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Computational Studies of gp41 6-Helix Bundle: Do Stabilized Energy of HIV Membrane Fusion Inhibitor C34 and Interaction Energy of gp41 6-Helix Bundle have Good Correlation with their Inhibitory Activity?

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Membrane fusion inhibitor is the active ingredient in some anti-HIV drugs, and is designed to work before HIV enters the host cell. Envelope protein gp41 plays an essential role in membrane fusion by forming a 6-helix bundle between N-terminal heptad repeat (N-HR) and C-HR. Enfuvitide and C34 which are synthetic peptides based on C-HR inhibit the formation of the 6-helix bundle. Therefore, if we found a correlation between the intensity of membrane fusion and the amino acid sequence by computational studies, we could design more potent peptides in silico. Based on the above background, we have calculated stable conformation of the 6-helix bundle by molecular dynamics, and have investigated the relationship between the intensity of membrane fusion and the interaction energy of the 6-helix bundle model consisting of gp41 N-HR and a variety of C34 peptides (Isarangkura et al., 2005), and found a moderate correlation between the calculated interaction energy and reported inhibitory activity. Our calculation method would be applied for these interaction systems. Now we carry out another calculation in order to find potent peptides by using this methodology.

Reference

Isarangkura, P., Ikuta, K., et al., 2005. Microbes Infect. 7, 356–364.

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Synthesis of Structural Analogues of dUY11, A Potent Rigid Amphipathic Fusion Inhibitor Nucleoside

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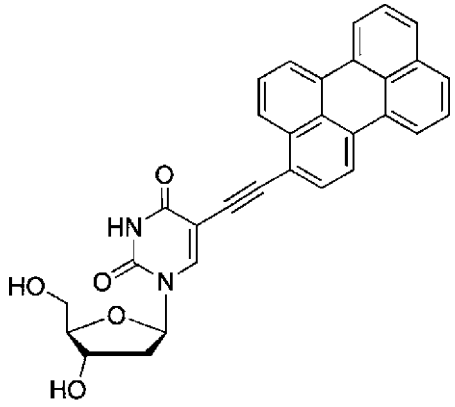
Recently, a unique non-nucleosidic mechanism of antiviral activity of certain 5-arylalkynylated deoxy- and arabino-uridines was suggested [Antiviral Res., 74 (2007), A87]. The preliminary SAR studies revealed that rigid ethynyl linkage and planar hydrophobic substituent at 5-position are essential for the fusion inhibitory antiviral activity. The most promising results were obtained for 5-(3-perylenylethynyl)deoxyuridine (dUY11). Thus, 3-perylenyl is the most appropriate aryl found until now.

Though some sites of the molecule appear critical, there is still potential for further structural analyses. Therefore, we have synthesized a series of compounds sharing some of the structural features of dUY11, in order to evaluate their antiviral activities, to therefore obtain an extended SAR profile.



dUY11

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Chemically Synthesized Tunicamycin Derivatives Effectively Inhibit the Propagation of Classical Swine Fever Virus—A Surrogate Model for Hepatitis C Virus

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Classical Swine Fever Virus (CSFV) is often used as a surrogate model to study the role of envelope glycoproteins of closely related human Hepatitis C Virus (HCV). The need to work with surrogate models for HCV is due to the fact that, until today, only a single isolate of this virus can be grown in in vitro cultures. CSFV glycoproteins, E2, E0 (E^{rns}) and E1, are detected on the external part of viral particles and play a major role in

the initial stages of viral infection. They form heterodimeric and homodimeric complexes needed to effectively infect host cells. The main aim of this work is to study the influence of different inhibitors of glycosylation on penetration and propagation of CSFV, and on maturation of its envelope glycoproteins. These results were later employed in the search for inhibitors interacting with HCV E2 glycoprotein which is crucial for initial stages of HCV infection. To this end we have investigated the formation of glycoprotein dimers by immunoperoxidase monolayer assay and by immunoblotting (Western blotting). By modifying the glycoprotein genes and by arresting *N*-glycosylation of E2 and E0 we have investigated which factors influence the formation of complexes. It has been found that some glycosylation inhibitors, such as tunicamycin and its derivatives, which act at the early stages of glycan chain processing, can influence, not only glycosylation, but also the stability of E2 protein, effectively inhibiting the formation of glycoprotein complexes and the yield of the virus. We have synthesized a number of inhibitors mimicking tunicamycin structure or a part of this structure. Some of them effectively arrested viral growth without significant toxicity for mammalian cells. These inhibitors were further studied in order to elucidate the molecular mechanism of their antiviral effect.

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QSAR Analysis of Cytotoxicity in HeLa Cells

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A lot of promising drug candidates was disqualified because of their toxicity. That is the reason why investigations of cytotoxicity are an integral part of development of a new drug despite it specific action. HeLa cells are widely used in such studies. Application of modern computer technologies allows substantially reducing duration and costs of cytotoxicity researches. Thus the aims of the present work are: the development of QSAR analysis of cytotoxicity in HeLa cells and obtaining of adequate and predictive QSAR models as a tool for consensus virtual screening of HeLa cytotoxicity. The objects of investigation are 93 structurally diverse compounds mainly represented by *N*,*N*′-(bis-5-nitropyrimidyl)dispirotripiperazine, [(biphenyloxy)propyl]isoxazole and 4H-pyrazolo[1,5-a]pyrimidin-7-one derivatives and several well-known antivirals including pleconaril, spirobromine, etc. The cytotoxicity in HeLa cells has been expressed in terms of 50% cytotoxic concentration (CC_{50}, μM). Hierarchic QSAR technology on the base of Simplex representation of molecular structure was a main tool of investigation. Three quite adequate PLS models ($R^2 = 0.89$–0.90, $Q^2 = 0.77$–0.82, $R_2^{test} = 0.78$–0.79) were used as a base of consensus prediction of cytotoxicity. The influence of different fragments into cytotoxicity was defined. It has been discovered that the presence of pyrimidine, naphthalene, *m*-nitrobenzene, *p*-bromobenzene and isoxazole groups is an important factor promoting with cytotoxicity. Vice versa, the insertion of *p*-vinyl-benzene and 1,2,3-trifluoro-benzene fragments into investigated compounds substantially decrease their cytotoxicity in HeLa cells. A tendency of increase in cytotoxicity along with lipophilicity has been revealed. A high impact of atom's individuality (∼40%), electrostatic (∼35%) and polarizability (∼35%) on cytotoxicity variation was found. Obtained models were used for consensus virtual screening of toxicity of compounds belonging to mentioned above structural classes possessing antiviral activity towards coxsackievirus B3 (pleconaril-sensitive 97-927 and pleconaril-resistant Nancy strains) and human rhinovirus 2.

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Peptide-based Entry Inhibitors for Paramyxoviruses

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Paramyxoviruses including respiratory syncytial virus (RSV) and measles virus (MV) are major caused of pediatric infectious disease with high morbidity (RSV) and mortality (MV) in infected infants and young children in developed and developing countries. The current standard of care for RSV infections include the use of Ribavirin or antibody-based therapies directed to severe cases of bronchiolitis. Measles, easily prevented by a licensed vaccine, remains the major viral cause of infant mortality in Africa due to lack of accessibility to vaccine and challenges with vaccination in the presence of maternal antibody in susceptible children. This reality has led to an increased interest in the development of therapeutics for RSV and measles aimed at a variety of targets including viral polymerase components and structural proteins. We have developed a peptide-based therapeutic platform targeting viral envelope glycoproteins for a number of human viruses including RSV and MV. In vitro studies show the prototype peptide to be effective at inhibiting a RSV and MV isolates at reasonable concentrations using a plaque inhibition assay. Additional studies support the proposed mechanism of action of these lead peptide candidates to be interaction with virus–cell fusion. Evaluation of the peptides in animal models of infection are proposed to further develop lead and second generation peptide inhibitors against RSV and MV. These data support further development of peptide-based therapeutics that target viral entry for RSV and MV for clinical use in infants and children susceptible to infection.

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