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Synthesis, Antiviral and Cytotoxic Activities of SomeNovel 2,3-Disubstituted Quinazolin-4(3H)-Ones

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2-Phenyl-benzoxazin-4-ones were condensed with primary amine to form the 2,3-disubstituted quinazolin-4(3H)-ones. Their chemical structure was elucidated by means of spectral (FT-IR, ^{1}H NMR, MS) and elemental analysis. The antiviral activity and cytotoxicity of the compounds were tested in HeLa cells (vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus), HEL cells [herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), vaccinia virus], Vero cells (parainfluenza-3, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus). Among the new derivatives evaluated, specific antiviral activity was noted with compounds QAA against vaccinia virus, parainfluenza-3 virus and Punta Toro virus, QOPD against HSV-1, HSV-2 and vaccinia virus, and QONA and PD-NFIN against Coxsackie virus B4.

doi:10.1016/j.antiviral.2008.01.138

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Combined Anti-Influenza Virus Effects of a Plant Polyphenol-rich Extract and E-aminocaproic Acid *In Vitro* and *In Vivo*

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doi:10.1016/j.antiviral.2008.01.139

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Differential Pathogenesis of Cowpox Virus Intranasal Infections in Mice Induced by Low and High Inoculum Volumes, and Effects of Cidofovir Treatment

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The causes of death from intranasal cowpox virus infections in mice remain unclear. Hypotheses include severe pneumonitis, hepatitis, and/or hyperproduction of cytokines and chemokines. We explored these hypotheses by studying the influence of low and high volume virus inoculums on viral pathogenesis, and examines the effects of cidofovir treatment on the infections. BALB/c mice were infected intranasally with a syncytium-forming variant of cowpox virus (Brighton strain) in 5- or 50-μl volumes containing the same infectious virus challenge dose; this resulted in different disease manifestations. The 50-μl infection produced a more rapidly lethal disease associated with severe pneumonitis, high lung and nasal virus titers, and increases in cytokine and chemokine levels in lungs and nasal tissue, while liver infection was minimal. The 5-μl inoculum infection was also lethal, but the infection was primarily confined to the upper respiratory tract, and included elevated cytokine and chemokine levels especially in nasal tissue. The pro-inflammatory cytokine, interleukin-6, was particularly high in both infections. Treatment of the infections with cidofovir (100 mg/kg/day for 2 days starting 24 h after virus exposure) led to survival and suppression of tissue virus titers. Treatment reduced pneumonitis in the 50-μl infection, and lessened cytokine hyperproduction in both types of infection. We conclude that 5-μl volume inoculum of cowpox virus results in a highly lethal infection restricted to the upper respiratory tract, with minimal pneumonitis, while the 50-μl inoculum leads to greater dissemination and more rapid lethality, with excessive release of systemic pro-inflammatory factors likely contributing to the accelerated time to death. Cidofovir effectively treated both infections and slowed viral replication sufficiently to subdue the exaggerated release of pro-inflammatory mediators.

Acknowledgement

Supported by contract NO1-AI-15435 from the Virology Branch, NIAID, NIH.

doi:10.1016/j.antiviral.2008.01.140

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Herpes Simplex Virus Type 2 Exposed to Rigid Amphipathic Fusion Inhibitors (RAFIs) are not Infectious in a Mouse Vaginal Model

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We presented in 2007 a novel family of fusion inhibitors, nucleoside derivatives with rigid polyaryl substituents in position 5, which target the lipid bilayers of several otherwise unrelated DNA and RNA viruses to inhibit their fusion with cellular membranes. Structure-Activity-Relationship (SAR) analyses indicate that amphipathicity and "inverted cone" shape, together with rigidity, planarity, size and hydrophobicity of the aryl group, are all important for antiviral activity. We named the compounds rigid amphipathic fusion inhibitors (RAFIs). We now tested whether the most potent RAFI, dUY11 (IC_{50}, 20 nM; IC_{99}, 700 nM; SI> 7,500), inhibits vaginal infection with a sexually transmitted virus. Synchronized 4–6 week old female mice were vaginally infected with $1–3 \times 10^3$ infectious particles of HSV-2 strain 186. As expected, all 10 mice infected with vehicle exposed virus shed $\sim 10^4$ infectious virions on days 2–4, and lower amounts until day 8, showed severe clinical signs,

and 8 had to be euthanized on days 7–11 due to disseminated infection, hind limb paralysis or more than 10% of body weight loss. None of the 10 mice infected with dUY11 exposed virus shed detectable infectious virus, became clinically ill, or had to be euthanized. Mice were next vaginally treated with dUY11 or vehicle before infection. The preliminary results of these experiments suggest that dUY11 also partially protects mice from infection. In conclusion, virion exposure to RAFIs protect female mice from vaginal infection with a sexually transmitted enveloped virus.

doi:10.1016/j.antiviral.2008.01.141

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Evaluation of C-5 Substituted Uracil Acyclic Phosphonates as Substrates or Inhibitors for DTMP and UMP–CMP Kinases and Potential Antipox Derivatives

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In the aim of designing nucleotide phosphonate analogs able to be activated by phosphorylation in cells and to be potential antipox agents, the reaction of uracil acyclic phosphonates with human cytosolic TMP and UMP-CMP kinases was evaluated as well as with TMP kinase from vaccinia virus. Surprinsingly uracil acyclic phosphonates, with an allyl or pentenyl group as acyclic moiety, were found to be substrates of TMP kinase, but not of UMP-CMP kinase, in contrast with dUMP, phosphorylated by both enzymes. The uracil acyclic phosphonates, with vinyl, allyl or pentenyl as acyclic moiety, were also modified on the C-5 of the base by an halogen (F, Cl and Br) or a phenyl group. Several 5-halogeno-uracil derivatives were substrates for recombinant TMP kinases, with a catalytic efficiency significantly higher than AZTMP. All derivatives were found to inhibit UMP–CMP kinase activity. As shown by fluorescent competition assays, some derivatives were found to bind to UMP site and/or in some cases, surprisingly, to ATP site. The 5-halogeno-uracil derivatives specificity is presumably due to the stacking interaction between uracil and Phe72 of human TMP kinase (Phe68 of vaccinia enzyme). These derivatives should now be tested as potential antiviral molecules after chemical modification to make them available inside cells.

Acknowledgement: These studies were supported by a grant from Sanofi-Aventis France and Bayer Pharma as part of a multi-organism call for proposals and by the french Agence Nationale pour la Recherche (ANR-05-BLAN-0368-02).

doi:10.1016/j.antiviral.2008.01.142

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Effects of PTU-23, HBBb, Ribavirin and Oxoglaucine on the Replication of Feline Calicivirus in CrFK Cells

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Effects of PTU-23, HBB, Ribavirin and Oxoglaucine on the Replication of Feline Calicivirus in CrFK Cells Julian D. Tumbarski and Angel S. Galabov Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria Searching for substances suppressing the replication of caliciviruses is of special interest due to their particular role in the human and veterinary infectious pathology. The investigations for a development of effective anti-calicivirus chemotherapy are important due to the lack of specific means for calicivirus infections treatment and prevention. Caliciviridae possess a RNA(+) genome and a virion structure close to one of Picornaviridae. Based on some similarities, investigations for anti-calicivirus activity of several highly efficient inhibitors of picornavirus replication were carried out. Research was done with feline calicivirus (FCV) F9 strain on Crandell's feline kidney cell line (CrFK). The following compounds were tested: PTU-23 (*N*-phenyl-*N*′-3-hydroxyphenylthiourea), HBB (2-a-hydroxybenzyl benzimidazole), known also as inhibitors of picornavirus-specific RNA synthesis, ribavirin (a broad-spectrum antiviral agent) and a recently described aporphinoid alkaloid oxoglaucine. Anti-calicivirus activity and citotoxicity were tested through CPE inhibition test and neutral red uptake assay (vs. virus inoculating doses ranging within 1 and 10,000 $CCID_{50}$). Kinetics of the effect of compounds was determined by timing-of-addition study in the one-step virus growth cycle experimental design. The compounds were added to DMEM in their maximal effective concentrations at 0 h, 1 h, 2 h, 3 h, 4 h and 5 h after the virus adsorption. The samples were freezed subsequently at 2 h, 3 h, 4 h, 6 h and 8 h. The infectious virus titer was determined by Reed and Muench. Anti-calicivirus effect in low virus inoculation doses possess all of the tested compounds. A marked activity of PTU-23 (when added during the latent period of virus replication cycle) was observed, while HBB and ribavirin (present at the same stage) showed a borderline effect.

doi:10.1016/j.antiviral.2008.01.143