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Phosphoramidate Dinucleosides as Inhibitors of Hepatitis C Virus Subgenomic Replicon and NS5B Polymerase Activity

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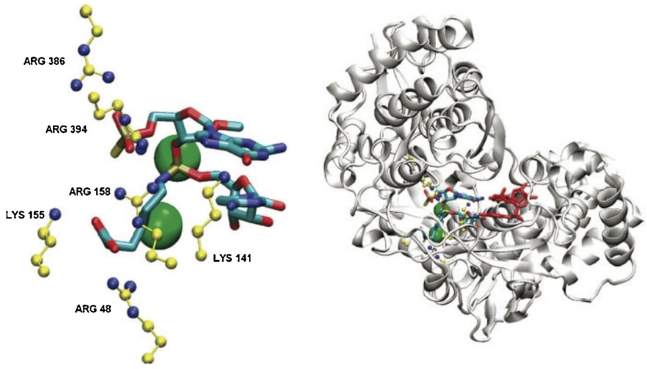
The hepatitis C virus (HCV) represents one of the major viral infections in the world. The development of new therapeutic strategies is urgently needed. HCV replicates its genomic RNA thanks to its own polymerase, so-called the HCV NS5B. This enzyme thus remains a target of choice for inhibitors. We have developed GC Dinucleosides exhibiting a internucleosidic linkage with neutral, amphiphile, positively or negatively charged amino side chains. A first series named "GC 3′-OH", carrying various linkages have been previously reported as hepatitis C virus inhibitors. In order to enhance the efficacy, we synthesized a novel "GC 3′-H" series as potential chain terminators with novel neutral and bis-negatively charged amino side chains. Their inhibitory effect on HCV NS5B polymerase was evaluated in vitro and in HCV subgenomic replicon containing Huh-6 cells. As expected, 3′-H compounds are more potent than their 3′-OH counterparts to inhibit HCV polymerase activity. The most potent inhibitor, a 5′-phosphorylated dinucleotide bearing a bis-negatively charged side chain, exhibits an IC_{50} value of 8 μM in vitro and EC_{50} value of 2.6 μM in the HCV subgenomic replicon system. A molecular structure model is presented to propose an interpretation of the gain afforded by the of 3′-H-cytidine modification.



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Tick-borne Flaviviruses Infection in Non-human Primates

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Employment of different animal models for tick-borne encephalitis (TBE) and Omskhemorrhagic fever (OHF) infection leaded to selection of particular lines of mice, as the most susceptible model. To investigate the virus pathogenesis, the safety and the protectiveness of vaccines and drugs sometimes it is necessary to use a model close to human by its susceptibility. Monkeys are not sensitive to peripheral inoculation with neurotropic flaviviruses. Subcutaneous (s/c), intramuscular or intraperitoneal injection of TBE virus does not cause encephalitis in these animals. However, in some studies it was shown that monkeys can have clinical manifestation of TBE and OHF and pathomorphological changes in CNS similar to human. A lot of epidemiological data accumulated about a high number of seropositive people among endemic territories population is the evidence for existence of inapparent forms of TBE in humans which are about 100 times more frequent than acute forms with clinical symptoms. Thus, monkeys can be very close to humans by their sensitivity to tick-borne flaviviruses.

In our study we particularly concentrated on the development of the most appropriate and informative model using s/c inoculation of two different monkeys' species (Macaca fascicularis and Cercopitecus aethiops) with two virulent strains of TBE virus and one strain of OHF virus. We have compared animal susceptibility, TBE virus strains virulence, and terms of experiments using following parameters: body temperature curve, level and duration of viremia, clinical manifestation, virus titers and lesions in CNS and viscera. Both viruses caused inapparent infection in monkeys of both species. We found which parameters and target organs can serve as the markers of infection at the different time points after inoculation.

The assessment of different viruses and two species of monkeys allowed us to choose monkeys of genus *Macaca* as the most susceptible model to peripheral inoculation of members of mammalian tick-borne flaviviruses group.

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Synthesis of Novel CADA Analog Prodrugs Designed as Down-Modulators of the CD4 Receptor

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Cyclotriazadisulfonamide (CADA) inhibits HIV replication by specifically down-modulating expression of the CD4 receptor protein on host cells. Many analogs of CADA have been synthesized in order to enhance potency, reduce toxicity, and improve physical properties, especially solubility and cell permeability (Bell et al., 2006). These analogs have also been used to develop a three-dimensional quantitative structure-activity relationship (3D-QSAR) computer model. Current studies are aimed at developing a prodrug approach involving novel CADA analog ES04. This compound is expected to have a CD4 down-modulation potency that is similar to that of CADA, according to our 3D-QSAR model. ES04 is the parent compound for prodrugs bearing dipeptide chains that are covalently bonded to the two amino groups of the aminomethylbenzenesulfonyl side arms. Cleavage of these chains by dipeptidyl-peptidase IV (Garcia-Aparicio et al., 2006) is expected to convert the prodrugs into ES04. The synthesis of ES04 involves reduction of the dicyano CADA analog ES03, which was prepared by means of a recently developed palladium-catalyzed macrocyclization method. The anti-HIV and CD4 down-modulation activities of the novel CADA compounds will also be presented.