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The Combination of Anti-poxvirus Compounds ST-246 and TTP-018 are Synergistic In Vitro

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ST-246 and TTP-018 are low molecular weight compounds that inhibit orthopoxvirus replication through distinct mechanisms of action (Yang et al., 2005; Bolken et al., 2006). The antiviral effects of each compound alone or in combination were evaluated in vitro using drug–drug combination analysis. Using two mathematically robust techniques (Loewe Additivity and Bliss Independence null reference models of additivity) to analyze the experimental data, significant synergistic effects were observed resulting in a decrease in the EC50 value for ST-246 and TTP-018 of 5-fold and 7.5-fold, respectively. The combination index (CI) was found to be 0.47 indicating a synergistic interaction between the two compounds. Evaluation of the data using a three-dimensional dose response model (MacSynergy II program) generated a synergy volume > 50 unit 2% at the 95% confidence level, implying moderate synergy with potential importance in vivo. Both analyses confirmed the synergistic interaction of ST-246 and TTP-018. In addition, there was no evidence of cytotoxicity with any of the compounds alone or in combination at the concentrations tested. Our findings suggest that the combination of ST-246 and TTP-018 produce greater than additive or synergistic antiviral effects in vitro.

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Thiazolobenzimidazoles, a Novel Class of Enterovirus Inhibitors, Target the 2C Protein

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Despite the fact that enteroviruses are implicated in a variety of human diseases, there is no approved therapy for the treatment of enteroviral infections. We previously reported on a series of 2,6-dihalophenyl-substituted 1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles with anti-enterovirus activity. In order to unravel the mechanism of action of these compounds, time-of-drug addition assays were performed, and virus, resistant to the most potent compound (CHI-033) was generated. Genotyping of drug-resistant strains revealed four amino acid mutations in protein 2C: K107R, A224V, I227V and A229V. Mutations at the latter two positions have been described earlier for echoviruses (Klein et al., 2000) that are resistant to [2-(*a*-hydroxybenzyl)-benzimidazole] (HBB), which suggests a similar mechanism of action Moreover, poliovirus, resistant to guanidine hydrochloride, another 2C inhibitor has been reported to carry mutations at positions 225 and 227 (Pincus et al., 1986). This suggests an important role for the 2C region encompassing amino acids 224–229 in enteroviral replication. To study whether or not individual mutations are sufficient to confer resistance, either single mutations or multiple mutations are being introduced in a full-length infectious clone of coxsackievirus B3. (Cross)-resistance profiles will be determined with the selected 2C inhibitors as well as for other known enterovirus inhibitors. It is our aim to unravel the precise molecular mechanism by which these compounds inhibit the function of 2C and thus viral replication.

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Selective Phosphorylation of Antiviral Drugs by Vaccinia Virus Thymidine Kinase

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The antiviral activity of a new series of thymidine analogs was determined against vaccinia virus (VV), cowpox virus (CV), herpes simplex virus, and varicella zoster virus. Several compounds were identified that had good activity against each of the viruses tested including (*N*)-methanocarbathymidine, and a series of 5-substituted thymidine analogs. To investigate the possibility that these drugs might be phosphorylated preferentially by the viral TK homologs, the antiviral activity of these compounds were also assessed using TK negative strains of some of these viruses. Some of these compounds were shown to be much less effective in the absence of a functional TK gene in CV, which was unexpected given the high degree of homology between this enzyme and its cellular homolog. This unanticipated result suggested