substantial toxicities and/or the development of drug-resistant viruses. Previously, we engineered a LTR-specific recombinase (Tre-recombinase) that can effectively excise integrated HIV-1 proviral DNA from infected human cell cultures, suggesting that customized enzymes might someday help to eradicate HIV-1 from the body. Here, we provide an update on our recent and further analyses of Tre-recombinase in various HIV-1 infection models. Moreover, we discuss potential future strategies to deliver Tre-recombinase into infected subjects.

doi:10.1016/j.antiviral.2010.02.367

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Small Molecules Targeting Protein–Protein Interactions: A Promising Anti-HIV Strategy

Alba Chimirri[1,*], Laura De Luca[1], Stefania Ferro[1], Rosaria Gitto[1], Anna Maria Monforte[1], Stefano Agnello[1], Maria Letizia Barreca[2], Frauke Christ[3], Zeger Debyser[3]

[1] *Department of Medicinal Chemistry, University of Messina, Messina, Italy;* [2] *Department of Pharmaceutical Chemistry and Technology, University of Perugia, Perugia, Italy;* [3] *Mol. Virology and Gene Therapy Mol. Medicine, Katholieke Universiteit Leuven and IRC KULAK, Leuven, Belgium*

The development of multidrug-resistant viruses compromises the efficacy of anti-human immunodeficiency virus (HIV) therapy and limits treatment options. Therefore, new targets with a different mechanism of action with respect to anti-AIDS drugs so far in therapy need to be identified. In recent years, many examples of protein–protein interactions in the HIV life cycle and related inhibitors is growing rapidly (Busschots et al., 2009). Thus, protein–protein interactions (PPIs) provide an important new approach for the drug design against HIV infection. In our previous paper, a structure-based 3D pharmacophore model for potential inhibitors of the interaction between HIV-1-IN and its cellular cofactor LEDGF/p75 was developed and used for virtual screening of chemical databases, leading to the identification of interesting hits for further optimization (De Luca et al., 2009). Consequently, the rational design, synthesis and biological tests of some derivatives have been carried out. Our studies resulted in the discovery of compounds able to interfere with the IN-LEDGF/p75 interaction at micromolar concentration. Docking simulations were also performed with the aim to investigate the possible binding mode of our new compounds.

Acknowledgement: Research supported by THINC project (HEALTH-F3-2008-201032).

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doi:10.1016/j.antiviral.2010.02.368

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A Cell Protection Screen Reveals Potent Inhibitors of Multiple Stages of the Hepatitis C Virus Life Cycle

Karuppiah Chockalingam[1,*], Rudo Simeon[1], Charles Rice[2], Zhilei Chen[1]

[1] *Texas A&M University, College Station, USA;* [2] *Rockefeller University, New York City, USA*

The hepatitis C virus (HCV) life cycle involves multiple steps, but most current drug candidates target only viral replication. Inability to systematically discover inhibitors targeting multiple steps of the HCV life cycle has hampered antiviral development. We describe a new screen for HCV antivirals based on the alleviation of a HCV-mediated cytopathic effect experienced by an engineered cell line—n4mBid. This approach obviates the need for a secondary screen to avoid cytotoxic false positive hits. Application of our screen to 1280 compounds, many in clinical trials or approved for therapeutic use, yielded >200 hits. Of the 55 leading hits, 47 inhibited one or more aspects of the HCV life cycle by >40%. Six compounds blocked HCV entry to levels similar to an antibody (JS-81) targeting the HCV entry receptor CD81. Seven hits inhibited HCV replication and/or infectious virus production by >100-fold, with one (quinidine) inhibiting infectious virus production by 450-fold relative to HCV replication levels. The described approach is simple and inexpensive, and should enable the rapid discovery of new classes of HCV life cycle inhibitors.

doi:10.1016/j.antiviral.2010.02.369

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Synthesis and Tissue Distribution Studies of Acyloxyalkyl Prodrug Derivative of an Anti-HBV Dinucleotide

John Coughlin[1,*], Kathleen O'Loughlin[2], Sung Rhee[2], Mary Tanga[2], Radhakrishnan Iyer[1], Anna Furimsky[2], Seetharamaiyer Padmanabhan[1], Judith Marquis[3], Amy Ta[2], Carol Green[2], Jon Mirsalis[2]

[1] *Spring Bank Pharmaceuticals, Inc., Milford, USA;* [2] *SRI International, Menlo Park, USA;* [3] *Genzyme Corporation, Cambridge, USA*

We have reported that phosphorothioate dinucleotides and trinucleotides are a new class of anti-HBV compounds with potent activity *in Vitro* and *in Vivo*. Recently, we had developed the acyloxyalkylester derivative as an oral prodrug of the model anti-HBV dinucleotide [R_p,S_p]-3′-dA-ps-$U_{2'OMe}$ (**1**). The current studies were undertaken to evaluate the distribution of the ^{35}S-labeled prodrug in the liver and other organs in rats. The ^{35}S-labeled prodrug was obtained as a solid in high specific activity (120 mCi/g; 84.9 mCi/mmol) by chemoselective *S*-alkylation of ^{35}S-1. The requisite ^{35}S-1 was synthesized using solid-phase phosphoramidite chemistry. Thus, controlled-pore-glass (CPG)-supported dA^{NBz} was coupled to 5′DMT-2′-OMe-uridine-3′-phosphoramidite to generate the intermediate dinucleoside phosphite. The sulfurization of the phosphite using ^{35}S-labeled 3H-1,2-benzodithiole-1,1-dioxide (independently synthesized) followed by deprotection of the CPG-bound dinucleoside phosphotriester, and HPLC purification gave ^{35}S-1. ^{35}S-1 was administered to rats, at a dose of 10 mg/kg, by intravenous (iv) and oral (po) routes. Radioactivity was readily detected in plasma at different time-points after both iv and po administration. Radioactivity concentrated in the liver and the ratio of liver to plasma concentration was as high as 2.9 (iv route) and 3.9 (po route) 1 h after dose administration. Other tissues – kidney, brain, spleen, and heart – contained minor amounts of radioactivity. The primary route of excretion of the radioactivity from the com-