines route to new virus inhibitors which block M2 ion channels.

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Oseltamivir-resistant Subpopulations of H5N1 Influenza Variants are Genetically Stable and Virulent in Ferrets

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H5N1 influenza viruses are emerging as human pathogens, and their high lethality warrants an urgent search for optimal antiviral therapy. While the neuraminidase (NA) inhibitor oseltamivir is currently our first line of defense against a pandemic threat, there is little information about mechanism(s) of emergence of drug-resistance, e.g. co-existence and selective advantage/disadvantage of H5N1 oseltamivir-resistant and sensitive viruses. Here we assessed the biological significance of minor subpopulations of oseltamivir-resistant H5N1 variants carrying H274Y NA mutation in a ferret model. Animals were inoculated with either recombinant wild-type A/Vietnam/1203/04 (clade 1) or A/Turkey/15/06 (clade 2.2) influenza viruses or mixtures containing different ratios of drug-resistant and sensitive variants, and their fitness was evaluated. Sequence analysis of individual clones obtained from nasal washes of ferrets (days 2, 4 and 6 p.i.) revealed genetic stability of the minor subpopulations of resistant and sensitive viruses for both H5N1 viruses. Ferrets inoculated with A/Vietnam/1203/04 oseltamivir-resistant variants were as virulent as sensitive viruses, e.g. animals experienced high fever, weight loss, anorexia, extreme lethargy, severe neurological impairment, and death. Titers of A/Vietnam/1203/04 oseltamivir-resistant and sensitive variants in the upper respiratory tract of ferrets did not differ significantly ($P < 0.05$). A/Turkey/15/06 (H5N1) virus is less pathogenic to ferrets and causes mild, non-lethal disease at infectious dose up to 10^6 EID_{50}/ferret. The animals inoculated with drug-resistant variants of A/Turkey/15/06 (H5N1) virus initially showed milder signs of disease on days 1–3 p.i., but at later time points the pathogenicity pattern was identical for resistant and sensitive variants. Our results suggest that minor subpopulations of oseltamivir-resistant H5N1 variants can be stably maintained in mammalian species and co-exist with drug-sensitive variants.

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Inhibition of Influenza Virus Replication: Discovery and Development of Therapeutic Compounds which Suppress Viral RNA Synthesis

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Although influenza outbreaks are usually self-limiting, over 500 million people are infected annually and significant morbidity and mortality result from these infections. Additionally, the potential appearance and spread of a highly virulent pandemic strain of influenza similar to outbreaks of H5N1 viruses in 1918 and 1997 emphasizes the need for new and novel inhibitors of influenza virus. At present, annual vaccinations based on predictions of expected circulating influenza viruses and the use of four approved compounds targeting ion channels and the viral neuraminidase represent the entire arsenal of available therapeutics and preventatives for influenza. Thus, continued development of new and novel antiviral agents for the control of influenza is urgently needed and these agents should be amenable for use in combination with the approved anti-influenza agents. ImQuest BioSciences has worked extensively to develop new anti-infective agents targeting the intracellular replication of RNA viruses, including HIV, hepatitis C virus and influenza/respiratory viruses. We have defined a specific series of compounds with activity against these pathogens that target novel and un-exploited viral replication pathways. These agents act as viral transcriptional inhibitors and appear to specifically interact with cellular microtubule macromolecule transport pathways specific to RNA viral pathogens. We have generated 110 compounds in a series of molecules identified as transcriptional inhibitors of HIV and HCV and these small molecules have been screened for activity against influenza A virus, resulting in the identification of three compounds with EC_{50} values in the ng/mL to low μg/mL range but with somewhat narrow therapeutic indices of approximately 25–50. Additional screening has been performed with a representative panel of influenza A and influenza B viruses, other respiratory viruses, including respiratory syncytial virus, rhinovirus, avian influenza virus and highly pathogenic human H5N1 influenza strains, as well as against other non-HIV RNA viruses in order to evaluate the breadth of activity and specificity of the active agents.

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Combinations of 5-Iodo-4′-thio-2′-deoxyuridine and ST-246 or CMX001 Synergistically Inhibit Orthopoxvirus Replication In Vitro

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The nucleoside analog, 5-iodo-4′-thio-2′-deoxyuridine (4′-thioIDU) has been reported to inhibit the replication of orthopoxviruses both in vitro and in vivo. This highly active compound appeared to be phosphorylated by the vaccinia virus thymidine kinase and acts by a mechanism distinct from that observed with either ST-246 or CMX001. Thus, combinations of 4′-thioIDU with these agents might be expected to result in the synergistic inhibition of viral replication. The evaluation of these

combinations is important since ST-246 and CMX001 are the most advanced candidates under development for the treatment of orthopoxvirus infections and potentially could be used together in the clinic to increase efficacy or minimize the emergence of drug resistance. Combination assays were performed in human foreskin fibroblast cells using the Western Reserve (WR) strain of vaccinia virus. Results from these studies revealed robust synergistic inhibition of viral replication with combinations of 4′-thioIDU and ST-246. Combinations of 4′-thioIDU and CMX001 also exhibited modest, but significant synergistic inhibition of vaccinia virus replication. Simultaneous cytotoxicity controls did not reveal any increased toxicity and suggested that the synergistic effects were not the result of increased toxicity. The use of drug combinations with different mechanisms of action is advantageous because the combinations can offer improved efficacy at lower dosages and minimize the development of drug resistance. The results of these experiments indicate that combinations of 4′-thioIDU with ST-246 or CMX001 are particularly effective in the treatment of orthopoxvirus infections in vitro and suggest that combined therapy may be useful in the treatment of these infections in animals and humans.

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Induction of Interferon Gamma Inducible Protein 10 by SARS-CoV Infection, Interferon Alfacon 1 and Interferon Inducer in Human Bronchial Epithelial Calu-3 Cells and BALB/c Mice

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SARS-CoV has been identified as the causative agent of an emerging human infectious disease, SARS. Its immunopathological mechanisms have not been fully characterized. One hypothesis is that the pathogenesis of SARS-CoV is caused by a disproportionate immune response, illustrated by elevated levels of inflammatory cytokines and chemokines, such as IP-10, MCP-1, IL-6 and IL-8. SARS-CoV has been shown in vitro to induce changes of cytokines and chemokines in various human and animal cells. We previously reported that interferon (IFN)-alfacon 1 was more active against SARS-CoV infection in Calu-3 cells than in African green monkey epithelial cells on day 3 post-infection. In the current study, we evaluated its efficacy of IFN-alfacon 1 in Calu-3 cells during the first 7 days of virus infection compared to its efficacy in Vero 76 cells, in which a more productive virus infection occurs. Calu-3 cells appeared to be more responsive to the antiviral effects induced by exogenous IFN than did Vero 76 cells. Furthermore, IP-10, an IFN-inducible white cell chemoattractant, was detected in Calu-3 cells after SARS-CoV infection. Interestingly, IP-10 expression was shown to be significantly elevated when SARS-CoV-infected Calu-3 cells were treated with IFN-alfacon 1. To our knowledge, this is the first time that the IP-10 expression has been clearly demonstrated in Calu-3 cells after SARS-CoV infection. Since IP-10 seems to be coordinated with a protective response in cells, we evaluated the efficacy of antivirals directed against SARS-CoV infection in BALB/c mice. IP-10 expression was detected in the lungs of SARS-CoV-infected BALB/c mice. Significantly high levels of mouse IP-10 in BALB/c mice was also detected when SARS-CoV-infected mice were treated with the interferon inducer, poly IC:LC. Our data might provide an important insight into the mechanism of pathogenesis of SARS-CoV and these properties might be therapeutically advantageous.

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Anti-influenza Efficacy of Combination Apply of Proteolytic Inhibitor E-aminocaproic Acid with Neuraminidase Inhibitor Tamiflu

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Our investigations have shown antiviral activity of proteolysis inhibitor E-aminocaproic acid (E-ACA). Tamiflu (Tm) is neuraminidase inhibitor and the most popular anti-influenza (AI) agents. But its toxicity is higher than E-ACA. We investigate efficacy of E-ACA and Tm combine action for optimization of AI therapy. AI activity in vitro was studied in tissue culture of chorioallantoic membranes chick embryos. Influenza virus strains A/HK/1/68 (H3N2), A/PR/8/34 (H1N1) and avian influenza H5N3 were used. Both Tm in doses 2 mkM/ml and 1 mkM/ml and E-ACA in doses 10 mg/ml and 15 mg/ml have displayed regular AI activity to A/PR/8/34 and H5N3 accordingly if the preparations were used separately. Combination action of these preparations was more effective. Combination action of Tm (1 mkM/ml) and E-ACA (10 mg/ml) has demonstrated synergistic effect on inhibition of reproduction A/HK/1/68 virus. Synergistic effect took place during experimental infection with influenza virus A/PR/8/34 in mice too. Only combination using of Tm and E-ACA have shown strongly protected effect. The results of this study have demonstrated the expediency of combination using of proteolytic and neuraminidase inhibitors for AI protection and therapy.

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Structure–Activity Relationship of a Novel Class of Aglycoristocetin Derivatives with Potent and Broad Activity Against Influenza Viruses

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Attachment of a hydrophobic substituent to a glycopeptide backbone structure was previously reported to offer favorable pharmacological (i.e. antibacterial and antiviral) properties. We here report on the in vitro anti-influenza virus activity of aglycoristocetin derivatives containing hydrophobic side chain-substituted cyclobutenedione. In Madin-Darby canine kidney (MDCK) cells, the lead compound 8e displayed an antivirally effective concentration (EC_{50}) of 0.4 μM, which was consistent amongst influenza A/H1N1, A/H3N2 and B viruses. The concentration producing 50% inhibition of cell proliferation was 67 μM, yielding an antiviral selectivity index (SI) of 167. Structural analogues derived from aglycovancomycin were completely inactive. The hydrophobic side chain