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Program and Abstracts

The Nineteenth International Conference on Antiviral Research

Sponsored by:

The International Society For Antiviral Research

Caribe Hilton Hotel

San Juan, Puerto Rico

May 7–11, 2006

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and
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Introduction to Sponsor
The International Society for Antiviral Research (ISAR)

The Society was organized in 1987 as a non-profit scientific organization for the purpose of advancing and disseminating knowledge in all areas of antiviral research. To achieve this objective, the Society organizes an annual meeting. The Society is now in its 20th year of existence, and has about 600 members representing 30 countries. For membership application forms or further information, please contact Dr. Amy Patick, Secretary, ISAR; Pfizer Global R&D, Department of Virology, 10777 Science Center Drive, San Diego, CA 92121; Phone +1 858 622 3117; fax +1 858 678 8182; E-mail amy.patick@pfizer.com. Membership application forms will also be available at the Conference Registration desk, or from our website www.isar-icar.com.

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SATELLITE SYMPOSIUM

Clinical Update on Antiviral Drugs

Sunday, May 7, 2006
14:00–17:00
San Gerónimo Ballroom
Caribe Hilton Hotel

SOCIAL EVENTS

Opening Reception

Sunday, May 7, 2006
18:00–20:00
Atlantico Pool Bar & Grill Area
Caribe Hilton Hotel

Conference Banquet

Wednesday, May 10, 2006

Reception

19:30
Peacock Alley

Dinner and Program

20:00–22:00
San Gerónimo Ballroom
Caribe Hilton Hotel

Final Program

Nineteenth International Conference on Antiviral Research

Sponsored by:

International Society for Antiviral Research

Caribe Hilton Hotel

San Juan, Puerto Rico

May 7–11, 2006

2006 International Conference on Antiviral Research

Monday, May 8, 2006

Opening Greetings

San Gerónimo Ballroom

08:45 Welcome to the 19th I.C.A.R., John A. Secrist, III, President I.S.A.R.
Welcome to San Juan, John C. Drach, Chair, I.S.A.R. Conference Committee

Oral Session I: Retroviruses

Chairs: Amy K. Patick and José A. Esté

09:00 Plenary Speaker
Robert T. Schooley, M.D., Professor and Head, Division of Infectious Diseases, University of California, San Diego, CA, USA
"Antiretroviral Therapy 2006: Where We Are and Where We're Going"

9:45 1. Conceptually Novel HIV Integrase Inhibitors with Nucleobase Scaffolds: Discovery of a Highly Potent anti-HIV Agent
Vasu Nair, Guochen Chi, Arthur Cox, Roger Ptak, Nouri Neamati
Department of Pharmaceutical and Biomedical Sciences and The Center for Drug Discovery, University of Georgia, Athens, GA 30602, USA; Department of Infectious Disease Research, Southern Research Institute, Frederick, MD 21701, USA; Department of Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90089, USA

10:00 2. Inhibition of Human Immunodeficiency Virus Type 1 Infection in Macrophages by Alpha-v Integrin Ligands
Berta Bosch, Imma Clotet-Codina, Julia Blanco, Eduard Pauls, Gemma Coma, Samandhy Cedeño, Francesc Mitjans, Anuska Llano, Margarita Bofill, Bonaventura Clotet, Jaume Piulats, José Esté
Retrovirology Laboratory, Fundacio IrsiCaixa; Laboratorio de Bioinvestigación, Merck Farma y Química, Catalonia, Spain

10:15 3. Phosphorothioate Oligonucleotides Inhibit HIV-1 Fusion By Blocking Gp41 Core Formation
Andrew Vaillant, Hong Lu, Shuwen Liu, Carol Lackman-Smith, Roger Ptak, Jean-Marc Juteau, Shibo Jiang
REPLICor Inc., Laval, Que., Canada; F. Lindsay Kimball Research Institute, New York Blood Center, New York, NY, USA; Southern Research Institute, Frederick, MD, USA

10:30 *Break*

11:00 4. Stereoselective Synthesis and Biological Evaluation of D- and L-*carba*-Nucleosides as Potential Antiviral Agents
Chris Meier, Soenke Jessel, Bastian Reichardt, Olaf Ludek, Jan Balzarini
University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany; Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

11:15 5. D-Ala-Peptide T-Amide (DAPTA) Strongly Suppresses HIV-1 Replication in Human Primary Macrophages and Prevents HIV-1-Related Neuronal Apoptosis
Michela Pollicita, Candace Pert, Maria-Teresa Polianova, Alessandro Ranazzi, Michael Ruff, Carlo-Federico Perno, Stefano Aquaro
University of Rome Tor Vergata, Italy; Georgetown University, Washington, DC, USA

11:30 6. Chemotherapy of Human Immunodeficiency Virus by Pradimicin A: A Novel Therapeutic Concept for Treatment of Glycosylated Enveloped Viruses
Jan Balzarini, Kristel Van Laethem, Sigrid Hatse, Antonella Bugatti, Marco Rusnati, Stefano Aquaro, Carlo-Federico Perno, Yasuhiro Igarashi, Toshikazu Oki, Dominique Schols
Rega Institute for Medical Research, K.U.Leuven, B-3000 Leuven, Belgium; Department of Biomedical Sciences and Biotechnology, University of Brescia, I-25123 Brescia, Italy; University of Rome Tor Vergata, I-00135 Rome, Italy; Biotechnology Research Center, Toyama Prefectural University, Kurokawa 5180, Toyama 939-0398, Japan; Tamagawa University Research Institute, Tamagawa-Gakuen 6-1-1, Machida-shi, Tokyo 194-8610, Japan

11:45 7. Identification and Optimization of VIRIP—A Natural Occurring Peptide Blocking HIV-1 Entry by Interfering with the Gp41 Fusion Peptide
Jan Muench, Ludger Ständker, Knut Adermann, Axel Schulz, Michael Schindler, Raghavan Chinnadurai, Wolf-Georg Forssmann, Frank Kirchhoff
Department of Virology University of Ulm, Albert-Einstein Allee 11, 89081 Ulm, Germany; IPF PharmaCeuticals GmbH, 30625 Hannover, Germany

12:00 8. Characterization of gp41 Evolution in a Large Cohort of HIV Infected Patients Receiving T20 Therapy as a Single Active Drug
Stefano Aquaro, Valentina Svicher, Roberta D'Arrigo, Ubaldo Visco-Comandini, Andrea Antinori, Mario Santoro, Giovanni Di Perri, Sergio Lo Caputo, Pasquale Narciso, Carlo-Federico Perno
University of Rome Tor Vergata, Italy; INMI L. Spallanzani, Italy; University of Turin, Italy; SM Annunziata Hospital Florence, Italy

12:15 *Lunch*
Local Restaurants

Monday, May 8, 2006

Elion Award Lecture

San Gerónimo Ballroom

14:00 Presentation of Award: John A. Secrist, III, President I.S.A.R.
Awardee Lecture: Robert W. Sidwell, Ph.D., Trustee Professor, Utah State University, Logan, UT, USA
"Influenza: Search for a Cure"

Oral Session II: Respiratory Viruses

Chairs: Donald F. Smee and Joseph M. Colacino

14:45 9. Oseltamivir Protects Ferrets Against Lethal H5n1 Influenza Virus Infection
Elena A. Govorkova, Natalia A. Ilyushina, James Smith, Robert G. Webster
St. Jude Children's Research Hospital, Memphis, TN 38105, USA; F. Hoffmann-La Roche, Basle, Switzerland

15:00 10. Advantages of Combination Chemotherapy for Highly Pathogenic A/Vietnam/1203/04 (H5n1) Influenza Virus in Mice
Natalia Ilyushina, Erich Hoffmann, Rachelle Salomon, Robert Webster, Elena Govorkova
St. Jude Children's Research Hospital, Memphis, TN 38105, USA

15:15 11. Proteomic Profiling of Host Cellular Proteins Incorporated by Severe Acute Respiratory Syndrome (SARS)-Associated Coronavirus Virions: Insights into Emerging Virus Biology and New Therapeutics Targets
François Jean, Reid Asbury, Meera Raj, David Lawrence, and Martin Petric
University of British Columbia, Vancouver, BC Canada V6T 1Z3, GE Healthcare Bio-Sciences, Piscataway, NJ USA 08855, British Columbia Center for Disease Control, Vancouver, BC, Canada V5Z 4R4

15:30 12. Inhibition of SARS-CoV Replication by Hydroxyethylrutosides and Mouse Interferon Alpha in a Mouse Model
Dale Barnard, Craig Day, Robert Montgomery, Kevin Bailey, Matt Heiner, Larry Lauridsen, Robert Sidwell, Kurt Berg
Institute for Antiviral Research, Utah State University, Logan, UT, USA; Panum Inst., IMMI, The IFN-lab, Copenhagen, Denmark

15:45 13. Small Molecule Inhibitors of Respiratory Syncytial Virus
J. Dyall, B. Buscher, J. Balsarotti, R. Roth, G. Starkey, A. O'Guin, P.D. Olivo
Apath LLC, Saint Louis, MO 63141, USA

Monday, May 8, 2006

Poster Session I: Retroviruses, Respiratory Viruses, Hepatitis Viruses, Prodrugs and Drug Delivery, Antiviral Methods
San Cristóbal Ballroom

16:00–18:00

Retroviruses

42. Synthesis of 4′-Carbon-Substituted Stavudine Analogues and SAR Studies on Their Anti-HIV Activity
Hiromichi Tanaka, Kazuhiro Haraguchi, Hiroki Kumamoto, Takao Nitanda, Masanori Baba, Ginger E. Dutschman, Yung-Chi Cheng
School of Pharmaceutical Sciences, Showa University, Tokyo, Japan; Center for Chronic Viral Diseases, Kagoshima University, Kagoshima, Japan; School of Medicine, Yale University, New Haven, CT, USA

44. Intramolecular Alicyclic Synergists for Polyanionic Antivirals
Y. Egorov, A. Serbin, L. Kasyan, I. Tarabara, O. Alikhanova
Health RDF, Moscow, Russia

46. Alkoxyalkyl Esters of Phosphonomethoxyethyl Purines: Synthesis and Antiviral Activity against HIV-1, in vitro
Nadejda R. Valiaeva, Kathy A. Aldern, Julissa Trahan, James R. Beadle, Karl Y. Hostetler
Department of Medicine, University of California San Diego and the Veterans Medical Research Foundation, San Diego, CA, USA

48. Influence of Artifical Ribonucleases Structure on Their Anti-HIV Activity
Victor Kuz'min, Eugene Muratov, Anatoly Artemenko, Ludmila Koroleva, Vladimir Silnikov, V. Lozitsky, A. Fedchuk
A.V. Bogatsky Physical-Chemical Institute, Odessa, Ukraine; Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation; Ukrainian Mechnikov Research Anti-Plague Institute, Odessa, Ukraine

50. Synthesis, Anti-HIV, and CD4 Down-Modulation Activities of Novel CADA Compounds
Sreenivasa Anugu, Thomas Bell, Noah Duffy, Erik De Clercq, Kurt Vermeire, Dominique Schols
Department of Chemistry, University of Nevada, Reno, NV, 89557, USA; Rega Institute for Medical Research, K.U. Leuven, B3000, Leuven, Belgium

52. Novel Inhibitors of Both the 3′-Processing and Strand Transfer Steps of HIV Integrase: Molecular Docking, Binding Poses, and Binding Affinities
Arthur Cox, Vasu Nair
Department of Pharmaceutical and Biomedical Sciences and The Center for Drug Discovery, University of Georgia, Athens, GA 30602, USA

54. Synthesis of Novel HIV Integrase Inhibitors with Highly Potent anti-HIV Activity
Guochen Chi, Vinod Uchil, Vasu Nair
Department of Pharmaceutical and Biomedical Sciences and The Center for Drug Discovery, University of Georgia, Athens, GA 30602, USA

56. Potent Inhibition of Both HIV and HCV in vitro by a Ring-Expanded ("Fat") Nucleoside: Part I. Mechanistic Studies of Anti-HIV Activity
Peng Zhang, Ning Zhang, Kalpana Ganjam, Vinayaka Prasad, Srinivas Kumar, Fenyong Liu, Venkata Yedavalli, Kuan-Teh Jeang, Ali Fattom, Scott Winston, Raafat Fahim, Ramachandra S. Hosmane
Laboratory for Drug Design & Synthesis, Department of Chemistry & Biochemistry, University of Maryland, Baltimore County, Baltimore, MD 21250, USA; Department of Microbiology & Immunology, Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, New York, NY 10461, USA; Division of Hematology & Oncology, Department of Medicine, Vanderbilt University, Nashville, Tennessee 37232, USA; School of Public Health, University of California, Berkeley, CA 94720, USA; Laboratory of Molecular Microbiology, National Institute of Allergy & Infectious Diseases (NIAID), National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892, USA; Nabi Biopharmaceuticals, 12280 Wilkins Avenue, Rockville, MD 20852, USA

58. Effect of Different Adamantane and Norbornene Derivatives on HIV-1 Infection in vitro
Marina Burshtein, Alexander Serbin, Alissa Bukrinskaya
D.I. Ivanovsky Institute of Virology, Moscow, Russia; Health Research and Development Found, Moscow, Russia

60. The Longer Intracellular Half-Life of Tenofovir Diphosphate Compared to Carbovir Triphosphate Correlates with Sustained Antiviral Persistence in vitro
Rebecca Ledford, Jennifer Vela, Adrian Ray, Christian Callebaut, Michael Miller, Damian McColl
Gilead Sciences, Inc, 333 Lakeside Drive, Foster City, CA 94404, USA

62. Development of the G-Quartet Possessing Phosphorothioate Oligonucleotide ISIS 5320 as a Potent Anti-HIV Topical Microbicide
Karen M. Watson, Tracy L. Hartman, Lu Yang, Robert W. Buckheit Jr.
ImQuest BioSciences, Inc., Frederick, MD, USA

64. Combination Topical Microbicide Therapy: Combination Efficacy in PBMCs, CEM-SS, and Virus Transmission Assays
Karen M. Watson, Tracy L. Hartman, Lu Yang, Robert W. Buckheit Jr.
ImQuest BioSciences, Inc., Frederick, MD, USA

66. Effects of Protease Inhibitors on Maturations, Production, Infectivity, and HIV-1-Induced T-cell Apoptosis of HIV-1 Following Drug Removal in Human Macrophages
Alessandro Ranazzi, Stefano Aquaro, Michela Pollicita, Andrea Modesti, Raffaele Caliò, Carlo Federico Perno
Department of Experimental Medicine University of Tor Vergata, Rome, Italy

68. In vitro Selection and Characterization of HIV Mutants Resistant to the NNRTI Capravirine
Weili Jin, Salvatore Santino, Michael Wang
Gilead Sciences, Foster City, CA, USA

70. Lack of Knowledge about Individual's HIV Status: The Obvious Risk Factor for the Spread of AIDS/HIV Among Youth in Developing Countries
Oluwafemi Olawuyi, Adeyemi Falegan
Medical Microbiology, University College Hospital, Ibadan, Nigeria; Dentistry, University College Hospital, Ibadan, Nigeria

72. The Second Generation RNAi Drug Agent Which Deals with RNAi Escape HIV-1 Variants
Yuichiro Habu, Jacob Barnor, Norio Yamamoto, Kahoko Hashimoto, Naoko Miyano-Kurosaki, Koichi Ishikawa, Naoki Yamamoto, David Ofori-Adjei, Hiroshi Takaku
Department of Life and Environmental Sciences, Chiba Institute of Technology, Chiba, Japan; High Technology Research Center, Chiba Institute of Technology, Chiba, Japan; Japan Foundation of AIDS Prevention; Department of Molecular Virology, Bio-Response, Tokyo Medical and Dental University, Tokyo, Japan; AIDS Research Center, National Institute of Infectious Disease, Tokyo, Japan; Department of Virology, Noguchi Memorial Institute for Medical Research Accra-Ghana, Accra, Ghana; Bach Tech Corp.

74. Engineered Stem Cell Vaccine Design: The Rescue for Immune System Against AIDS
Oluwafemi Olawuyi, Adeyemi Falegan, Ebiere Egbejule
University College Hospital, Ibadan, Nigeria; University College Hospital, Ibadan, Nigeria; Lagos State University, Lagos, Nigeria

Respiratory Viruses

76. Increase of Amantadine Resistance Among Porzine but not Avian Influenzavirus A Strains Isolated in Germany Between 1981 and 2002
Michaela Schmidtke, Roland Zell, Katja Bauer, Andi Krumbholz, Jochen Suess, Christina Schrader, Ortrud Werner, Peter Wutzler

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Periyasamy Selvam, Narayanan Murugesh, Markandavel Chandramohan, Robert W. Sidwell, Miles K. Wandersee, Donald F. Smee
A. K. College of Pharmacy, Anand Nagar, Krishnankoil 626 190, India; Institute of Pharmacology, Madurai Medical College, Madurai 625 020, India; Bharat Ratna Kamarajar Liver Hospital and Research Centre, Madurai 625 001, India; Institute of Antiviral Research, Utah State University, Logan, Utah, USA

80. Inhibition of Multiple Influenza A Subtypes in Cell Culture with Antisense Phosphorodiamidate Morpholino Oligomers
Patrick Iversen, D. Stein, Q. Ge, P. Puthavathana, D. Kobasa, D. Burger, M. Pastey, R. Bestwick, J. Chen
AVI BioPharma, Inc. Corvallis, OR, USA; Massachusetts Institute of Technology, Cambridge, MA, USA; Mahidol University, Bangkok, Thailand; Public Health Agency of Canada, Winnipeg, Canada; Oregon State University, Corvallis, OR, USA

82. Antiherpetic and Anti-Influenza Activity of Aza-Crown Ethers
V. Lozitsky, S. Basok, A. Fedchuk, L. Shitikova, L. Mudrik, T. Gridina
Anti-Plague Research Institute, Odessa, Ukraine; Physico-Chemical Institute, Odessa, Ukraine

84. Effect of Single i.m. or i.v. Injection of Peramivir on an Influenza A (H5N1) Virus Infection in Mice
Robert W. Sidwell, Kevin W. Bailey, Min-Hui Wong, Donald F. Smee, Dale L. Barnard, Shanta Bantia
Inst. for Antiviral Research, Utah State University, Logan, UT, USA; BioCryst Pharmaceuticals, Inc., Birmingham, AL, USA

86. Inhibition of Influenza Virus A and B Production by RNA Interference
Hiroshi Saitoh, Naoko Miyano-kurosaki, Hiroshi Takaku
Department of Life and Environmental Sciences, Faculty of Engineering,Chiba Institute of Technology, Chiba, Japan; Department of Life and Environmental Sciences, Faculty of Engineering and High Technology Research Center, Chiba Institute of Technology, Chiba, Japan

88. Antioxidant and Radical Scavenging Activity of a Plant Polyphenol-Rich Extract in the Murine Experimental Influenza Virus Infection
Julia Serkedjieva, Ekaterina Krumova, Tsvetanka Stefanova, Nadja Nikolova, Maria Angelova
Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

90. The Antiviral Activity of S11, a Natural Herb Extract, Against Influenza Virus Infections in Mice
Ji-Sun Kwon, Hyun-Jeong Lee, Chi-Ung Moon, Jong-Hwan Kwak, Youn-Jeong Lee, Chang-Seon Song
Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, Seoul, Korea; Hanyang University, Seoul, Korea; Sungkyunkwan University, Seoul, Korea; National Veterinary Research and Quarantine Services, Seoul, Korea

92. Baculovirus (CpG motifs) Induces an Innate Immune Response and Confers Protection from Lethal Influenza Virus A and B Infection in Mice
Hiroshi Takaku, Takayuki Abe, Hitoshi Takahashi, Naoko Miyano-Kurosaki
Department Life Environ. Sci., and High Tech. Res. Center, Chiba Inst. Tech., Chiba, Japan; Res. Inst. Microbial Dis., Osaka University, Osaka, Japan; Bach Tech Corp.

94. Rep 9: A Potent Broad Spectrum Aerosol Prophylaxis and Therapy Against Infuenza Infection in vivo
Andrew Vaillant, Annie Lebel, Nathalie Goyette, Guy Boivin, Jean-Marc Juteau, Phil Wyde
REPLICor Inc., Laval, Quebec, Canada; CHUQ-CHUL and Laval University, St. Foy, Quebec, Canada; Baylor College of Medicine, University of Texas, Houston, TX, USA

96. Inability to Select in vitro and in vivo of Parainfluenza Virus Variant Resistant to Novel Hemagglutinin-Neuraminidase Inhibitor BCX 2798
Irina V. Alymova, Y. Sudhakara Babu, Allen Portner
Virology Division, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, BioCryst Pharmaceutical, Inc., Birmingham, AL 35244, USA

98. In vitro Inhibition of Maporal Hantavirus
Kie-Hoon Jung, Michelle Mendenhall, Lawrence M. Blatt, Robert W. Sidwell, Brian B. Gowen
Institute for Antiviral Research, Utah State University, Logan, UT, USA; InterMune, Brisbane, California, USA

Hepatitis Viruses

100. Design, Synthesis, and Biological Evaluation of Novel Nucleoside Phosphoramidates as Potential Anti-HCV Agents
P. Perrone, S. Rajyaguru, M.R. Kelleher, G. Luoni, F. Daverio, S. Mulready, C. McGuigan, J.A. Martin, S. Le Pogam, I. Najera, K Klumpp, D.B. Smith
Cardiff University, Cardiff, UK; Roche Palo Alto, Palo Alto, CA, USA

102. Design and Synthesis of Novel Anthranilic Acid Analogs as HCV Polymerase Inhibitors
Kevin Curran, Tom Nittoli, Shabana Insaf, Amar Prashad, Brawner Floyd, Marc Orlowski, Anita Howe, Rajiv Chropra, Atul Agrawal, Jonathan Bloom
Chemical and Screening Sciences, Medicinal Chemistry, Pearl River, NY 10965, USA; Biology, Infectious Diseases, Pearl River, NY 10965, USA

104. Withdrawn

106. Synthesis and Evaluation of Novel Potential HCV Helicase Inhibitors
Andrea Brancale, Dimitrios Vlachakis, Maria Chiara Barbera, Romano Silvestri, Colin Berry, Johan Neyts
Cardiff University, The Welsh School of Pharmacy, Cardiff CF10 3 XF, UK; Universita' degli Studi "La Sapienza," Dipartimento di Studi Farmaceutici, 00185 Roma, Italy; Cardiff University, Cardiff School of Biosciences, Cardiff CF10 3US, UK; Rega Institute for Medical Research, K.U. Leuven, B 300 Leuven, Belgium

108. Identification of Novel HCV Inhibitors Utilizing Virtual Screening
Dale R. Cameron
MIGENIX Inc., 3650 Wesbrook Mall, Vancouver, BC, Canada V6S 2L2

110. Synthesis, Antiviral Activity, and Cytotoxicity of Some Novel Quinazolin-4(3h)-One Derivatives
Periyasamy Selvam, Narayanan Murugesh, Markandavel Chandramohan, Erik De Clercq, Christophe Pannecouque, Johan Neyts
A.K.College of Pharmacy, Anand Nagar, Krishnankoil 626190, Tamilnadu, India; Institute of Pharmacology, Madurai Medical College, Madurai 625 001, Tamilnadu, India; Bharat Ratna Kamarajar Liver Hospital and Research Centre, Madurai 625 001, Tamilnadu, India; Rega Institute for Medical Research, Katholieke Universiteit Leuven

112. HCV NS5B Nonnucleoside Inhibitors Specifically Block Synthesis of Single-Stranded Viral RNA Catalyzed by HCV Replication Complexes in vitro
Wengang Yang, Yongnian Sun, Avinash Phadke, Milind Deshpande, Mingjun Huang
Achillion Pharmaceuticals, New Haven, CT 06511, USA

114. Potent Inhibition of Both HIV and HCV in vitro by a Ring-Expanded ("Fat") Nucleoside: Part II. Mechanistic Studies of Anti-HCV Activity
Ning Zhang, Peng Zhang, Brent Korba, David Oldach, Stephanie Lagrange, Lucyna Cova, Andrea Baier, Peter Borowski, Ali Fattom, Scott Winston, Raafat Fahim, Ramachandra S. Hosmane
Laboratory for Drug Design & Synthesis, Department of Chemistry & Biochemistry, University of Maryland, Baltimore County, Baltimore, MD 21250, USA; Division of Molecular Virology & Immunology, Georgetown University Medical Center, Rockville, MD 20850, USA; Institute of Human Virology, University of Maryland Biotechnology Institute, Baltimore, MD 21201, USA; INSERM U271, Virus des Hépatites et Pathologies Associées, Lyon, France; Catholic University of Lublin, 20-950 Lublin, Poland; Nabi Biopharmaceuticals, 12280 Wilkins Avenue, Rockville, MD 20852, USA

116. Comparison of the Antiviral Activity of Amantadine Against Hepatitis C Virus of Different Genotypes in Cell-Based Replicon and Infectious Virus Assays
Nigel Bourne, Ronald Veselenak, Richard Pyles, MinKyung Yi, Stanley Lemon
University of Texas Medical Branch, Galveston, TX, USA

118. Celgosivir and Castanospermine are Highly Synergistic Against Bovine Viral Diarrhea Virus when Combined with Interferon Alpha 2b or with Interferon Alpha 2b and Ribavirin
Dominique Dugourd, Raymond Siu, Jeremy Fenn
MIGENIX Inc., Vancouver, BC, Canada

120. Antiviral Activity of the α-Glucosidase Inhibitors Celgosivir and Castanospermine Combined with NM-107, Amantadine, and NB-DNJ
Dominique Dugourd, Raymond Siu, Jacob Clement, Jeremy Fenn
MIGENIX Inc., Vancouver, BC, Canada

122. Interference of Hepatitis C Virus Replication by Combination of Protein Kinase C-like 2 Inhibitors and Interferon-α
Seong-Jun Kim, Jina Yun, Jong-Won Oh
Department of Biotechnology, Yonsei University, Seoul 120-749, Korea

124. Hepatitis B Virus e Antigen Production is Dependent upon Covalently Closed Circular (ccc) DNA in HepAD38 Cell and may Serve as a cccDNA Surrogate in Antiviral Screening Assays
Haitao Guo, Tianlun Zhou, Ju-tao Guo, Andrea Cuconati, Anand Mehta, Timothy Block
Drexel University College of Medicine, Doylestown, PA, USA; Nucleonic Inc., Irvine, CA, USA; Hepatitis B Foundation, Doylestown, PA, USA

Prodrugs and Drug Delivery

126. Comparison of the Uptake and Intracellular Metabolism of Hexadecyloxypropyl Esters of (S)-HPMPA and Cidofovir.
Kathy Aldern, James Beadle, Karl Hostetler
University of California, San Diego and the Veterans Medical Research Foundation, San Diego, CA, USA

128. Antiviral Activity and Metabolic Stability of Branched Methyl Alkoxyalkyl Esters of Cidofovir against Vaccinia, Cowpox, and Ectromelia Viruses, in vitro
Jaqueline C. Ruiz, James R. Beadle, W. Brad Wan, Julissa Trahan, Kathy A. Aldern, Kathy A. Keith, Jill Schriewer, R. Mark Buller, Earl R. Kern, Karl Y. Hostetler
University of California San Diego and the Veterans Medical Research Foundation, San Diego, CA, USA; University of Alabama School of Medicine, Birmingham, AL, USA; Department of Molecular Microbiology and Immunology, St. Louis University, St. Louis, MO, USA

130. Ethylene Glycol-Linked Amino Acid Conjugates of Cyclic Cidofovir: Synthesis and Biological Activity
Ulrika Eriksson, Larryn W. Peterson, Jae-seung Kim, Stefanie Mitchell, Paul Kijek, John M. Hilfinger, John C. Drach, Boris A. Kashemirov, Charles E. McKenna
Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA; TSRL, Inc., Ann Arbor, MI 48108, USA; Department of Biologic & Materials Science, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA

132. Stability, Transport, and Activity of Cidofovir Peptide Prodrugs
Jae-seung Kim, Stefanie Mitchell, Paul Kijek, Charles McKenna, Boris Kashemirov, Ulrika Eriksson, Julie Breitenbach, Kathy Borysko, Gordon Amidon, John Drach, John Hilfinger
TSRL, Inc. Ann Arbor, MI, USA; Department of Chemistry, University of Southern California, Los Angeles, CA, USA; Pharmaceutical Sciences, College of Pharmacy and School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA

134. CMX052, An Orally Available Lipid Conjugate of Foscarnet for the Treatment of Drug Resistant HIV
Lawrence Trost, Bernhard Lampert, Lloyd Frick, Merrick Almond, George Painter
Chimerix, Inc. (DMPK Advisor), Durham, NC, USA

136. Amino Acid Ester Prodrugs of Vidarabine: Stability, Permeability, and Activity Against Vaccinia Virus
Zhiqian Wu, Julie Breitenbach, Ulrika Erickson, John Hilfinger, John Drach, Gordon Amidon
Department of Pharmaceutical Sciences, College of Pharmacy and School of Dentistry, University of Michigan, Ann Arbor, MI, USA; TSRL, Inc., Ann Arbor, MI, USA

138. Vidarabine Prodrugs as Anti-Pox Virus Agents
John Hilfinger, Zhiqian Wu, Jae-Seung Kim, Stefanie Mitchell, Julie Breitenbach, Gordon Amidon, John Drach
TSRL, Inc. Ann Arbor, MI, USA; Pharm. Sciences and Medicinal Chemistry, College of Pharmacy and Biologic & Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA

140. cycloSal-Monophosphate Prodrugs with an Optimized Mask
Ulf Goerbig, Anne Baum, Jan Balzarini, Chris Meier
University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany; Rega Institute for Medical Research, Katolieke Universiteit Leuven, Leuven, Belgium

142. Synthesis and Properties of Intrinsically Fluorescent cycloSal-Pronucleotides
Henning Jessen, Wolfgang Fendrich, Tilmann Schulz, Jan Balzarini, Chris Meier
University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany; Rega Institute for Medicinal Research, Katholieke Universiteit Leuven, Leuven, Belgium

144. Enzymatically Activated cycloSal-Pronucleotides
Nicolas Gisch, Jan Balzarini, Chris Meier
University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany; Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

146. GS9131, a Phosphoramidate Prodrug of Novel Cyclic Nucleotide Analog, Exhibits Favorable in vitro and in vivo Pharmacological Profile
Tomas Cihlar, Richard Mackman, Adrian Ray, Dean Boojamra, Lijun Zhang, Deborah Grant, Hon Hui, Jennifer Vela, Neil Parkin, Yolanda Lie, Kirsten White, Michael Miller, Gerry Rhodes, Manoj Desai
Gilead Sciences, Foster City, CA, USA; Monogram Biosciences, So. San Francisco, CA, USA

148. Cathepsin A is the Major Hydrolase Catalyzing the Intracellular Activation of Nucleotide Phosphoramidate Prodrugs GS-7340 and GS-9131
Martin McDermott, Gabriel Birkus, Ruth Wang, Holly MacArthur, Xiaohong Liu, Nilima Kutty, Tomas Cihlar, Craig Gibbs, Swami Swaminathan, Arnold Fridland, William Lee
Gilead Sciences, Inc., Foster City, CA, USA

150. Aerosolic Poloxagel-Loaded Triphosphate Antivirals against Influenza Infection
Serguei Vinogradov
Center for Drug Delivery and Nanomedicine, University of Nebraska Medical Center, Omaha, NE, USA

152. Evaluation of Interferon (IFN) Bioavailability by Relative Quantitation of MxA mRNA Expression Using TaqMan RT-PCR
Edwin Gong, Ebrima Gibbs, Joel Oger
Department of Pharmacology and Therapeutics and NeuroImmunology Lab, UBC Hospital, Department of Medicine, University of British Columbia, Vancouver, BC, Canada

154. Influence of Some Permeability Enhancers on Anti-Influenza Efficacy of Transdermal Delivery System Containing Rimantadine during Experimental Infection
V. Lozitsky, I. Kravchenko, V. Larionov
Research Anti-Plague Institute, Odessa, Ukraine; National University, Odessa, Ukraine

156. Antiviral Properties of the α-*p*-borano-2′,3′-dideoxy Nucleotide Analogues
Mikhail Dobrikov, Serguei Vinogradov, Barbara Ramsay Shaw
Department of Chemistry, Duke University, Durham, NC, USA; Center of Drug Delivery and Nanomedicine, and College of Pharmacy, University of Nebraska, Omaha, NE, USA

Antiviral Methods

158. Development, Validation, and Optimization of a Luminescence-Based High Throughput Screen for Inhibitors of Influenza
James Noah, William Severson, Lynn Rassumussen, Lucile White, Colleen Jonsson
Biochemistry and Molecular Biology, Southern Research Institute, Birmingham, Alabama, USA; High Throughput Screening Center, Southern Research Institute, Birmingham, AL, USA

160. A Novel Viral Protease Assay Using a Bacteriophage Lambda-Based Genetic Screen
Mariona Parera, Bonaventura Clotet, Miguel Angel Martinez
Fundacio irsiCaixa, Hospital Universitari Germans Trias i Pujol, 08916 Badalona, Spain

162. Development of a Virus Transmission and Rapid Resistance Selection Assay to Evaluate and Prioritize Antiviral Agents for Systemic and Microbicide Use
Karen M. Watson, Todd B. Parsley, Robert W. Buckheit Jr.
ImQuest BioSciences, Inc., Frederick, Maryland, USA

164. Antisense Morpholino-Oligomers Directed Against Bunyavirus Genome Segments Inhibit Replication and Proliferation
Laure Deflubé, Kerstin Angner, Anna Overby, David Stein, Patrick Iversen, Ramon Flick
UTMB, Department of Pathology, Galveston, Texas, USA; AVI BioPharma, Inc., Corvallis, Oregon, USA

166. Fractal Microscopic Description of Herpes Virus-Cell Dynamic System
Oleksandr Fedchuk, Andriy Fedchuk, George Bartsykovsky, Alla Fedchuk
Physical, I. I. Mechnikov Odesa National University, Odesa, Ukraine; Computer Sciences, I. I. Mechnikov Odesa National University, Odesa, Ukraine; Chemotherapy, I. I. Mechnikov Ukrainian Research Anti-Plague Institute, Odesa, Ukraine

168. Generalized Fourier Image Processing in Fractal Microscopy for Virus-Cell Interaction Imaging
Andriy Fedchuk, George Bartsykovsky, Alla Fedchuk, Oleksandr Fedchuk
Computer Sciences, I. I. Mechnikov Odesa National University, Odesa, Ukraine; Chemotherapy, I. I. Mechnikov Ukrainian Research Anti-Plague Institute, Odesa, Ukraine; Physical, I. I. Mechnikov Odesa National University, Odesa, Ukraine

Tuesday, May 9, 2006

Mini-Symposium: Emerging Infections and Biodefense

San Gerónimo Ballroom

Chairs: Earl R. Kern and Richard J. Whitley

09:00 Arnold S. Monto, M.D., Professor of Epidemeology and Director of Bioterrorism Initiative, University of Michigan, School of Public Health, Ann Arbor, Michigan, USA
"Spread of Avian Influenza: Lessons from Past Pandemics"

09:30 Kwok-Yung Yuen, M.D., Chair of Infectious Diseases and Department of Microbiology, Hong Kong University, Hong Kong, China
"Bats as a Continuing Source of Emerging Pathogens"

10:00 Dennis E. Hruby, Ph.D., Chief Scientific Officer, SIGA Technologies, Inc., Corvallis, OR, USA
"Development of Poxvirus Antivirals: Trials and Tribulations"

10:30 *Break*

11:00 Adolfo García-Sastre, Ph.D., Department of Microbiology, Mount Sinai School of Medicine, New York, New York, USA
"Archeobiology of the Pandemic Influenza Virus of 1918"

11:30 Andrew T. Pavia, M.D., Presidential Professor and Chief, Division of Pediatric Infectious Diseases, University of Utah, Salt Lake City, Utah, USA
"Opportunities for Antiviral Therapy for Pandemic and Avian Influenza"

12:00 General Panel Discussion

12:30 *Adjourn*

Free Afternoon and San Juan Tours

Wednesday, May 10, 2006

Prusoff Young Investigator Award Lecture

San Gerónimo Ballroom

09:00 Presentation of Award: John A. Secrist, III, President I.S.A.R.
Awardee Lecture: Tomás Cihlar, Ph.D., Principal Scientist, Gilead Sciences, Foster City, CA, USA
"Understanding the Biological Attributes of Nucleoside Phosphonates: From the First (Cidofovir) to the Newest (GS-9131)"

Oral Session III: Herpesviruses

Chairs: Johan Neyts and Deborah A. Quenelle

09:45 14. PreClinical Development of the BCNAs: The Most Potent Anti-VZV Agents Reported to Date
Christopher McGuigan, Ranjith Pathirana, Marco Migliore, Rina Adak, Annette Angell, Geoffrey Henson, Robert Snoeck, Graciela Andrei, Erik De Clercq, Jan Balzarini
Welsh School of Pharmacy, Cardiff University; FermaVir Pharmaceuticals, New York, NY, USA; Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

10:00 15. Organotypic Epithelial Raft Cultures as a Model for Evaluation of Antivirals Against Varicella-zoster Virus (VZV)
Graciela Andrei, Joos Van Den Oord, Pierre Fiten, Ghislain Opdenakker, Erik De Clercq, Robert Snoeck
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium; Pathology Department, U.Z. Leuven, Leuven, Belgium

10:15 16. Identification of New Benzimidazole D-Ribonucleosides that Inhibit HCMV Packaging Proteins
Jae-Seon Hwang, Oliver Kregler, John C. Drach, Leroy B. Townsend, Elke Bogner
Institut für Klinische und Molekulare Virologie, Erlangen, Germany; Department of Biologic and Materials Sciences, School of Dentistry and; Interdepartmental Graduate Program in Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA

10:30 *Break*

10:50 Invitation to 20th ICAR, Palm Springs, California, USA, John C. Drach

11:00 ISAR Business Meeting

11:15 17. Foldamer-Based Inhibitors of Cytomegalovirus Entry
Emily English, Rene Roy, Samuel Gellman, Teresa Compton
Department of Chemistry, University of Wisconsin – Madison, Madison, WI, USA; McArdle Laboratory for Cancer Research, University of Wisconsin – Madison Medical School, Madison, WI, USA

11:30 18. Nonsteroidal Anti-Inflammatory Drugs, Indomethacin and Aspirin, Inhibit Herpes Simplex Virus Replication and Block Activation of Nuclear Factor-KappaB
Seth A. Faith, Erin Bailey, John J. Docherty
Department of Microbiology, Immunology and Biochemistry, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272, USA

11:45 19. Increasing the Relevance of Animal Models of Cytomegalovirus (CMV) Infection: A Chimeric Human-Guinea Pig CMV is Ganciclovir and Maribavir Susceptible and Produces Disease in Animals
Mark Schleiss, Alistair McGregor, Jodi Anderson, Xiaohong Cui, Michael McVoy
University of Minnesota Medical School, Division of Pediatric Infectious Diseases, Minneapolis, Minnesota, USA; Virginia Commonwealth University, Division of Pediatric Infectious Diseases, Richmond, VA, USA

12:00 20. Potent Antiviral Activity of REP 9 and Analogs Against Vaginal HSV-2 Infection
James Ireland, E. Guzman, A. Vaillant, J.-M. Juteau, R. Cardin, B.C. Herold, D.I. Bernstein
Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; Mount Sinai School of Medicine, New York, NY, USA; Replicor Inc., Laval, Que., Canada

12:15 *Lunch*
Local Restaurants

Wednesday, May 10, 2006

Oral Session IV: Hepatitis Viruses
San Gerónimo Ballroom
Chairs: Diana S. Berard and Michael Murray

14:00 Plenary Speaker
Jane A. McKeating, Ph.D., Professor of Molecular Virology, Institute Biomedical Research, Medical School, University of Birmingham, Birmingham, UK
"*In Vitro* System to Study Hepatitis C Virus Replication"

14:30 21. AVI-4065, an Antisense Approach to Active HCV Infection; Preclinical and Clinical Evaluation
Patrick Iversen, A. Amantana, D. Stein, R. Rjinbrand, N. Borne, S. Dagan, E. Ilan, J. Christensen, D. Burger, P. O'Hanley
AVI BioPharma, Inc. Corvallis, OR; University of Texas Galveston, Galveston, TX; XTL Biopharmaceuticals, Rehovot, Israel

14:45 22. Synthesis and Anti-HCV Activity of 4′-Substituted Ribonucleosides
David B. Smith, Joseph A. Martin, Mark Smith, Chris J. Hobbs, John H. Merritt, Keshab Sarma, Klaus Klumpp, Vincent Leveque, Isabel Najera, Wen-Rong Jiang, Rene Devos, Nick Cammack
Roche Palo Alto, Department of Medicinal Chemisty; Department of HCV Biology; Department of Viral Biochemistry; Viral Diseases Therapy Area, Palo Alto, CA 94304, USA

15:00 23. NIM811, an HCV Replication Inhibitor of Novel Mechanism, Exhibits Potent Antiviral Activities Alone or in Combination with a Non-nucleoside HCV Polymerase Inhibitor
Joanna E. Boerner, Sue Ma, ChoiLai TiongYip, Michael P. Cooreman, Teresa Compton, Kai Lin
Novartis Institutes for Biomedical Research, 100 Technology Square, Cambridge, MA 02139, USA

15:15 24. R1479 is a Highly Selective Inhibitor of NS5B-Dependent HCV Replication and Does Not Inhibit Human DNA and RNA Polymerases
Han Ma, Sophie Le Pogam, Vincent Leveque, Weixing Li, Wen-Rong Jiang, Isabel Najera, Julian Symons, Nick Cammack, Klaus Klumpp
Roche Palo Alto LLC, Palo Alto, CA 94304, USA

15:30 25. Application of Stable Hepatitis C Virus (HCV)-Secreting Human Hepatoma Cell Lines for Antiviral Drug Discovery
Guangxiang Luo, Zhaohui Cai, Chen Zhang, Kyung-Soo Chang, Jieyun Jiang
Microbiology, Immunology, and Molecular Genetics, University of Kentucky College of Medicine, Lexington, KY 40536, USA

15:45 26. Novel Small Molecule Inhibitors of Hepatitis B Virus Surface Antigen Secretion
Andrea Cuconati, Haitao Guo, Gael Westby, Anand Mehta, Timothy Block
Institute for Hepatitis and Virus Research; Drexel Institute for Biotechnology and Virology Research, Doylestown, PA, USA

16:00 27. Mechanistic Characterization of Entecavir Resistance in Lamivudine Resistant Hepatitis B Virus
A.W. Walsh, D.R. Langley, R.E. Rose, C.J. Baldick, S.M. Levine, K.A. Pokornowski, C.F. Yu, C.E. Mazzucco, M.J. Plym, B.J. Eggers, R.J. Colonno, D.J. Tenney
Bristol Myers Squibb Pharmaceutical Research Institute, Wallingford, CT, USA

Wednesday, May 10, 2006

Poster Session II: Herpesviruses, Poxviruses, Other Viruses, Antiviral Targets, and Natural Products
San Cristobal Ballroom
16:00–18:00

Herpesviruses

43. SAR of Alkyloxyphenyl Furano Pyrimidines: Potent and Selective Anti-VZV Agents
R. Adak, B. Mollwitz, M. Migliore, C. McGuigan, R. Snoeck, G. Andrei, E. De Clercq, J. Balzarini
Cardiff University, Cardiff, Wales, UK; Rega Institute for Medical Research, Leuven, Belgium

45. Withdrawn

47. Some Modifications to the Bicyclic Pyrimidines: A Novel Class of Anti-Viral Nucleosides
A. Angell, C. McGuigan, L. Avanzi, L.G Sevillano, R. Snoeck, G. Andrei, E. De Clercq, J. Balzarini
Welsh School of Pharmacy, Cardiff University, UK; Rega Institute for Medical Research, Leuven, Belgium

49. SAR of Monosubstituted Phenyl Furano Pyrimidine as Potent and Selective anti Varicella-Zoster Virus (VZV) compounds
M. Migliore, O. Bidet, C. McGuigan, R. Snoeck, G. Andrei, E. De Clercq, J. Balzarini
Welsh School of Pharmacy, Cardiff University, Cardiff, UK; Rega Institute for Medical Research, Leuven, Belgium

51. Influence of Structure of *N*,*N*′-(bis-5-nitropyrimidyl)dispirotripiperazine Derivatives on Their Antiherpetic Activity
Eugene Muratov, Anatoly Artemenko, Victor Kuz'min, Vladimir Makarov, Olga Riabova, Peter Wutzler, Michaela Schmidtke, V. Lozitsky, A. Fedchuk

A.V. Bogatsky Physical-Chemical Institute, Odessa, Ukraine; Research Center for Antibiotics, Moscow, Russia; Institute of Virology and Antiviral Therapy, Friedrich Schiller University, Jena, Germany; Ukrainian Mechnikov Research Anti-Plague Institute, Odessa, Ukraine

53. Synthesis and Antiviral Activity of Methylene-3-Fluorocyclopropane Analogues
Jiri Zemlicka, Shaoman Zhou, Earl R. Kern, John C. Drach, Hiroaki Mitsuya
Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, USA; University of Alabama School of Medicine, Birmingham, AL, USA; School of Dentistry, University of Michigan, Ann Arbor, MI, USA; National Cancer Institute, Bethesda, MD, USA

55. 5-Arylethynyl Derivatives of 2′-Deoxyuridine and *Arabino*-uridine: Synthesis and Antiviral Evaluation
Olga Valueva, Stanislav Korkach, Irina Astakhova, Irina Stepanova, Vladimir Korshun, Alexey Ustinov
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Miklukho-Maklaya 16/10, Moscow 117997, Russia

57. Inhibition of Murine Cytomegalovirus by Second Generation Ribonucleotide Reductase Inhibitors Didox and Trimidox
Mohammed Inayat, Simon Cooper, Donald Smee, Vincent Gallicchio, Beth Garvy, Howard Elford, Oliver Oakley
Department of Clinical Sciences and Department of Infectious Disease, University of Kentucky, Lexington, KY, USA; Arizona Cancer Center, University of Arizona, Tucson, AZ, USA; Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA; Department of Biological Sciences, Clemson University, Clemson, SC, USA; Molecules for Health Inc. Richmond, VA, USA

59. Inhibition of Herpesvirus Replication by a Series of Alkoxyalkyl Esters of Purine- and Pyrimidine-Based Nucleoside Phosphonates
Caroll Hartline, Emma Harden, Shannon Daily, Mary Johnson, Mark Prichard, Kathy Aldern, Karl Hostetler, Earl Kern
Department of Pediatrics, University of Alabama School of Medicine, Birmingham, AL, USA; VA Medical Center and University of California, San Diego, CA, USA

61. Resistance of Human Cytomegalovirus with Single and Double Mutations in UL97 to First and Second Generation Methylenecyclopropane Purines
Julie M. Breitenbach, Katherine Z. Borysko, Jiri Zemlicka, John C. Drach
Biologic & Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan, USA; Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan, USA

63. Studying of Anti Epstein-Barr Virus Activity of New Triazine Bearing Tricyclic Bases and their *N*-Glycosidic Derivatives
Svitlana Zagorodnya, Nadiya Nesterova, Inna Alexeeva, Larisa Palchikovskaya, Galina Baranova, Alexander Kobko, Anna Golovan
Zabolotny Institute of Microbiology and Virology of NAS of Ukraine and Institute of Molecular Biology and Genetics of NAS of Ukraine, Kiev, Ukraine

65. Antiviral Activity of a GSH Derivative of Glutathione in HSV 1-Induced Keratitis in Rabbits
R. Sgarbanti, L. Nencioni, G. Macrì, C. Nucci, U. Benatti, M. Magnani, E. Garaci, A.T. Palamara
Dep. Public Health Sciences, University Rome “La Sapienza,” Rome, Italy; Dep. Biopathology, Physiopathological Optics, University Rome “Tor Vergata,” Rome, Italy; Dep. Exp. Medicine Biochemistry Section, University Genova, Genoa, Italy; Inst. Biochemistry, University Urbino, Urbino, Italy; Dep. Exp. Med. Biochem. Sciences, University Rome “Tor Vergata,” Rome, Italy

67. Effect of Oral Treatment with (S)-HPMPA, HDP-(S)-HPMPA, or ODE-(S)-HPMPA on Replication of Human Cytomegalovirus (HCMV) or Murine Cytomegalovirus (MCMV) in Animal Models
Debra Quenelle, Deborah Collins, Latisha Pettway, Caroll Hartline, James Beadle, W. Wan, Karl Hostetler, Earl Kern
University of Alabama School of Medicine, Birmingham, AL, USA; Department of Medicine, University of California, San Diego and Veterans Medical Research Foundation, San Diego, CA, USA

69. Potent, Antiviral Activity of REP 9 and Analogs Against Systemic MCMV Infection
R.D. Cardin, A.P. Sewell, F.J. Bravo, A. Vaillant, J.-M. Juteau, D.I. Bernstein
Division of Infectious Diseases, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA; REPLICor, Inc., Laval, Quebec, Canada

Poxviruses

71. Design and Synthesis of Novel Phosphonomethoxyethyl Adenine Analogs for Treatment of Orthopoxvirus Infections
Kathy Keith, Joseph Maddry, Namita Bansal, Kochurani Jacob, Secrist John, Earl Kern
Department of Pediatrics, University of Alabama School of Medicine, Birmingham, AL, USA; Southern Research Institute, Birmingham, AL, USA

73. Inhibitory Activity of Isatine-Sulphonamide Derivatives Against Orthopoxvirus Replication in vitro
Periyasamy Selvam, Narayanan Murugesh, Markandavel Chandramohan, Kathy A. Keith, Earl R. Kern
A. K. College of Pharmacy, Anand nagar, Krishnankoil 626 190, India; Institute of Pharmacology, Madurai Medical College, Madurai 625 020, India; Bharat Ratna Kamarajar Liver Hospital and Research Centre, Madurai 625 001, India; University of Alabama School of Medicine, Birmingham, AL 35233, USA

75. Anti-Orthopoxviruses Activity of the New Class of 1,2,4-Benzotriazine Derivative
Evgeny Belanov, Svetlana Kotovskaya, Nikolay Bormotov, Sergey Balakhnin, Olga Serova, Nataliya Perova, Zina Baskakova, Galina Dzhumbaeva, Valerii Charushin, Oleg Chupakhin
State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Reg., Russia; Ural State Technical University, Yekaterinburg, Russia; Institute of Organic Syntheses, Yekaterinburg, Russia

77. ST-246 Inhibits Vaccinia Virus IMV Wrapping in the Intracellular Vesicles
Yali Chen, Guang Yang, Kady Honeychurch, Dennis Hruby, Robert Jordan
SIGA Technologies, Inc., Corvallis, OR 97333, USA

79. Characterization of Virus Variants Resistant to the Antiviral Effects of ST-246
Guang Yang, Chris Harver, Dennis Hruby, Robert Jordan
SIGA Technologies, Inc., Corvallis, OR 97333, USA

81. Activity of 5-Subsituted Pyrimidine Analogs Against Herpesvirus and Orthopoxvirus Replication in vitro
Emma Harden, Caroll Hartline, Mark Prichard, Kathy Keith, Angela Williams, Xuesan Fan, Paul Torrence, Earl Kern
Department of Pediatrics, University of Alabama School of Medicine, Birmingham, AL 35233, USA; Department of Chemistry and Biochemistry, Northern Arizona University, Flagstaff, AZ 86011, USA

83. In vitro and in vivo Activity of *N*-methanocarbathymidine Against Herpesvirus and Orthopoxvirus Infections and its Mechanism of Action
Mark Prichard, Kathy Keith, Debra Quenelle, Earl Kern
Department of Pediatrics, University of Alabama School of Medicine, Birmingham, AL 35233, USA

85. Drug Susceptibility Profiles of Recombinant Vaccinia Virus Harboring Mutation(s) Conferring Resistance to Cidofovir
Graciela Andrei, Don Gammon, Pierre Fiten, Ghislain Opdenakker, Erik De Clercq, Robert Snoeck, David Evans
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium; Department of Medical Microbiology & Immunology, University of Alberta, Edmonton, Alberta, Canada

87. Evaluating the Use of CpG DNA as an Antiviral Therapy
Amanda Phelps, Linda Eastaugh, Amanda Gates, David Ulaeto, Arthur Krieg
Defence Science and Technology Laboratories (Dstl), Salisbury, Wiltshire, UK; Coley Pharmaceuticals Ltd., Ottawa, Ontario, Canada

89. Characterization of the Lister Strain of Vaccinia Virus Used for Vaccination Against Smallpox
Irina Gurt, Ihab Abdalrhman, Ehud Katz
Department of Virology, Hebrew University, Hadassah Medical School, Jerusalem, Israel

91. Cell Line Dependency for Antiviral Activity of *N*-Methanocarbathymidine Against Orthopoxvirus Infections
Donald F. Smee, Miles K. Wandersee, Kevin W. Bailey, Min-Hui Wong, Chung K. Chu, Srinivas Gadthula, Robert W. Sidwell
Institute for Antiviral Research, Utah State University, Logan, UT 84322, USA; College of Pharmacy, University of Georgia, Athens, GA 30602, USA

93. Antiviral Efficacy of ST-246 in a Ground Squirrel Model of Severe Monkeypox Virus Infection
Chelsea Byrd, Elena Sbrana, Shu-Yuan Xiao, Marina Siirin, Robert Tesh, Dennis Hruby, Robert Jordan
SIGA Technologies, Inc., Corvallis, OR, USA; University of Texas Medical Branch, Galveston, TX, USA

95. ΔF13L-Vac (p37-Deleted Vaccinia Virus) is Attenuated in Mice and Protects Against Infection with Wild-Type Virus
Inge Vliegen, Guang Yang, Dennis Hruby, Erik De Clercq, Robert Jordan, Johan Neyts
Rega Institute for Medical Research, K.U. Leuven, Belgium; Siga Technologies, Inc., Corvallis, OR, USA

Coxsackie, West Nile, Flavi, Arena, and Other Viruses

97. Inhibition of Coxsackievirus B3 Replication in HeLa Cells and Cardiomyocytes by Peptide-Conjugated Phosphorodiamidate Morpholino Oligomers
Ji Yuan, Travis Lim, Shuan Coughlin, Dexin Qiu, Zhen Liu, Dave Stein, Decheng Yang
The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, University of British Columbia, Vancouver, British Columbia, Canada; AVI BioPharma, Inc., Corvallis, OR, USA

99. QSAR Studies Demonstrate the Influence of Structure of [(Biphenyloxy)Propyl]Isoxazole Derivatives on Inhibition of Coxsackievirus B3 (CVB3) Replication
Eugene Muratov, Anatoly Artemenko, Victor Kuz'min, Vladimir Makarov, Olga Riabova, Peter Wutzler, Michaela Schmidtke, V. Lozitsky, A. Fedchuk
A.V. Bogatsky Physical-Chemical Institute NAS of Ukraine, Odessa, Ukraine; Research Center for Antibiotics, Moscow, Russia; Institute of Virology and Antiviral Therapy, Friedrich Schiller University, Jena, Germany; Ukrainian Mechnikov Research Anti-Plague Institute, Odessa, Ukraine

101. Molecular Genetic Study of the Disoxaril Mutants of Coxsackievirus B1
Ivanka Nikolova, Roumena Petkova, Boris Atanassov, Stoyan Chakarov, Angel S. Galabov
Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; Scientific Technological Service, Ltd., Sofia, Bulgaria; Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

103. Characterization of Triple Combinations of Enteroviral Replication Inhibitors Effective Against Experimental Neurotropic Coxsackievirus B1 Infection in Newborn Mice
Ralitsa Vassileva-Pencheva, Angel S. Galabov
Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

105. Isoxazolecarbonitriles: A Novel Class of AntiCoxsackievirus Compounds Blocking Virus Adsorption
Rossella Timpanaro, Adriana Garozzo, Aldo Stivala, Gianna Tempera, Angelo Castro
Departments of Microbiological Sciences, University of Catania, Via Androne 81, 95124 Catania, Italy

107. Transforming Growth Factor-Beta 1 Improves Blood-Brain Barrier Properties in Mice Infected with West Nile Virus
Aaron Olsen, Venkatraman Siddharthan, John Morrey
Institute for Antiviral Research, Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT 84322, USA

109. Treatment of West Nile Disease with Neuroprotective Agents
John D. Morrey, Aaron L. Olsen, Justin G. Julander, Robert W. Sidwell, Grant J. Roper, Michael R. Fetell
Institute for Antiviral Research, Department of Animal Dairy and Veterinary Sciences, Utah State University, Logan, Utah, USA; IVAX Research, Miami, FL, USA

111. Identification of West Nile Virus-Infected Cells in the Central Nervous System of Rodents Early in Infection: Implications for Treatment
Venkatraman Siddharthan, Grant J. Roper, Hong Wang, Aaron L. Olsen, Landon Preece, Christensen Andy, Brandon Taro, John D. Morrey
Institute for Antiviral Research, Department of Animal Dairy and Veterinary Sciences, Utah State University, Logan, Utah, USA

113. Antiviral Mode of Action of Carrageenans Against Dengue Virus in Vero and HepG2 Cells
Laura Talarico, Elsa Damonte
Laboratory of Virology, Department of Biological Chemistry, School of Sciences, University of Buenos Aires, Argentina

115. Antiviral Activity of Iminosugar Compounds with Modified Alkyl Side Chains
Baohua Gu, Peter Mason, Liguan Wang, Nigel Bourne, Anand Mehta, Timothy Block
Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, Doylestown, PA, USA; Department of Pathology and Department of Pediatrics and Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX, USA

117. A Nucleoside Inhibitor of Hepatitis C Virus Replication Efficiently Inhibits the Replication of Flaviviruses in vitro
Pieter Leyssen, Xavier De Lamballerie, Erik De Clercq, Johan Neyts
Rega Institute for Medical Research, K. U. Leuven, B-3000 Leuven, Belgium; Unité des Virus Emergents EA3292, Etablissement Francais du Sang Alpes-Mediterranée and Faculté de Médecine de Marseille, 13005 Marseille Cedex 5, France

119. Cage Compounds as Inhibitors of Arenaviruses Reproduction
Yuri Klimochkin, Andrew Shiryaev, Igor Moiseev, V Sabynin, Larisa Rustamova, Alexandr Petkevich
State Technical University, Samara, Russia; Institute of Epidemiology and Microbiology, Minsk, Belaruss

121. Ribavirin and Consensus Interferon-Alpha Combination Therapy of Acute Arenaviral Disease
Brian Gowen, Donald Smee, Min-Hui Wong, Anne Pace, Kie-Hoon Jung, Kevin Bailey, Lawrence Blatt, Robert Sidwell
Institute for Antiviral Research, Utah State University, Logan, UT, USA; InterMune, Brisbane, CA, USA

123. REP 9, a Degenerate Phosphorothioate Oligonucleotide that Inhibits Rift Valley Fever Viral Infection in vivo
Slobodan Paessler, Laure Deflubé, Andrew Vaillant, Jean-Marc Juteau, Ramon Flick
University of Texas Medical Branch, Department of Pathology, Galveston, TX, USA; REPLICor Inc., Laval, Quebec, Canada

125. Antipoliovirus Activity and Mechanism of Action of 3-methylthio-5-phenyl-4-isothiazolecarbonitrile
Adriana Garozzo, Rossella Timpanaro, Aldo Stivala, Gianna Tempera, Christian C.C. Cutrì, Angelo Castro
Department of Microbiological Sciences, University of Catania, Via Androne 8, 95124 Catania, Italy; Department of Pharmaceutical Sciences, University of Catania, Viale A. Doria 6, 95125 Catania, Italy

127. Efficacy of Exogenous Interferon Treatment of Venezuelan and Western Equine Encephalitis Viruses in vivo
Justin Julander, Aaron Olsen, John Morrey, Lawrence Blatt, Kristiina Shafer, Robert Sidwell
Institute for Antiviral Research, Utah State University, Logan, UT, USA; InterMune, Brisbane, CA, USA

129. Comparison of the Inhibitory Effects of Ribavirin and Interferon Alfacon 1 on a Yellow Fever Virus Infection in Syrian Golden Hamsters
Justin Julander, Kristiina Shafer, John Morrey, Lawrence Blatt, Robert Sidwell
Institute for Antiviral Research, Utah State University, Logan, UT, USA; InterMune, Brisbane, CA, USA

131. Activity of 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines Against Polyomaviruses
Ilya Lebeau, Graciela Andrei, Antonin Holý, Erik De Clercq, Robert Snoeck
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium; Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Praha, Czech Republic

Antiviral Targets

133. 7-Deaza Carbocyclic N-3 Isonucleosides as SAHase Inhibitors for Antiviral Chemotherapeutics
Naresh Sunkara, Sylvester Mosley, Brian Bakke, Joshua Sadler, Katherine Seley(Radtke), Sunny Zhou
University of Maryland-Baltimore County, Baltimore, MD, USA; Washington State University, Pullman, WA, USA

135. Phosphorylation of α-*p*-Borano Substituted Nucleoside Diphosphates
Charlotta Wennefors, Mikhail Dobrikov, Barbara Ramsay Shaw
Chemistry Department, Duke University, Durham, NC 27708, USA

137. Design of DNA Binding Agents that Target the Origin of Replication (ori) of HPV31
James Bashkin, Megh Singh, Kathleen Crowley, Barbara Schweitzer, Scott Woodard, Peggy Garner-Hamrick, Chris Fisher
Pharmacia, Kalamazoo, MI, USA; Pharmacia, St. Louis, MO, USA; University of Missouri-St. Louis, St. Louis, MO, USA; NanoVir LLC, Kalamazoo, MI, USA; Pfizer, St. Louis, MO, USA; Monsanto, St. Louis, MO, USA

139. 2-(4-Phenyl-4-Piperidinyl)Ethyl Amine-Based CCR5 Antagonists: Derivatizations at the N-terminal of the Piperidine Ring
Maosheng Duan, Christopher Aquino, Brian Chauder, Robert Ferris, Wieslaw Kazmierski, Terry Kenakin, Cecilia Koble, Jennifer Peckham, Jim Thompson, Pat Wheelan, Chris Watson, Michael Youngman
Department of Medicinal Chemistry, Department of Virology, Department of DMPK, MV CEDD, GlaxoSmithKline, Research Triangle Park, NC, USA

141. Two Forms of HIV-1 Matrix Protein as Targets for Antiviral Compounds
Alissa Bukrinskaya, Alexandr Serbin, Galina Vorkunova, Marina Burstein
D. I. Ivanovsky Institute of Virology, Moscow, Russian Federation; Health Research and Development Foundation, Moscow, Russian Federation

143. The Candidate Microbicide CADA, an Entry Inhibitor that Specifically Targets the Cellular CD4 Receptor, Prevents HIV and SIV Infection of Human and Simian Cells
Kurt Vermeire, Thomas Bell, Sreenivasa Anugu, Noah Duffy, Roger Le Grand, Erik De Clercq, Dominique Schols
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium; Department of Chemistry, University of Nevada, Reno, USA; Service de Neurovirologie, Fontenay-aux-Roses, France

145. Varicella Zoster Virus is Sensitive to Compounds that Target Host Cell Cycle Functions
Rebecca Greenblatt, Dongmei Liu, Jennifer Moffat
Department of Micro & Immuno, SUNY Upstate Medical University, Syracuse, NY, USA

147. Nano-Responsible Multifunctional Antivirals
A. Serbin, Y. Egorov, S. Tykvinski, O. Alikhanova
Health RDF, Moscow, Russia

149. Antiviral Activity of [(Z)- and (E)-9-[3-(phosphonomethoxy)prop-1-en-1-purines] in Cell Cultures and Evaluation of their Diphosphates in Cell-Free System
Marina Kukhanova, Alexander Ivanov, Georgii Galegov, Valeria Andronova, Maxim Jasko
Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia; Centre for Medical Studies, University of Oslo, Moscow, Russia; Ivanovsky Institute of Virology, Russian Academy of Medical Sciences, Moscow, Russia

151. Inhibition of Japanese Encephalitis Virus RNA Replication by the Peptide Nucleic Acids Targeted to the *Cis*-Acting Element of the Viral Genomic RNA
Jee-Yon Kim, Ji-Seung Yoo, Yeon-Gu Kim, Jung-Hee Kim, Jong-Won Oh
Department of Biotechnology, Yonsei University, Seoul 120-749, Korea

Natural Products

153. Evaluation of Some Pharmacological Activities of Selected Bulgarian and Turkish Medicinal Plants
Julia Serkedjieva, Reneta Toshkova, Milena Nikolova, Reneta Tsvetkova, Stefka Antonova, Ivana Roeva, Munnever Sokmen, Bektas Tepe, Medine Gulluce, Fikrettin Sahin, Atalay Sokmen
Institute of Microbiology, Institute of Experimental Pathology and Parasitology, Institute of Botany, Bulgarian Academy of Sciences, Faculty of Biology, Department of Microbiology, Sofia University, Sofia, Bulgaria; Faculty of Art and Science, Department of Biology, Cumhuriyet University, Sivas, Turkey; Faculty of Art and Science, Department of Biology, Atatürk University, Erzurum, Turkey

155. Antiviral Properties of Proteolysis Inhibitors and of Compounds Containing their Fragments
Alla Fedchuk, Regina Lozitska, Tetyana Gridina, Victor Kuzmin, Victor Lozitsky
I. I. Mechnikov Ukrainian Research Anti-Plague Institute, Odesa, Ukraine; O. V. Bogatsky Physico-Chemical Institute, Odesa, Ukraine

157. Oxoglaucine—A Broad-Spectrum Anti-Enteroviral Inhibitor
Lubomira Nikolaeva-Glomb, Angelina Trifonova, Stephan Filipov, Angel S. Galabov
Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

159. Effect of Plant Polyphenols Quercetin and Rutin on Oxidative Stress and CYP Dependent Monooxygenases in Liver of Influenza Virus-Infected Mice
Milka Mileva, Angel S. Galabov
Department of Medical Physics and Biophysics, Medical University, Sofia, Bulgaria; Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

161. Antiviral Activity of S22, Natural Herb Extract, Against Influenza A Virus Infection in vitro and in vivo
Hyun-Jeong Lee, Ji-Sun Kwon, Chi-Ung Moon, Jong-Hwan Kwak, Youn-Jeong Lee, Chang-Seon Song
Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, Seoul, Korea; Hanyang University, Seoul, Korea; Sungkyunkwan University, Seoul, Korea; National Veterinary Research and Quarantine Services, Seoul, Korea

163. Antiviral Effect of Some Nigerian Herbal Extracts on Newcastle Disease Virus
George Ezeifeka, S.U. Ekeoma, C.O. Okeke, V.N. Akpunonu
Nnamdi Azikiwe University, Awka, Nigeria

Thursday, May 11, 2006

Oral Session V: Pox, West Nile, Hemorrhagic Fever, and Papilloma Viruses

San Gerónimo Ballroom

Chairs: Roger G. Ptak and Brian B. Gowen

09:00 Plenary Speaker
Ehud Katz, Ph.D., Chanock Chair in Virology,Hebrew University - Hadassah Medical School, Jerusalem, Israel
"In Vitro and In Vivo Characterization of Spontaneous and Engineered Mutants of Vaccinia Virus"

09:30 28. Sequential Determination of Virus in Blood and Tissues of the Variola Cynomolgus Monkey Model of Classical Smallpox Reveals that IV Cidofovir can Effectively Treat Monkeys with Extensive Viral Burden
John Huggins, JoLynn Raymond, Robert Fisher, Peter Jahrling, Lisa Hensley
Viral Therapeutics Branch, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD 21702, NIAID, NIH, Bethesda, MD, USA

09:45 29. Evaluation of ST-246 in Vaccinia or Cowpox Virus Infections of Mice
Earl Kern, Kathy Keith, Robert Jordan, Dennis Hruby, Debra Quenelle
Department of Pediatrics, University of Alabama School of Medicine, Birmingham, AL, USA; SIGA Technologies, Inc., Corvallis, OR 97333, USA

10:00 30. Cidofovir and Foscarnet Peptide Prodrugs
Charles E. McKenna, Boris A. Kashemirov, Larryn W. Peterson, Ulrika Eriksson, Kanokkarn Saejueng, Jae-seung Kim, Stefanie Mitchell, Paul Kijek, John M. Hilfinger, John C. Drach
Department of Chemistry, University of Southern California, Los Angeles, CA 90089; TSRL, Inc., Ann Arbor, MI 48108; School of Dentistry and College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA

10:15 31. Treatment of West Nile Disease with Humanized Monoclonal Antibody After the Virus is in the Brain
John D. Morrey, Venkatraman Siddharthan, Aaron L. Olsen, Grant J. Roper, Thomas J. Baldwin, Scott Koenig, Syd Johnson, Jeffrey L. Nordstrom, Michael S. Diamond
Institute for Antiviral Research, Department of Animal Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA; MacroGenics, Inc., Rockville, MD, USA; Departments of Molecular Microbiology, Medicine, and Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA

10:30 *Break*

11:00 32. Identification and Characterization of Antiviral Drugs for Lassa Fever Virus
Sean M. Amberg, Tove C. Bolken, Ryan A. Larson, Dongcheng Dai, Kevin F. Jones, Travis K. Warren, S. Amanda Lund, Dana L. Kirkwood-Watts, David S. King, William C. Weimers, Amy C. Shurtleff, Kathleen A. Kashman, Philip J. Ferro, Mary C. Guttieri, Dennis E. Hruby
SIGA Technologies, Inc. 4575 SW Research Way, Corvallis, OR 97333, USA; USAMRIID, Department of Mol. Virol., Bldg. 1301, Fort Detrick, Frederick, MD 21702, USA

11:15 33. Antiviral Strategies Against Nipah and Ebola Virus: Exploring Gene Silencing Mechanisms to Identify Potential Antiviral Targets
Sven Enterlein, Pramila Walpita, Allison Groseth, Heinz Feldmann, Ramon Flick
University of Texas Medical Branch, Department of Pathology, Galveston, TX, USA; National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada

11:30 34. Identification of Inhibitors of Ebola Virus with a Subgenomic Replication System
J. Dyall, J. Balsarotti, B. Buscher, R. Roth, G. Starkey, A. O'Guin, J. Paragas, P. Olivo
Apath, LLC, Saint Louis, MO 63141, USA; United States Army Medical Research Institute for Infectious Diseases, Virology Division, Fort Detrick, MD 21702, USA

11:45 35. Targeting of HPV31 Episomal DNA with Compounds Designed to Bind the Origin of Replication (ori)
Chris Fisher, Peggy Garner-Hamrick, Megh Singh, Kathleen Crowley, Barbara Schweitzer, Scott Woodard, James Bashkin
Pharmacia, Kalamazoo, MI, USA; Pharmacia, St. Louis, MO, USA; University of Missouri – St. Louis, St. Louis, MO, USA; NanoVir, LLC; Pfizer, St. Louis, MO; Monsanto, St. Louis, MO, USA

12:00 36. High Potency Silencing by Boranophosphate Sirna: Synthesis, Properties, and Silencing Activities
Jing Wan, Zinaida Sergueeva, Allison Hall, Kenneth Alexander, Barbara-Ramsay Shaw
Department of Chemistry, Duke University, Durham, NC 27708, USA; Department of Pediatrics, Duke University Medical School, Durham, NC 27708, USA

12:15 Late-breaker Presentation

12:30 *Lunch*
Local Restaurants

Thursday, May 11, 2006

Oral Session VI: Retroviruses II, Coxsackie, and Late Breaker Presentations
San Gerónimo Ballroom
Chairs: Susan B. Cox and Robert W. Eisinger

14:00 37. Discovery of GS9148, a Novel Nucleotide HIV Reverse Transcriptase (RT) Inhibitor
R. Mackman, C. Boojamra, J. Chen, J. Douglas, D. Grant, C. Kim, K.-Y. Lin, D. Markevitch, V. Prasad, A. Ray, L. Zhang, T. Cihlar
Gilead Sciences, Foster City, CA 94030, USA

14:15 38. Synthesis and Structure Activity Relationship of a Novel Series of Cyclopropyl-based CCR-5 Antagonists
Christopher Aquino, Jennifer Peckham, Robert Ferris, Wieslaw Kazmierski, Terrence Kenakin, Ed McLean, Angilique Svolto, Christian Watson, Michael Youngman
Department of Medicinal Chemistry; Department of Virology; Assay Development and Compound Profiling, Glaxo-SmithKline, Research Triangle Park, NC 27709, USA

14:30 39. *N*-Aminoimidazole Derivatives Inhibit HIV-1 Gene Expression by an Unexploited Mechanism of Action
Miguel Stevens, Irene Lagoja, Jan Balzarini, Arthur Van Aerschot, Piet Herdewijn, Erik De Clercq, Christophe Pannecouque
Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

14:45 40. Key Mutations of the 69 Insertion Complex of Multidrug-Resistant Hiv-1 Reverse Transcriptase
Clara E. Cases-González, Sandra Franco, Miguel A. Martínez, Luis Menéndez-Arias
Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid, Spain; Fundació irsiCaixa, Hospital University Germans Trias i Pujol, Badalona, Spain

3:00 41. Thiazolobenzimidazoles as Potent Inhibitors of the In Vitro Replication of Coxsackie B Virus
Armando M. De Palma, Ward Heggermont, Pieter Leyssen, Erik De Clercq, Angela Rao, Anna-Maria Monforte, Alba Chimirri, Johan Neyts
Rega Institute for Medical Research, University of Leuven, Leuven, Belgium; Dipartimento Farmaco-Chimico, Università di Messina, Messina, Italy

3:15 Late Breaker Presentation

3:30 Late Breaker Presentation

3:45 Adjournment of 19th I.C.A.R.

Oral Session I: Retroviruses

1

Conceptually Novel HIV Integrase Inhibitors with Nucleobase Scaffolds: Discovery of a Highly Potent Anti-HIV Agent

Vasu Nair[1], Guochen Chi[1], Arthur Cox[1], Roger Ptak[2], Nouri Neamati[3]

[1]Department of Pharmaceutical and Biomedical Sciences and The Center for Drug Discovery, University of Georgia, Athens, GA 30602, USA; [2]Department of Infectious Disease Research, Southern Research Institute, Frederick, MD 21701, USA; [3]Department of Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90089, USA

Enzymes of the pol gene of HIV have been identified as important viral targets for the discovery anti-HIV therapeutic agents. While the viral targets, HIV reverse transcriptase and HIV protease, have been successfully investigated for the development of clinically useful therapeutic agents, research efforts on drug discovery on the third enzyme of the pol gene, HIV integrase, have not resulted in a single FDA-approved drug. Nevertheless, as integrase is essential for HIV replication, it remains an attractive target for the discovery of new anti-HIV agents. In this presentation, we report the discovery of a conceptually new beta-diketo acid, constructed on a nucleobase scaffold, that is a potent inhibitor of both the 3′-processing and strand transfer steps of recombinant HIV integrase. This inhibitor and the positive control compound, AZT, were tested in a PBMC cell-based, microtiter anti-HIV assay against the clinical isolate, HIV-1TEKI (NSI phenotype) and HIV-1NL4-3 (SI phenotype), and in a MAGI-X4 assay against HIV-1NL4-3 with HeLa-CD4-LTR-beta-gal cells. Our integrase inhibitor was found to have highly potent in vitro anti-HIV activity and efficacy. The discovery of this remarkably active molecule, representative of a unique set of diketo acids bearing nucleobase scaffolds, has uncovered a new chapter in the chemistry and biology of integrase inhibitors and their potential therapeutic applications.

2

Inhibition of Human Immunodeficiency Virus Type 1 Infection in Macrophages by Alpha-v Integrin Ligands

Berta Bosch[1], Imma Clotet-Codina[1], Julia Blanco[1], Eduard Pauls[1], Gemma Coma[1], Samandhy Cedeño[1], Francesc Mitjans[2], Anuska Llano[1], Margarita Bofill[1], Bonaventura Clotet[1], Jaume Piulats[2], Jose Este[1]

[1]Retrovirology Laboratory, Fundacio IrsiCaixa, Badalona, Spain; [2]Laboratorio de Bioinvestigación, Merck Farma y Química, Barcelona, Spain

Macrophages are key cells for HIV infection and spreading inside the organism. Macrophages cultured in vitro can be successfully infected after differentiation with cytokines such as macrophage colony stimulating factor (M-CSF). In the monocyte to macrophage differentiation process with M-CSF, av-integrins are upregulated concomitantly to the capacity of HIV to generate a productive virus infection. In the present study we show that an anti-av antibody, 17E6, inhibited HIV-1 infection of primary macrophages. The effect of 17E6 on R5 or X4 HIV-1 replication in acutely infected macrophages was dose-dependent, with a 50% effective concentration (EC50) of $17 \pm 2\,\mu$g/ml in the absence of cytotoxicity. Similarly, a monoclonal antibody targeting the avb6 integrin (14D9.F8) also inhibited HIV-1 infection in this cell type. 17E6 reduced the detection of HIV-1 BaL proviral DNA in acutely infected macrophages but was completely ineffective against HIV-1 BaL production in chronically infected macrophages, suggesting that 17E6 inhibited HIV infection at an early stage of the virus cycle. Finally, a small molecular weight antagonist of the avb6 integrin reduced HIV replication at subtoxic concentrations. Therefore, our results suggest that av-containing integrins could play a role in HIV replication in macrophages and indicate that small molecular weight compounds may be developed to interfere with HIV replication in macrophages through the interaction with av integrins.

3

Phosphorothioate Oligonucleotides Inhibit HIV-1 Fusion By Blocking Gp41 Core Formation

Andrew Vaillant[1], Hong Lu[2], Shuwen Liu[2], Carol Lackman-Smith[3], Roger Ptak[3], Jean-Marc Juteau[1], Shibo Jiang[2]

[1]REPLICor Inc., Laval, Que., Canada; [2]F. Lindsay Kimball Research Institute, New York Blood Center, New York, NY, USA; [3]Southern Research Institute, Frederick, MD, USA

The sequence independent antiviral activity of phosphorothioate oligonucleotides in inhibiting HIV-1 by blocking interactions between the v3 loop and CD4 has been previously described. This activity was attributed to their polyanionic activity. Here we show that PS–ONs (and their fully 2′-*O*-methylated derivatives) are also potent inhibitors of HIV-1-mediated membrane fusion and HIV-1 replication in a sequence-independent, size-dependent (optimal size ~50–60 bases) and phosphorothioation dependent manner (independent of stabilization). PS–ONs interact with the heptad repeat regions of gp41 and the HIV-1 fusion inhibitory activity of PS–ONs is closely correlated with their ability to bind to these heptad repeats and block gp41 six-helix bundle formation, a critical step during the process of HIV-1 fusion with the target cell. The requirement for PS–ON interaction was also found to be dependent on phosphorothioation, suggesting that the v3 loop/PS–ON interaction may also have a hydrophobic component. The increased hydrophobicity of longer ($\geq$40 base) PS–ONs may contribute to their inhibitory activity against HIV-1 fusion and entry because these longer PS–ONs have a greater hydrophobicity and are more potent in blocking the hydrophobic interactions involved in the gp41 six-helix bundle formation than shorter PS–ONs (<30 bases). This novel antiviral mechanism of action of long PS–ONs has important implications for therapy against infection by HIV-1 and other enveloped viruses with type I fusion proteins.

4

Stereoselective Synthesis and Biological Evaluation of D- and L-*carba*-Nucleosides as Potential Antiviral Agents

Chris Meier[1], Soenke Jessel[1], Bastian Reichardt[1], Olaf Ludek[1], Jan Balzarini[2]

[1]University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany; [2]Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

Carbocyclic nucleoside analogues like abacavir showed very interesting antiviral properties. Therefore, we are interested in a convenient stereoselective access to this class of compounds as potential antiviral agents.

By using a new convergent synthetic strategy, starting from a chiral cyclopentenol, enantiomerically pure carbocyclic thymidine (*carba*-dT) was obtained as a key intermediate for further variations at the 3′-position. This pathway allows an entry to D- and L-configurated nucleoside analogues. However, using this approach a mixture of side products avoids the formation of the product in very high yields. However, we will present that the side products can be recovered by a stereoselective hydroboration leading to one intermediate only that can be use as well for the synthesis of carbocyclic nucleosides. The condensation of the carbocyclic moiety and different pyrimidine and purine nucleobase was achieved by a Mitsunobu reaction. Various analogues have been prepared via this strategy, e.g. D- and L-*carba*-BVDU, nucleoside analogues known to be antivirally active against HSV-1. Additionally, carbocyclic α-nucleosides and carbocyclic *iso*-nucleosides are accessible by this reaction sequence. All new nucleoside analogues were tested for their antiviral activity. Particularly *carba*-dT was found to be a potent anti-HIV active derivative showing no toxicity. However, it can not be excluded that a non-activity of a compound is related to a missing phosphorylation to the monophosphate. In order to prove that, all nucleosides were converted into their *cyclo*Sal-phosphate trimesters and transferred into the nucleotides. Detailed chemistry, enzymatic and antiviral activity data will be presented. In some cases the nucleotide releasing system showed improved antiviral activity as compared to the parent nucleoside.

5

D-Ala-Peptide T-Amide (DAPTA) Strongly Suppresses HIV-1 Replication in Human Primary Macrophages and Prevents HIV-1-Related Neuronal Apoptosis

Michela Pollicita[1], Candace Pert[2], Maria-Teresa Polianova[2], Alessandro Ranazzi[1], Michael Ruff[2], Carlo-Federico Perno[1], Stefano Aquaro[1]

[1]University of Rome Tor Vergata, Italy; [2]Georgetown University, Washington, DC, USA

Monocytes/macrophages (M/M) are a strategic reservoir of HIV-1 commonly infected by CCR5-using (R5) strains of HIV-1. CCR5 is an attractive target for inhibition of CCR5 mediated HIV-1 entry. Thus, CCR5 antagonists are expected to be a powerful new class of receptor-based therapeutic agents against HIV-1 infection. D-Ala-Peptide T-Amide (DAPTA) is an octapeptide derived from the gp120 V2 region of HIV-1, able to bind CCR5. DAPTA acts as selective viral entry inhibitor, displacing the binding of gp120 with CCR5. DAPTA anti-HIV-1 activity was evaluated in M/M infected with two different HIV-1 R5 strains, BaL and 81A, in presence of several doses of the compound. DAPTA inhibited HIV-1 replication in M/M (>80% compared to control), measured by the p24 gag Ag released in the cell culture supernatants, at concentration of 10-3 nM. PCR analysis of integrated HIV-1 proviral DNA on cultured M/M proved that DAPTA is able to block HIV entry and so, to prevent HIV infection in M/M. Moreover, the capability of different HIV-1 R5 strains produced and released by infected M/M in affecting neuronal homeostasis was assessed in a neuroblastoma cell line, SK-N-SH, expressing CCR5. In SK-N-SH were evaluated cell morphology, propidium iodide binding and fluorescence-activated cell sorting (FACS) analysis. DAPTA, at concentration of 10-3 and 10-4 nM, strongly inhibited apoptosis in SK-N-SH of 58 and 56%, respectively, compared to control. Unexpectedly, TAK-779 (a nonpeptidic CCR5 antagonist with potent anti-HIV-1 activity) inhibited apoptosis only of 30% compared to control. Our results suggest that the development of new anti-HIV-1 compounds, such as DAPTA, could be important in synergistic combination with other antiretroviral treatments in prevent both central nervous system HIV-infection and the consequent neural damage. The mechanisms of DAPTA inhibition may include both suppression of HIV-1 R5 strains in the brain as direct inhibition of HIV-1 replication in M/M and gp120 related damage by CCR5 binding.

6

Chemotherapy of Human Immunodeficiency Virus by Pradimicin A: A Novel Therapeutic Concept for Treatment of Glycosylated Enveloped Viruses

Jan Balzarini[1], Kristel Van Laethem[1], Sigrid Hatse[1], Antonella Bugatti[2], Marco Rusnati[2], Stefano Aquaro[3], Carlo-Federico Perno[3], Yasuhiro Igarashi[4], Toshikazu Oki[5], Dominique Schols[1]

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Pradimicin A (PRM-A) is an antifungal non-peptidic benzonaphtacenequinone antibiotic that specifically inhibits human immunodeficiency virus (HIV) in cell culture. It markedly suppresses a variety of different HIV-1 clades in PBMCs, HIV-1 (BaL) in primary macrophages and several HIV-2 and SIV strains in laboratory cell lines (range of 50% effective concentrations: 0.69–11 μg/ml; 50% cytostatic concentration: >50 μg/ml). PRM-A also inhibits syncytium formation between persistently HIV-1-infected HUT-78 cells and uninfected Sup

T1 cells. PRM-A behaves as an artificial lectin that selectively binds mannose-containing glycans. Consequently, Biacore experiments revealed that it binds to gp120 of HIV-1/MN in the presence of Ca^{2+}. PRM-A is endowed with a high genetic barrier with regard to drug resistance development against HIV-1. A variety of multiple mutations at *N*-glycosylation sites in HIV-1 gp120 are required before the virus looses marked sensitivity to the drug. There is no clustered pattern of HIV-1 gp120 glycan deletions that occur under PRM-A drug pressure. The resistance spectrum and mode of action is unique among any of the existing anti-HIV drugs and warrant further (pre)clinical investigations.

Acknowledgement: This research was supported by the Flemish "Fonds voor Wetenschappelijk Onderzoek," the Centers of Excellence of the K.U. Leuven (No. EF/05/15), and the European Commission (EMPRO).

7

Identification and Optimization of VIRIP—A Natural Occurring Peptide Blocking HIV-1 Entry by Interfering with the Gp41 Fusion Peptide

Jan Muench[1], Ludger Ständker[2], Knut Adermann[2], Axel Schulz[2], Michael Schindler[2], Raghavan Chinnadurai[2], Wolf-Georg Forssmann[2], Frank Kirchhoff[2]

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A variety of components in human blood might influence HIV-1 replication in infected individuals. Peptide libraries derived from hemofiltrate (HF), an aqueous blood solution, contain essentially all circulating blood peptides with a molecular mass below 30 kDa, including chemokines, defensins, and cytokines.

To identify the most potent natural occurring factors inhibiting HIV-1 replication, we screened a HF-derived peptide library for antiviral activities. The most active fraction contained a 20-residue peptide corresponding to a C-terminal fragment of α1-antitrypsin (α1-AT), a highly abundant serine proteinase inhibitor. Further analysis of the corresponding chemically synthesized peptide, termed virus inhibitory peptide (VIRIP), demonstrated that it inhibits infection by all HIV-1 variants tested, independently of their subtype and coreceptor usage. Notably, VIRIP also blocked multi-resistant HIV-1 variants and primary isolates. VIRIP specifically inhibited HIV-1 Env function, and did not affect infection by virions containing HIV-2, SIV, MLV, HCV, Ebola or VSV Env proteins. The antiviral activity proved to be highly specific for the 20-residue VIRIP sequence since structurally closely related peptides were inactive. We found that VIRIP inhibits hemolysis of erythrocytes induced by the HIV-1 gp41 fusion-peptide (FP). NMR spectroscopy confirmed that VIRIP interacts directly with synthetic gp41 FP. Our observations are evidence that a naturally occurring human substance inhibits HIV-1 infection by a new mode of action, i.e. binding of the highly conserved FP. Furthermore, we performed a structure-activity-relation study with more than 350 VIRIP analogs and found that specific amino acid changes enhanced the antiviral potency of VIRIP by up to two orders of magnitude. Experiments in cell culture and animal models further demonstrated that VIRIP exerted no cytotoxic effects. Thus, VIRIP derivatives might become a new class of HIV-1 entry inhibitors.

8

Characterization of gp41 Evolution in a Large Cohort of HIV Infected Patients Receiving T20 Therapy as a Single Active Drug

Stefano Aquaro[1], Valentina Svicher[1], Roberta D'Arrigo[2], Ubaldo Visco-Comandini[2], Andrea Antinori[2], Mario Santoro[1], Giovanni Di Perri[3], Sergio Lo Caputo[4], Pasquale Narciso[2], Carlo-Federico Perno[1,2]

[1]University of Rome Tor Vergata, Italy; [2]INMI L. Spallanzani, Italy; [3]University of Turin, Italy; [4]SM Annunziata Hospital, Florence, Italy

To investigate gp41-variability and correlation with viro-immunological parameters in 54 HIV-infected patients (pts) receiving T20 added as a single active drug to a failing regimen.

Two hundred and ten HIV-gp41 sequences and clinical follow-up from 54 T20-treated patients were analyzed from baseline up to 48 weeks (weeks) of treatment. The association of mutations with viremia (VL)/CD4 count (c/ul) was assessed by Mann–Whitney test.

The addition of T20 to the failing antiretroviral regimen induced at 4 weeks a significant VL decrease from 5.1 log (stable in the last 12 weeks prior T20) to 4.3 log ($p = .0002$) and a significant CD4 increase from 48 c/ul (decreasing in the last 12 weeks prior T20) to 106 c/ul ($p = .008$). While VL rebounded to 4.7–5 log at 12–48 weeks, respectively, CD4 increased to 129 c/ul at 48 weeks. T20 resistance mutations, absent at BL, occurred shortly after treatment and usually alone. V38A was the most common sign of T20 failure (27.8% of pts). The viro-immunological outcome of T20-treated pts varied according to gp41-mutations. V38A/E (38.5% of pts) was associated with a CD4 increase from BL (48 c/ul) of 4.5-fold (142 c/ul) at 24 weeks and 6.0-fold (196 c/ul) at 36 weeks ($p = .004$ and .02 compared without V38A/E, respectively). No significant correlation with VL was observed (from 4.9 log at BL to 4.4–4.8 at 24–36 weeks). By contrast, Q40H + L45M (11.1% of pts) was associated with CD4 loss from 71 c/ul at BL to 26 c/ul at 36 weeks ($p = .02$), without significant changes in VL (from 5 log at BL to 5 log at 36 weeks). Mutation N126K (observed in 6 pts, but never found at BL) abrogates the 4th gp41-glycosylation site and correlated with 1.7-fold CD4 increase at 24 weeks.

Conformational changes induced by V38A/E in the highly conserved GIV motif of gp41-HR1, are tightly related with a loss of HIV-induced damage of immune system. This facilitates CD4-recovery through mechanisms that can be virus-(loss of fusion efficiency) and immune-mediated (exposure of new epitopes) not applicable to Protease/RT-inhibitors, and thus important for innovative therapeutic strategies.

Oral Session II: Respiratory Viruses

9

Oseltamivir Protects Ferrets Against Lethal H5n1 Influenza Virus Infection

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The spread of highly pathogenic H5N1 influenza viruses in humans in Asia, with high mortality rates among infected individuals is a major public health concern. In the absence of a vaccine antigenically matching the pandemic virus, antiviral drugs can play an important role. In the present study we reported the antiviral activity of neuraminidase inhibitor oseltamivir against lethal H5N1 influenza virus infection in ferrets, an appropriate animal model that closely resembles clinical signs of human influenza. Inoculation of young adult ferrets with a viral dose as low as 10^2 EID_{50} of A/Vietnam/1203/04 (H5N1) influenza virus caused high fever (39.8–41.8 °C), weight loss (25.4% of initial), anorexia, extreme lethargy and death of animals on days 7–10 post-virus inoculation (p.i.). Oral administration of oseltamivir at a dose of 5 mg/kg/day for 5 days twice daily initiated 4 h p.i. inhibited the febrile response, reduced weight changes (14.6% of initial) and, most importantly, completely protected ferrets from lethal H5N1 infection. In the treatment groups, virus replication in the upper respiratory tract of ferrets was prevented, whereas untreated animals shed virus at titers of 2.8–6.5 $\log_{10}EID_{50}$/ml on days 3, 5 and 7 p.i. Systemic spread of the H5N1 virus was observed in untreated ferrets: virus was detected in multiple internal organs, including the brain. Treatment with oseltamivir resulted in complete inhibition of virus replication in the lungs and small intestine on day 5 p.i. In the brains of treated animals virus was detected in one of the two animals tested with >100-fold reduction of titer. Sequence analysis showed no amino acid substitutions at conserved residues in NA or HA1 subunit in viruses isolated from ferret's internal organs after treatment. These results suggest that oseltamivir earlier treatment can prevent H5N1 mortality in ferrets, however, further studies investigating optimal doses and treatment durations required to achieve protection against infection with highly pathogenic influenza viruses are much needed.

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Advantages of Combination Chemotherapy for Highly Pathogenic A/Vietnam/1203/04 (H5n1) Influenza Virus in Mice

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In the present study we tested in the mouse model the hypothesis that combination chemotherapy with drugs targeting different virus proteins may lead to more potent and beneficial effects. We applied plasmid-based reverse genetics technique to generate two recombinant A/Vietnam/1203/04-like (H5N1) viruses. One virus possessed asparagine at position 31 of the M2 protein that was found in the naturally circulating virus (rgVN-1203) and confers resistance to amantadine. The other recombinant virus possessed serine at that position and was sensitive to amantadine (rgVN-1203sens). BALB/c mice were administered oseltamivir (1 or 10 mg/kg/day) and amantadine (1.5 or 15 mg/kg/day) twice daily for 5 days by oral gavage; the first doses were given 24 h before inoculation with 10 MLD50 of H5N1 virus. Combination treatment with 10 mg/kg/day oseltamivir and 15 mg/kg/day amantadine was given on the same schedule. Single-drug oseltamivir produced a dose-dependent antiviral effect against both recombinant H5N1 viruses ($p < 0.01$). Treatment with oseltamivir at dosage of 10 mg/kg/day significantly inhibited virus replication in the lungs, brain, spleen, and blood of mice at days 3, 6, and 9 after inoculation ($p < 0.05$), but resulted in low survival rate (20%). Single-drug amantadine showed dose-dependent effect only against rgVN-1203sens strain. Notably, risk of death for mice that received 15 mg/kg/day of amantadine or 10 mg/kg/day of oseltamivir was similar ($p < 0.05$). In contrast, prophylactic treatment of mice with combinations of oseltamivir and amantadine completely inhibited virus replication in the animals infected with rgVN-1203sens ($p < 0.05$) compared to single-drug usage and protected 90% of animals. Importantly combination chemotherapy completely protected H5N1 virus spread to the brain of the mice: virus was not detected in brain of treated animals on days 3, 6, and 9 after inoculation and neurological symptoms were not observed. Our results suggest that combination chemotherapy provides an advantage over single-agent treatment. This strategy could be an option for the control of influenza virus infection, and combinations with other novel drugs should be explored.

11

Proteomic Profiling of Host Cellular Proteins Incorporated by Severe Acute Respiratory Syndrome (SARS)-Associated Coronavirus Virions: Insights into Emerging Virus Biology and New Therapeutics Targets

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In late 2002, severe acute respiratory syndrome (SARS) became the first new severe and easily transmissible human disease to emerge in the 21st century. Although it abated after six months, SARS serves as a modern paradigm for human emerging infections with 772 deaths reported from 29 countries. The causative agent was found to be a new SARS-associated coronavirus (SARS-CoV). While the sequence of SARS-CoV genome was first reported by the BC Genome Sciences Center, the full set of

viral and cellular proteins that compose the SARS-CoV virion remains unknown.

To approach this problem, we have utilized two-dimensional gel electrophoresis and liquid chromatography-tandem MS (LC–MS/MS) to identify the viral and cellular proteins in purified SARS-CoV virions obtained from human infected cells [Huh7: human liver] and primate (VeroE6: monkey kidney) infected cells. Interestingly, analysis of the proteins from purified SARS-CoV preparations has revealed that the enveloped virions contain not only the predicted viral structural proteins (e.g. spike glycoprotein, nucleocapsid protein, and membrane glycoprotein) but also an important number of differentially incorporated host cellular proteins into or onto the newly formed viruses. We have unambiguously identified over 50 host cellular proteins in SARS-CoV virions by LC–MS/MS. These proteins include members of the annexin superfamily, cytoskeletal proteins, chaperones, vesicular transport proteins, uracyl-DNA glycosylases, and aldehyde oxidoreductases.

This study provides the first comprehensive and comparative analysis of the viral and cellular proteins that compose infectious particles of SARS-CoV obtained from human and primate infected cells. The functions of these newly identified host-specific proteins are currently being investigated using RNA interfering systems; their contributions to structure, viral productive replication, and pathogenicity will be discussed.

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Inhibition of SARS-CoV Replication by Hydroxyethylrutosides and Mouse Interferon (MuIFN-α) in a Mouse Model

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Severe acute respiratory syndrome (SARS) is a life-threatening respiratory illness caused by SARS-CoV. There are no approved therapies for SARS. Some drugs inhibit SARS-CoV replication in vitro including human interferons and selected anti-inflammatory agents (Chihrin and Loufty, 2005. 3, 251–262). Interferons are very promising because of their potent in vitro inhibition of SARS-CoV. Although anti-inflammatory agents are not very active in vitro, it is thought that they might be efficacious in reducing any deleterious inflammatory response associated with virus infections such as SARS infections in humans. For example, troxerutin, a flavenoid with anti-inflammatory properties, is in clinical trials for treating rhinovirus (RV) infections, ameliorating RV-induced inflammation (Turner et al., 2004. APMIS 112, 605–611). Therefore, troxerutin was tested for inhibition of SARS-CoV replication in the lungs of infected mice using a mix of four hydroxyethylrutosides that included troxuretin. In addition, mouse interferon-alpha, used as a model compound for human interferon-alpha, was evaluated for inhibition of virus lung titers. Both mouse interferon-alpha administered i.p. daily beginning 12 h pre virus exposure at doses of 100,000 and 10,000 IU and the hydroxyethylrutoside mix (100 and 10 mg/kg) administered i.p using the same schedule reduced virus replication in the lungs of mice to below detectable limits. When a hydroxyethylrutoside mix was given to mice in the drinking water at 1.32 mg/ml (likely equivalent to an i.p. dose of 100 mg/kg, assuming that the mice drank freely), virus lung replication was also completely inhibited. All treatments appeared to be well tolerated, since all groups of mice gained weight. We also report on the efficacy of various combinations of two doses of these drugs administered i.p., using the same dosing regimen as described. These data support the supposition that interferon might be a useful therapy for treating human SARS infections and that hydroxyethylrutosides should be investigated further as a potential therapy.

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Small Molecule Inhibitors of Respiratory Syncytial Virus

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Treatment options for human respiratory syncytial virus (RSV) are limited. An effective vaccine is not yet available. Neutralizing polyclonal antibody (RespiGam^{TM}, MedImmune) and a humanized monoclonal antibody (Synagis^{TM}, MedImmune), are licensed for prophylactic use. Ribavirin is the only approved antiviral against RSV, but its efficacy is controversial and its use is limited to treatment of high-risk patients. There is a clear need for new anti-RSV therapeutics with improved efficacy and ease-of-use. Many early efforts to identify anti-RSV compounds focused on blocking the process of fusion. We have developed a cell-based screening platform to identify antivirals that inhibit RSV transcription and replication. The assay does not require infection with wild-type virus. It is based on an RSV subgenomic replication system in baby hamster kidney (BHK-21) cells that express the essential viral replication proteins (N, P, L and M2-1). The readout is expression of the reporter gene *lacZ* from a subgenomic RNA. Screening of the Apath small molecule library yielded 596 compounds (hit rate = 0.75%) with EC50 values $\leq$10 μM and with selectivity index (SI) values $\geq$10. Seventy-two compounds demonstrated antiviral activity against wild-type RSV (strain A2) in a cytopathic effects inhibition assay (EC50 < 10 μM; SI > 10). These anti-RSV compounds represent nine different chemical classes. Two compounds, a 4-aminoquinoline (EC50 = 0.25 μM) and a thienopyrimidine (EC50 = 0.82 μM), were shown to have desirable pharmacokinetic profiles and were chosen for efficacy testing in the cotton-tail rat model of infection. SAR studies to identify the pharmacophore of the compounds have been initiated. Preliminary studies to characterize the mechanism-of-action in virological assays will be discussed.

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Oral Session III: Herpesviruses

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Pre-Clinical Development of the BCNAs: The Most Potent Anti-VZV Agents Reported to Date

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We have previously reported bicyclic furano pyrimidine nucleoside analogues (BCNAs) as exquisitely potent and selective inhibitors of Varicella Zoster Virus (VZV) (McGuigan et al., 1999), with subnanomolar activity for *p*-alkylphenyl substituted analogues such as lead compound Cf1743(CF-1743) (**1**) (McGuigan et al., 2000). These compounds have entered preclinical development with FermaVir Pharmaceuticals.

We now report the first chromatography-free synthesis of these agents, their scale up to multi-gramme amounts, and their pre-clinical characterisation.

In addition, we were keen to address potential solubility and bioavailability issues of these highly lipophilic agents by the synthesis of more polar analogues in two categories; side-chain ethers (**2**) as new analogues in their own right, and 5′-phosphates (**3**) as potential more soluble ProDrugs. We report data on both of these new families at this meeting.

Finally, we note the application of our phosphoramidate ProTide approach to this family, with a series of BCNA ProTides (**4**) designed as intracellular phosphate delivery forms to bypass the essential VZV thymidine kinase-mediated first phosphorylation step.

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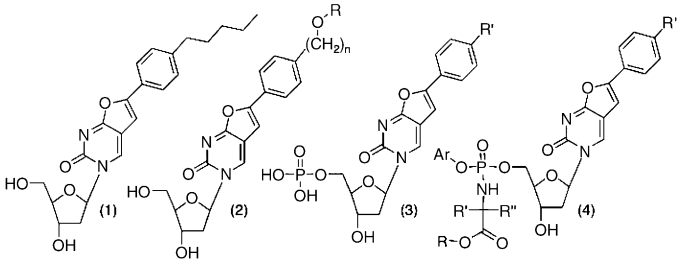

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Organotypic Epithelial Raft Cultures as a Model for Evaluation of Antivirals Against Varicella-Zoster Virus (VZV)

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Varicella (chickenpox), the primary infection caused by VZV, is characterized by viremia and skin lesions. Reactivation of the latent virus results in skin lesions characteristic of herpes zoster (shingles). As keratinocytes are one of the main target cells for productive infection in vivo for VZV, human epithelial cells represent a relevant model for the study of VZV pathogenesis and evaluation of antiviral compounds. Organotypic epithelial raft cultures permit full differentiation of keratinocytes via culturing of the cells on collagen matrix at the air–liquid interface. We have previously shown that the susceptibility of cultures to infection with VZV depends on the stage of differentiation of the rafts. We have now quantified the activity of reference anti-VZV compounds by measuring viral DNA load by real-time PCR. Quantitative PCR for VZV DNA was performed by using specific primers and a MGB-probe for the ORF29 gene (single-stranded DNA binding protein) by the Taqman method. Two series of raft cultures were infected with the wild-type Oka strain after 4 days of differentiation and treated with serial dilutions of the test compounds. At 12 days post-differentiation one series of the cultures was processed for histology and the other one for viral DNA quantification. Acyclovir (ACV), Penciclovir (PCV) and Brivudin (BVDU) at 4 and 0.4 μg/ml, foscarnet (PFA) at 40 μg/ml and cidofovir (CDV) at 4, 0.4 and 0.04 μg/ml inhibited viral DNA content by more than 95%. These results were in agreement with histological examination of the rafts, no cytopathic effect being observed at these concentrations. As expected, only CDV and PFA inhibited the replication of the thymidine-kinase deficient (TK-) 07-1 strain. A correlation between the degree of protection as determined by histological examination and viral quantification could also be demonstrated for CDV and PFA against the TK-07-1 strain. Since no animal model is available for the in vivo evaluation of antiviral agents against VZV, the organotypic cultures may be considered as a valuable ex vivo model to evaluate the efficacy of new anti-VZV antivirals.

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Identification of New Benzimidazole D-Ribonucleosides that Inhibit HCMV Packaging Proteins

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DNA packaging is the key step in viral maturation and involves binding and cleavage of viral DNA containing specific DNA-packaging motifs. This process is mediated by a group of specific enzymes called terminases. We have previously demonstrated that the HCMV terminase is composed of two subunits, the large one encoding pUL56 and the small pUL89, where each protein has a different function. While the large subunit mediates sequence specific DNA binding and ATP hydrolysis, pUL89 is only required for duplex nicking. Inhibitors targeting pUL56 and/or pUL89 are attractive alternatives as HCMV antivirals since mammalian cell DNA replication does not involve cleavage of concatameric DNA. We now have screened several members of the benzimidazole ribonucleoside class of replication inhibitors in order to determine if a compound has the capacity to block the ATPase activity of the large terminase subunit pUL56. Analysis by bioluminometric ATPase activity assays identified BDCRB and one more compound [2-bromo-4,5,6-trichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (BTCRB)] with inhibitory effects. Although only BTCRB and BDCRB were inhibitors of the ATPase activity, two other compounds, dBDCRB and Cl4RB, inhibited virus replication in a plaque-reduction assay, thus indicating that those have a different mode of action. In addition, by electron microscopy of thin sections we observed that in the presence of BTCRB only B-capsids and dense bodies were formed. Furthermore, spherical capsids accumulated in the perinuclear cisternae indicating a block in nuclear egress thereby providing additional evidence that closely-related benzimidazole D-ribonucleosides may have differences in their antiviral modes of action.

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Foldamer-Based Inhibitors of Cytomegalovirus Entry

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Human cytomegalovirus (HCMV) is the cause of significant morbidity and mortality in a variety of immunocompromised patients. Currently available anti-HCMV drugs interfere with DNA replication; however, these drugs are highly toxic, precluding their long-term use in humans. Interrupting HCMV viral entry is largely unexplored as an antiviral drug development strategy and is potentially an ideal and tractable goal. HCMV is believed to rely upon formation of α-helical coiled coils in the viral glycoproteins gB and gH to promote virus–host membrane fusion; peptides encompassing heptad repeat sequences in these two proteins inhibit viral infection. We have explored non-natural oligomeric molecules ("foldamers") that are designed to mimic elements of the putative α-helical segment of gB. This effort has led to the discovery of oligomers of β-amino acids ("β-peptides") that block HCMV infection. The β-peptide scaffold offers several advantages for the design of protein-protein interaction inhibitors, as β-peptides are amenable to modular synthesis, resist proteolytic degradation, and can display large and tailored molecular surfaces. The most potent β-peptide inhibitor blocks HCMV infection with a micromolar IC_{50} in a cell-based assay. These compounds show specificity for HCMV relative to closely related viruses. Mechanistic studies suggest that these inhibitors interfere with membrane fusion between HCMV particles and host cells. Current efforts are focused on understanding in greater detail the origin of the observed biological activity, exploring other foldamer scaffolds as bases for inhibitor design, and developing specific fusion inhibitors for other herpesviruses.

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Nonsteroidal Anti-Inflammatory Drugs, Indomethacin and Aspirin, Inhibit Herpes Simplex Virus Replication and Block Activation of Nuclear Factor-KappaB

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Previous reports have indicated that herpes simplex virus (HSV) activates nuclear factor-kappaB (NF-kB) during productive infections. Nonsteroidal anti-inflammatory drugs (NSAIDs) have significant inhibitory effects on NF-kB. Therefore, two NSAIDs, indomethacin and aspirin, were assayed for anti-herpetic effects and utilized as tools to further study the role of NF-kB in HSV-1 infection. We report that indomethacin and aspirin inhibited HSV-1 replication at non-cytotoxic doses. In Vero cells, 500 uM indomethacin and 20 mM aspirin reduced HSV-1 titers 99.999 and 99.5%, respectively. Electromobility shift assays revealed that HSV-1 activation of NF-kB is inhibited by the NSAIDs at doses that coincide with reduction of HSV-1 titers. To investigate a pathway for NF-kB inactivation, protein levels of IkB-alpha, a cytoplasmic NF-kB inhibitor, were examined. IkB-alpha protein was present in uninfected samples, but decreased over time in all HSV samples, regardless of chemical treatment, suggesting localization of NF-kB to the nucleus. Immunohistochemistry studies verified that p65, a component of the dimeric NF-kB complex, translocated to the nucleus of HSV-1 infected cells in the presence or absence of the NSAIDs. Finally, direct effects on viral gene activity were assayed by real-time RT-PCR analysis. Indomethacin and aspirin reduced

mRNA for ICP4, an essential HSV immediate-early gene, 2.9- and 2.5-fold, respectively, resulting in significant decreases of ICP4 protein. But transcriptional analysis revealed that synthesis of mRNA for thymidine kinase, an HSV early gene, was unaffected by chemical treatment. However, mRNA for glycoprotein C, an HSV late gene was undetectable in indomethacin and aspirin treated samples. Cumulatively, these data indicate that: (i) indomethacin and aspirin block HSV-1 replication and (ii) the in vitro anti-herpetic effects of NSAIDs may reside in their ability to block NF-kB activity within the nucleus, impairing activation of essentials HSV genes.

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Increasing the Relevance of Animal Models of Cytomegalovirus (CMV) Infection: A Chimeric Human-Guinea Pig CMV is Ganciclovir and Maribavir Susceptible and Produces Disease in Animals

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Species-specificity constraints preclude study of human cytomegalovirus (HCMV) in animals, necessitating the use of rodent CMVs to model human disease. However, the susceptibility of animal CMVs to clinically useful antivirals is unpredictable. For example, the guinea pig CMV (GPCMV), a uniquely valuable virus for modeling congenital CMV infection, is highly resistant to ganciclovir (GCV) at medically relevant doses. We used a molecular virological approach to test the hypothesis that GCV susceptibility could be conferred on GPCMV by insertion of the human *UL97* phosphotransferase gene into the GPCMV genome. The GPCMV genome, cloned as a bacterial artificial chromosome in *E. coli*, was modified by site-specific recombination, using a shuttle plasmid targeting the *GP97* locus, and carrying the *UL97* gene from HCMV strain Towne. The resultant chimeric virus was replication competent, and was found to contain the HCMV *UL97* by Southern-blot and sequence analyses. Northern-blot revealed that a HCMV *UL97*-specific transcript was expressed with late gene kinetics. Western-blot, using a HCMV *UL97*-specific polyclonal antibody, detected protein in virus-infected cells. The chimeric virus was GCV-susceptible, compared to wild-type GPCMV, with an IC_{50} of 15 μM. Chimeric virus also exhibited increased sensitivity to maribavir (MBV), exhibiting a 3-log reduction (compared to wild-type virus) in the presence of 50 μM MBV, and an IC_{50} of 5 μM. To study the in vivo pathogenesis of chimeric virus, cyclophosphamide-immunocompromised strain two guinea pigs were challenged intraperitoneally, resulting in evidence of disseminated infection, and mortality. Ganciclovir treatment (25 mg/kg/day) resulted in reduced weight loss, and mortality, compared to placebo. These studies confirm the key role of *UL97* in CMV antiviral therapy, and demonstrate that a 'humanized' GPCMV can be generated with altered antiviral susceptibilities. The use of BAC-based mutagenesis to generate chimeric CMVs may open new avenues into animal model evaluation of HCMV antivirals.

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Potent Antiviral Activity of REP 9 and Analogs Against Vaginal HSV-2 Infection

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Genital herpes infections are a global health problem and impact HIV/AIDS epidemic. Strategies to prevent transmission include treatment of infected subjects to suppress shedding and prophylaxis with vaginally-applied microbicides. We examined the in vitro and vivo activity of REP 9, a fully degenerate 40 mer phosphorothioated oligonucleotide against HSV-2 infection of human cervical cells and in a vaginal murine model. REP 9 has broad-spectrum anti-herpetic activity with potent in vitro activity against HSV-1, HSV-2, HCMV, VZV, EBV, and HSV-6 (Vaillant et al., submitted for publication). At a concentration of 100 μM, REP 9 inhibited 6-logs of HSV-2 infection if present during the entire experiment. Synchronized infectivity assays demonstrate that, unlike sulfonated polyanions in clinical trials, which primarily block HSV attachment, REP 9 acts at multiple steps and inhibits binding, entry and post-entry gene expression. In our in vivo studies, mice were treated once intravaginally with REP 9 or PBS control at various times prior to vaginal challenge with a lethal dose of HSV-2 strain 186 (10 log 4 pfu). REP 9 prophylaxis provided protection to mice from HSV-2 infection and disease. Protection was significant when challenged 30 min after treatment ($p < 0.01$). Additionally, treatment with an analog of REP 9, which cannot activate TLR-9 mediated immune stimulation, was at least as active as REP 9, suggesting that direct antiviral activity and not stimulation of innate immunity is the mechanism of action in vivo. Utilizing this analog, protection was significant when challenged 30 min after treatment ($p < 0.001$) with a trend toward protection when administered 60 min prior to challenge ($p = 0.07$). In summary, treatment with the REP 9 analog which has superior resistance to low pH and nuclease degradation was more effective than REP 9, in some experiments protecting 100% of mice from viral infection and disease. The testing of this pH resistant REP 9 analog in a gel formulation is currently underway.

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Oral Session IV: Hepatitis Viruses

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AVI-4065: An Antisense Approach to Active HCV Infection; Preclinical and Clinical Evaluation

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A phosphorodiamidate morpholino oligomer (PMO) designed to hybridize to a highly conserved region including the AUG translation start site of HCV, called AVI-4065, has been evaluated for efficacy, toxicity, and pharmacokinetic properties. AVI-4065 inhibits translation initiated at the AUG start site with EC50 of 308 nM (2.1 ug/ml) and shows positive cooperativity. This PMO retains most of the activity in the presence of point mutations in the HCV genome. Huh-7 cells were incubated with normal human serum (NHS) or HCV infected human serum (IS) and HCV replication observed by RT-PCR. AVI-4065 produced robust inhibition of HCV in a dose and sequence-specific manner. Studies conducted in vivo with AVI-4065 in the HCV infected Trimera mouse (XTL) show reduction in viral titer which is dose dependent with approximately 90% of mice with undetectable viral titer and the remaining mice show 1 log reduction in viral titer with 0.1 mg/mouse/day for 7 consecutive days. The fractional bioavailability of AVI-4065 from a SQ dose is approximately 1. The apparent elimination half life in rat, non-human primate and humans was 2.3, 4.5 and 11.4 h, respectively. The volume of distribution ranged from 0.6 to 1.0 l/kg and the Cmax is linearly related to the dose in mg/m^2. A phase I study in healthy volunteers in which 14 daily SQ doses of 50 and 100 mg has been completed. No serious adverse events have been observed. Treatment of infected patients is currently planned. Inhibition of HCV polyprotein synthesis is anticipated to contain therapeutic benefis of both protease inhibitors and polymerase inhibition.

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Synthesis and Anti-HCV Activity of 4′-Substituted Ribonucleosides

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Hepatitis C virus (HCV) is the causative agent of chronic liver disease, estimated to affect over 170 million people worldwide. HCV infection can progress to fibrosis, reduced liver function, hepatocellular carcinoma, and death. Currently, the standard treatment for HCV infection involves treatment with pegylated interferon in combination with the nucleoside analogue ribavirin. This treatment regimen effects a cure in approximately 40–60% of the genotype-1 (GT-1) population; therefore a significant unmet clinical need exists in HCV therapy.

Virus-encoded polymerases have proven to be excellent molecular targets for chemotherapeutic intervention in numerous viral mediated diseases. In the case of HIV, HBV and herpes virus infections, deoxy-nucleoside analogues, which act as chain terminating agents, have been shown to have invaluable clinical utility. By analogy, appropriate ribonucleoside analogues might be expected to inhibit the essential RNA polymerase (NS5B) encoded by HCV. Here we describe the preparation of nucleoside analogues as inhibitors of the HCV polymerase.

In our design of nucleoside analogs as potential anti-HCV agents, we chose to investigate the effect of 4′-substituted ribonucleoside derivatives. We reasoned that after incorporation of a ribonucleoside containing a 4′-substituent, a disruption in elongation of the growing RNA could be effected through either steric hindrance or via a conformational change of the carbohydrate moiety. Our investigations on several such analogues will be presented. Of particular interest is 4′-azido-cytidine, which shows good activity in the genotype 1b sub-genomic replicon (IC50 = 1.28 μM) with no measurable cytotoxic or cytostatic behavior. In addition, we have shown that the triphosphate of 4′-azido-cytidine is a potent and highly selective inhibitor of NS5B (IC50 = 0.32 μM).

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NIM811: An HCV Replication Inhibitor of Novel Mechanism, Exhibits Potent Antiviral Activities Alone or in Combination with a Non-nucleoside HCV Polymerase Inhibitor

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Current drug discovery efforts for hepatitis C virus (HCV) focus on developing specific inhibitors of two viral enzymes, NS5B polymerase and NS3-4A protease. However, resistant viral mutants are likely to emerge during therapy, compromising the effectiveness of these inhibitors. An alternative and complementary strategy is to target host factors that are also essential for viral replication. Cyclophilins, a family of peptidyl-prolyl isomerases and the cellular targets of cyclosporin A (CsA), present such an opportunity. It was reported recently that cyclophilin B bound to HCV NS5B polymerase and stimulated its RNA-binding activity, and that these functions were blocked in the presence of CsA (Watashi K. et al., Molecular Cell 2005). NIM811, a CsA derivative, is a more suitable candidate for HCV therapy because it binds to cyclophilins with higher affinity than CsA while lacking the immunosuppressive activity associated with CsA. Using the HCV replicon system we demonstrated that NIM811 exhibited potent anti-HCV activities in vitro. Moreover, the combination of NIM811 with a specific non-nucleoside inhibitor of HCV polymerase led to synergistic antiviral effects

with no significant increase of cytotoxicity. Resistant clones against both inhibitors were obtained in vitro, however, it was much more difficult to generate resistance against NIM811 than the polymerase inhibitor. Also, there was no cross-resistance between the two inhibitors. Finally, addition of NIM811 to the HCV polymerase inhibitor drastically reduced the emergence of resistance compared to polymerase inhibitor alone. Taken together, NIM811, with a novel mechanism of action and a favorable pharmacokinetics and safety profile, represents a promising clinical candidate for treating hepatitis C and provides a rationale for specific combination therapy.

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R1479 is a Highly Selective Inhibitor of NS5B-dependent HCV Replication and Does Not Inhibit Human DNA and RNA Polymerases

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The nucleoside analog R1479 was identified as a specific inhibitor of HCV replication in subgenomic HCV replicon cells. R1479-TP is a competitive inhibitor of CMP incorporation by HCV polymerase NS5B. In a transient replicon system R1479 inhibited HCV RNA replication driven by genotype 1b polymerase with similar potency as compared to that driven by genotype 1a polymerase. R1479-TP inhibited native HCV replicase and recombinant NS5B from genotype 1a and 1b with similar potency. In contrast, R1479-TP did not inhibit human DNA polymerases alpha, beta or gamma, including reverse transcriptase activities of DNA polymerases beta and gamma, which were highly sensitive to inhibition by AZT-TP and 3TC-TP. No significant inhibition was observed with human RNA polymerases I, II and III derived from HeLa cells. In addition, the functionally related native influenza virus RNA dependent RNA polymerase (RdRp) activity in vitro was not inhibited by R1479-TP at concentrations up to 1 mM, suggesting high selectivity for the HCV RdRp. Thus, R1479 was identified as a potent and highly selective inhibitor of HCV polymerase mediated RNA synthesis.

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Application of Stable Hepatitis C Virus (HCV)-Secreting Human Hepatoma Cell Lines for Antiviral Drug Discovery

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The study of hepatitis C virus (HCV) replication and the search for specific antiviral agents against HCV infection have been hampered by the lack of an efficient stable cell culture system of HCV infection and propagation. We have successfully constructed stable human hepatoma cell lines that contain a chromosomally integrated-genotype 2a HCV cDNA and constitutively produce and secrete high titers of infectious virus into the culture media. Transcriptional expression of the full-length HCV RNA genome is under the control of a cellular Pol II polymerase promoter at the 5′ end and a hepatitis delta virus ribozyme at the 3′ end. The resulting HCV RNA was expressed and replicated efficiently, as shown by the presence of high levels of HCV proteins as well as HCV RNA in the stable Huh7 cell lines. HCV secreted from the stable cell lines was infectious, as determined by antibody neutralization, blockage of putative HCV receptors, and inhibition of HCV replication by interferon. Our findings demonstrate the establishment of a stable cell culture system of infectious HCV production and propagation, which allows the study of the entire HCV infectious cycle. The stable HCV-secreting cell lines are now being pursued to develop high throughput screens for effective HCV inhibitors. Additionally, we established a novel and powerful HCV replication system in the mouse hepatocyte and mouse embryo fibroblasts (MEF). HCV RNA was found to replicate efficiently in both $PKR^{+/+}$ and $PKR^{-/-}$ MEF cells, demonstrating that HCV RNA replication in MEF cells is a powerful system to study host-virus interaction by using diverse gene-knockout animals. Interestingly, HCV RNA replicates more efficiently in the $PKR^{-/-}$ cell than in the $PKR^{+/+}$ cell, suggesting a role of PKR in the control of HCV RNA replication. However, IFN inhibited HCV RNA replication in the $PKR^{-/-}$ cell with an efficacy similar to that in the $PKR^{+/+}$ cell, suggesting a PKR-independent antiviral mechanism. Clearly both PKR-dependent and PKR-independent antiviral mechanisms are important for the control of HCV replication and the mediation of the IFN-induced anti-HCV response. Our studies set a stage for the development of transgenic mouse models of HCV replication and open up new avenues to study HCV and host interactions in MEFs derived from diverse gene-knockout animals.

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Novel Small Molecule Inhibitors of Hepatitis B Virus Surface Antigen Secretion

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The high levels of hepatitis B surface antigen (HBsAg)-bearing non-infectious particles in the serum of infected individuals is thought to play a role in suppressing hepatitis B virus (HBV)-specific immune response by titering out HBV-specific antibodies and lymphocytes. Current HBV therapeutics do not directly reduce this viral antigenemia. Our group has focused on the enhancement of the immune response through the inhibition of viral antigen secretion in the infected hepatocytes, with the therapeutic goal being the use of HBV vaccination for the treatment of acute and chronic infection. The high-throughput screening of a small molecule library of 80,288 drug-like compounds was undertaken to discover novel inhibitors of HBsAg secretion. Using the stably HBV-transfected, human hepatoma cell line HepG2.2.15, we developed an HTS-compatible ELISA protocol for the detection of HBsAg secreted in the culture media. The

screen resulted in 1758 initially positive hits, a hit rate of 2.2%. Subsequent retesting for activity and toxicity by MTT assay has narrowed the number of confirmed, non-toxic hits to 77, currently categorized in twelve chemical series. We have previously reported on a trio of related pyrazolo-pyridines with EC50 measurements below 5.0 μm and CC50 measurements >50.0 μm. Nascent structure-activity relationship (SAR) suggests that a central moiety of the molecules is essential to activity, with an aromatic side group contributing to potency. Among recently confirmed inhibitors, two currently under investigation include: (1) An isobutyl-acetamide with an EC50 of 87.0 nanomolar, and a CC50 of >50 μm, and (2) a carbothiamide with an EC50 of 1.6 micromolar and a CC50 of >50 μm. Measurement of secreted HBV L and M antigens and cellular markers indicated that the pyrazolo-pyridines are not specific inhibitors of viral antigens, while the isobutyl-acetamide and the carbothiamide are indeed specific. Measurement of intracellular viral DNA indicated that none of these molecules are inhibitors of replication. We will be reporting on our studies of the potency, specificity, and potential mechanisms of action of these novel anti-HBV compounds.

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Mechanistic Characterization of Entecavir Resistance in Lamivudine Resistant Hepatitis B Virus

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Background: Entecavir (ETV) is a potent competitive inhibitor of hepatitis B virus (HBV) polymerase with activity versus all three enzymatic functions including priming, minus, and plus strand DNA synthesis. Virologic rebound due to ETV resistance (ETVr) has only been observed in lamivudine resistant (LVDr) HBV (M204V/I ± L180M), and requires at least one additional change in the reverse transcriptase domain (RT) at residues T184, S202, or M250. These substitutions surround the dNTP binding site or primer grip of RT. The objectives of this work were to further characterize ETVr and its mechanism(s) using cell culture, in vitro enzyme, and molecular modeling studies.

Methods: HBV cell culture assays used transfected HepG2 cells and quantitation of released, immunocaptured HBV nucleocapsids. Gradient-purified intracellular nucleocapsids were used for in vitro RT assays. A 3D homology model based on the HIV-1 RT structure was used to model resistance changes in HBV.

Results: Reduced ETV susceptibility of ETVr HBV was observed both in culture and enzymatically in vitro. Kinetic studies showed various ETVr substitutions in LVDr HBV selectively reduced ETV-triphosphate (ETV-TP) binding (K_i) to RT without markedly changing the affinity for dGTP (K_m) or inhibition by ddGTP. ETVr RTs also displayed reduced enzymatic activity (k_{cat}) relative to wildtype and ETVr HBV appeared growth impaired. Modeling studies suggested a novel ETV-TP binding pocket in HBV RT that became constrained with ETVr changes. M250 changes in the primer grip region of RT were unique in that resistance was primarily seen during synthesis of minus strand DNA. ETVr changes in the absence of LVDr substitutions had greatly reduced impacts on ETV susceptibility, confirming models suggesting ETVr is imparted through LVDr changes.

Summary: ETV provides a high genetic barrier to resistance, requiring additional changes at residues T184, S202 or M250 along with pre-existing LVDr substitutions M204V/I ± L180M. Kinetic parameters and molecular modeling indicated that ETVr substitutions selectively affected ETV-TP binding and reduced the replication capacity of HBV.

Oral Session V: Pox, West Nile, Hemorrhagic Fever, and Papilloma Viruses

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Sequential Determination of Virus in Blood and Tissues of the Variola Cynomolgus Monkey Model of Classical Smallpox Reveals that IV Cidofovir can Effectively Treat Monkeys with Extensive Viral Burden

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A nonhuman primate (NHP) model of classical, lesional smallpox has been used to test the efficacy of intravenous (IV) cidofovir treatment. Cynomolgus macaques were infected with a high dose (10^8 PFU IV) of variola to produce an artificial primary viremia, and then treated with cidofovir at 0, 24, or 48 h postinfection (PI). Later treatment times were not evaluated. Treatment at 24 or 48 h PI halted increases in peak blood viral genome titers measured by quantitative TaqMan-MGB real-time PCR, which were more than 10-fold less in CDV-treated animals compared to placebo. Historically, the number of pox lesions provided the best correlation with human smallpox clinical severity, and CDV treatment in our model significantly reduced maximum pox lesion counts by >90%; the number and size of skin lesions, and in untreated animals contributed significantly to the total viral burden with lesions containing 10^9–10^{10} genomes/g. To better understand the role of viral burden and disease progression in major organ systems, a serial sample study was undertaken. In untreated animals at 24 h PI, viral replication in spleen exceeded 10^9 genomes/g while liver and bone marrow yielded 10^8 genomes/mL. In comparison, titers in other tissues ranged between 10^5 and 10^6 genomes/g and blood yielded 10^4 genomes/mL at 24 h, suggesting that the liver, spleen, and marrow may be initial sites of replication. Levels of virus in the bone marrow reached a peak of approximately 10^{10} genomes/g at day 5, then decreased to quantities consistent with those in blood. Viral load in the blood increased with time, peaking around days 7–9 at 10^8 genomes/mL. Virus was also detected in intestine, skeletal muscle, and late in infection, testes. The ability to successfully treat with CDV 24 h PI despite early extensive

organ infection in the accelerated NHP variola model suggests that this treatment could be effective in reducing viremia and mortality after onset of symptoms in human smallpox, which demonstrates a more protracted disease course.

Work involving variola virus conducted in WHO-sanctioned CDC, Atlanta BSL-4 laboratory.

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Evaluation of ST-246 in Vaccinia or Cowpox Virus Infections of Mice

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Although cidofovir (CDV) has been approved as an investigational new drug for emergency treatment of smallpox, its lack of oral activity and dose limiting toxicity dictates a need for continued development of better therapeutic agents for this potential bioterror disease. It has been reported previously that ST-246, a low-molecular weight compound, inhibits replication of all the orthopoxviruses in vitro and protects mice infected with vaccinia or ectomelia virus. In the present study, we have utilized cowpox virus (CV) and vaccinia virus (VV) infections in vitro and in vivo to evaluate the efficacy of ST-246 for treatment of orthopoxvirus infections. In plaque reduction assays in Human Foreskin Fibroblast cells, both CV and VV were inhibited by about 0.1–0.5 uM of ST-246. For in vivo studies, ST-246 was administered once daily by oral gavage to mice using 100 mg/kg for 5, 7, 10, or 12 days beginning 4 or 24 h after intranasal inoculation with VV or CV. ST-246 was highly effective ($p < 0.001$) in preventing mortality due to VV or CV even when treatment was delayed up to 24 h post-infection. A dosing duration of 5 days was adequate for VV infected mice, but duration of 7 days or longer was required for efficacy in CV infected mice. When ST-246 was given once daily for 14 days at 100, 30, or 10 mg/kg daily at 24, 48, or 72 h post-CV inoculation, mortality was significantly altered at all dosage levels and time points. To determine the effect of treatment on virus replication in target tissues, mice were inoculated with CV or VV and treated once daily with 50 mg/kg of ST-246. On various days post-infection tissues were harvested and assayed for virus. In CV or VV-infected mice, ST-246 treatment successfully reduced virus titers from 3 to 5 $\log_{10}$ in liver, spleen, and kidney. Little effect was noted in lung tissue. These results indicate that ST-246 has significant activity against VV and CV infections in vitro and in vivo and may be a potential chemotherapeutic agent for treatment of human orthopoxvirus infections.

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Cidofovir and Foscarnet Peptide Prodrugs

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Cidofovir (HPMPC) is a broad-spectrum anti-viral agent that is used (Vistide®) to treat AIDS-related CMV retinitis. Currently, cidofovir is of particular interest as a potential therapy for orthopox virus infections, including smallpox. An important limitation of cidofovir and analogous nucleotide drugs in a therapeutic role is their low oral bioavailability and poor transport into cells. In principle, bioavailability of a drug can be improved by structural modification targeting transporters expressed in human intestine. To be effective, the transported prodrug must be cleaved by endogenous enzymes to its parent compound. We will present synthetic studies of novel cidofovir and cyclic cidofovir (cHPMPC) prodrugs incorporating amino acids or small peptides, comparing different drug-amino acid linkage strategies. The compounds were evaluated for transporter-mediated uptake and cellular and plasma hydrolysis. The results will be compared with similar studies carried out on a series of peptidomimetic conjugates of foscarnet, the trisodium salt of phosphonoformic acid (PFA), an anti-viral agent that also has very low oral bioavailability and poor cell penetration.

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Treatment of West Nile Disease with Humanized Monoclonal Antibody After the Virus is in the Brain

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People infected with West Nile virus (WNV) most often see their physicians after showing symptoms suggestive of neurological infection. The question addressed in this study is if WNV-reactive antibody can improve disease signs in a hamster model after the virus is demonstrated to be in the brain. The hypothesis is based on the high activity of a humanized monoclonal antibody, hE16, in a mouse model when administered later in infection (Oliphant et al., 2005. Nat. Med. 11, 522). In this study, virus was demonstrated to be in the brains of hamsters at 5 days post-viral injection (dpi) by cell culture assay, quantitative RT-PCR, and immunohistochemical staining of WNV

in neurons. Eighty percent of hamsters treated i.p. 5 dpi with 100 mg/kg of humanized monoclonal antibody, hE16, survived WNV disease, whereas, 37% of placebo-treated hamsters survived ($^{***}p < 0.001$). If administered at 2 dpi, 100% survived. We tested the hypothesis that hE16 is effective if delivered directly into the brain instead of by peripheral administration. The antibody was delivered into the brain 5 dpi using convection-enhanced delivery through a cannula implanted into the brain. The hE16 was detected in the CNS, but none was detected in the kidney. The survival of hE16-treated hamsters was 88% as compared to 22% of placebo-treated animals ($^{***}p < 0.001$). For additional proof, the majority of hamsters having WNV in their cerebrospinal fluid, a marker for CNS infection, were protected with hE16 administered i.p. at 5 dpi. This humanized monoclonal antibody, therefore, is a possible treatment for the post-exposure, WNV-infected humans that develop signs of neuroinvasive disease.

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Identification and Characterization of Antiviral Drugs for Lassa Fever Virus

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Hemorrhagic fever viruses are of serious worldwide health concern as well as potential biological weapons. Lassa fever virus in particular annually infects several hundred thousand individuals in West Africa, and the export of this pathogen outside of this region, either intentionally or unintentionally, presents a serious risk to the developed countries of the world. The CDC and NIAID have identified Lassa fever virus as a Category A priority pathogen, indicating the highest degree of threat to public health. No arenavirus-specific antiviral drugs are currently approved for use in humans. The purpose of SIGA's biodefense program is to develop safe and effective drugs for preventing and treating diseases caused by Category A viruses. To that end, a large and diverse library of small molecule compounds was screened using a viral pseudotype assay to identify inhibitors that target the essential Lassa surface glycoprotein (GP) and thus block viral entry into the host cell. Twenty-six compounds were identified as quality hits, as defined by potency, selectivity, and chemical tractability. Antiviral activity against authentic Lassa fever virus was assessed in cell culture through a collaboration with colleagues at USAMRIID. A number of these potent antiviral compounds and their related analogs have exhibited informative chemical structure-biological activity relationships (SAR). Two potential lead compound series have emerged from these studies, each with 50% effective concentrations (EC50s) of less than 100 nM against Lassa fever virus and with EC50s of less than 2 nM against Lassa GP-pseudotyped virus. Characterization of the in vivo properties of these compounds is underway. The in vitro antiviral potency and selectivity, animal pharmacokinetics, and the development process will be presented. These inhibitors represent an important step toward the development of a small molecule antiviral drug for Lassa fever virus.

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Antiviral Strategies Against Nipah and Ebola Virus: Exploring Gene Silencing Mechanisms to Identify Potential Antiviral Targets

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Nipah (NiV) virus (family *Paramyxoviridae*) is a recently emerged human and animal pathogen that can cause severe encephalitis with fatality rates of up to 70%. Since no treatment or vaccination is available, and cross-species spread was observed, the virus has been classified as biosafety level 4 (BSL-4) agent. To avoid BSL-4 containment for the study of *cis*-acting signals as target for antiviral strategies, we used an optimized plasmid-driven T7 minigenome rescue system (without the need for recombinant vaccinia virus MVA-T7) as well as an newly established RNA polymerase I-based approach. Minigenome rescue is based on transfection of the minigenome NiV-CAT and the plasmids encoding for the three nucleocapsid proteins N (nucleoprotein), L (polymerase), and P (phosphoprotein) and measured by enzymatic CAT assays.

We used the established plasmid-based minigenome rescue systems to screen for potential antiviral compounds. In a first step we tried to determine the optimal strategy for the delivery of small hairpin (sh) interfering RNA molecules. For this we compared three shRNA delivery systems against another BSL4 agent—Reston ebolavirus (family *Filoviridae*); (i) plasmid-mediated pol I and (ii) pol III-driven shRNAs, and (iii) exogenously (T7) produced shRNA, for their ability to induce gene silencing. Interestingly, beside the in vitro-generated or pol III-driven shRNAs, pol I transcripts showed very efficient inhibition of minigenome rescue. However, the most efficient delivery method was transfection of in vitro transcribed shRNAs. We will present the results of this comparison and, based on the most efficient approach, also first results of shRNAs targeted either to NiV N, P, and L genes or to the leader/trailer noncoding regions to interfere with minigenome replication. Conformative data with live virus experiments under BSL4 conditions will be included.

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Identification of Inhibitors of Ebola Virus with a Subgenomic Replication System

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Filoviruses, which include Ebola virus and Marburg virus, are among the most notorious human pathogens because they cause sporadic outbreaks of severe hemorrhagic fever. Unfortunately, very few therapeutic agents are available to treat infections with these viruses. Antiviral screening methods that determine the effect of compounds on viral replication involve working with infectious virus, which is obviously not practical for these biosafety level 4 (BSL-4) agents. We developed an antiviral screening method based on a cell-based, infection-independent, Ebola subgenomic replication system in which the expression of an easily measurable enzyme is dependent on the RNA replication and transcription factors of Ebola virus. Using this system we screened a synthetic compound library for antiviral activity against Ebola virus and have identified a number of inhibitors. We also used it to identify a peptide inhibitor directed against VP30. Anti-Ebola virus activity for many of the inhibitors was confirmed in a viral replication assay using a GFP-expressing Zaire '76 strain of Ebola virus. Fifty-two small molecule inhibitors from at least six classes of compounds had EC50 values in the low micromolar range and good selectivity. Several of these compounds have promising chemical, biological, and pharmacological profiles to pursue as potential anti-filovirus drugs. We are currently preparing to test these compounds in a mouse model of Ebola virus. We have also begun a lead optimization program to improve antiviral potency and selectivity of aryl sulfonamide and 4-aminoquinoline compounds.

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Targeting of HPV31 Episomal DNA with Compounds Designed to Bind the Origin of Replication (ori)

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Human papillomavirus (HPV) has been a difficult virus to target by traditional antiviral methods due to its small size, its small number of obvious therapeutic targets, and its resistance to propagation in vitro. Nevertheless, antiviral compounds that reduce HPV DNA load have the potential to prevent carcinogenic progression in infected patients. To that end, we developed an approach that dramatically reduces the HPV episomal DNA load of keratinocytes in vitro by targeting viral DNA sequences. Pyrrole-imidazole polyamides, with some containing fluorescent probes to aid in cell localization studies, were designed to recognize the HPV31 ori. All fluorescent compounds rapidly localized to the nucleus of cultured keratinocytes following addition to the culture media. The compounds were then tested for their ability to alter keratinocyte HPV31 episomal DNA content. Two of the 19 compounds caused a dose-dependent reduction in HPV31 episomes as measured by Taqman™ real-time PCR. While control and vehicle-treated cells maintained ~1000 copies of HPV31 per cell, compounds 2-Ta and 4-Ta both reduced HPV31 DNA levels to below 50 copies per cell after 48 h incubation with 10 μM compound. An alternative Taqman™ amplicon within the HPV31 E7 gene produced identical results. A multiplexed Taqman™ real-time PCR reaction that followed the ratio of HPV31 DNA to the human ApoE gene also demonstrated dramatic loss of HPV31 DNA copies, further confirming our initial observations. Finally, cells were treated with polyamides for 48 h, polyamide-containing media was removed, and episome levels were followed for 8 days. At day 6, 4 days after removal of polyamide and 3 days after sub-culturing of the cells, viral episome levels remained approximately 60% lower than control samples. By day 8, 6 days after removal of polyamide, viral DNA levels were beginning to recover but still remained significantly lower than control samples. Together our results demonstrate that targeting the HPV origin of replication with DNA-binding compounds dramatically reduces episomal DNA levels.

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High Potency Silencing by Boranophosphate Sirna: Synthesis, Properties, and Silencing Activities

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Small interfering RNAs (siRNAs) are potent tools for gene down-regulation but are minimally stable in cells. To improve the efficacy of siRNA, we replaced non-bridging oxygens in the phosphodiester linkages of natural RNAs with BH3 groups. The resulting boranophosphates have unique properties, including enhanced nuclease resistance, altered hydrogen bonding of the phosphate, different interactions with metal ions, and increased thermal stability of RNA:RNA and RNA:DNA duplexes.

Anti-EGFP siRNAs containing boranophosphate modifications were prepared by in vitro transcription with T7 RNA polymerase from ribonucleoside 5′-(alpha-*p*-borano)triphosphates, as well as normal and phosphorothioate siRNAs. After confirming the presence of the borane modifications with MALDI-MS, several properties of borano-modified siRNAs were investigated: (1) the double stranded RNA with borane modifications maintained the A-form conformation characteristics according

to the circular dichroism (CD) spectra; (2) the borane groups in the siRNAs increased the thermal stability, with an enhancement of T_m by 0.5–0.8 °C per modification; and (3) siRNAs with borano-modifications were shown to be at least 10-fold more resistant to RNase A digestion than normal ones.

When these modified siRNAs were used to down-regulate EGFP expression in HeLa cell cultures, it was found that: (1) borano-modified siRNAs were consistently more effective than siRNAs containing the corresponding phosphorothioate modifications; (2) borano-siRNAs were more effective than normal siRNAs provided that the center of the antisense strand was not heavily modified; (3) borano-siRNAs were more potent than normal or phosphorothioate siRNAs at lower concentrations; and (4) finally, the silencing activity of boranophosphate single-stranded siRNA (ss-RNA) was comparable to that of unmodified ds-siRNA. The borano ss-RNA had excellent maximum silencing activity and was highly effective at low concentrations, and silencing activity was durable up to one week after transfection. Results with anti-HPV siRNAs will be discussed. Boranophosphate modification is a potential new class of anti-viral therapeutic agents.

Oral Session VI: Retroviruses II and Late Breaker Presentations

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Discovery of GS9148, a Novel Nucleotide HIV Reverse Transcriptase (RT) Inhibitor

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Background: Nucleos(t)ide RT inhibitors [N(t)RTIs] that possess improved activity toward clinically relevant resistant viruses have utility in the optimal treatment of HIV-infected patients. This report describes the antiviral structure activity relationships that led to the discovery of phosphonomethoxy-2′-fluoro-2′,3′-dideoxydidehydroadenosine (Fd4AP, GS9148), a novel NtRTI, with an excellent resistance profile toward HIV-1 variants containing major N(t)RTI resistance mutations.

Methods: Phosphonomethoxy analogs on purine and pyrimidine dideoxydidehydro (d4) and dideoxy (dd) ribose scaffolds were prepared. Antiviral activity was measured against wild-type and N(t)RTI-resistant recombinant viruses using cytopathic assay in MT-2 cells. Mitochondrial toxicity was assessed in HepG2 cells by measuring mitochondrial DNA content.

Results: The d4 scaffolds displayed superior antiviral activity compared to the dd scaffold and adenine was superior to other nucleobases. Phosphonomethoxy-2′,3′-dideoxydidehydroadenosine (d4AP) inhibited HIV-1 replication with a mean EC50 of 2.1 μM and an 0.8-, 2.9-, and 2.9-fold change in potency against viruses containing M184V, K65R, and 6 thymidine analog mutations (TAMs), respectively. Further exploration of d4AP was limited by its mitochondrial toxicity, which was then addressed in 2 ways: (i) preparation of L-d4AP or (ii) 2′ fluorine substitution. L-d4AP exhibited an EC50 of 5.9 μM but had substantially reduced potency (14-fold) toward M184V mutant viruses. Fd4AP exhibited an EC50 of 12.3 μM, with 0.8-, 1.2-, and 3.5-fold change in potency against viruses containing M184V, K65R, and 6 TAMs, respectively. No cytotoxic effects were measured up to 1 mM in MT-2 cells and no effects on mitochondrial DNA were detected up to 300 μM in HepG2 cells for both Fd4AP and L-d4AP.

Conclusion: Fd4AP is a novel phosphonate NtRTI with antiretroviral activity toward wild-type and resistant mutant HIV-1 strains. Compared to d4AP, the 2′-fluorine atom significantly improved the in vitro toxicity profile while retaining the favorable resistance profile. In subsequent studies, the monoamidate prodrug strategy was applied to Fd4AP to achieve optimal in vivo pharmacokinetic properties.

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Synthesis and Structure Activity Relationship of a Novel Series of Cyclopropyl-Based CCR-5 Antagonists

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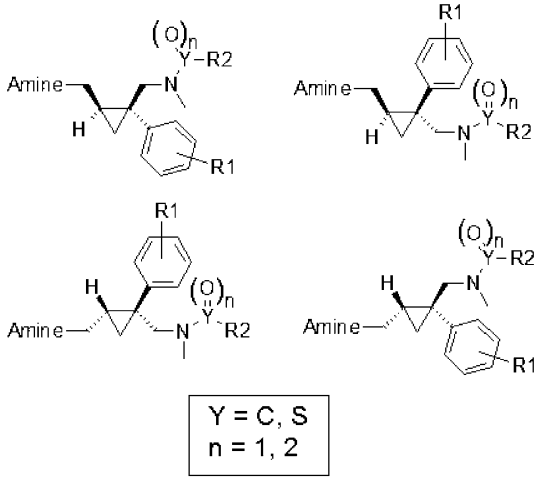
Entry inhibitors, and CCR-5 antagonists in particular, have become one of the most actively pursued treatments for HIV within the pharmaceutical industry. Recently, multiple groups have disclosed piperidine-based CCR-5 antagonists that – to the medicinal chemist's eye – might appear to share a common three-point pharmacophore comprised of a tertiary amine, a phenyl ring, and a carboxamide or sulfonamide group. In several of these cases, these pharmacophoric elements are tethered together by a flexible, aliphatic chain. We sought to improve the potency of and introduce structural novelty into this class of compounds by rigidifying this tether. Herein, we describe stereoselective syntheses and SAR of a series of CCR-5 antagonists wherein the tether has been replaced with four stereochemical isomers of a rigidified cyclopropyl scaffold.



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N-Aminoimidazole Derivatives Inhibit HIV-1 Gene Expression by an Unexploited Mechanism of Action

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The regulation of HIV transcription is a complex, multistage process that requires the concerted action of viral and cellular proteins. We discovered the *N*-aminoimidazoles (NAIMs) as a unique class of HIV inhibitors targeted at the viral transcription level. A prototype NAIM, NR-818, prevents the reactivation of dormant virus by inhibiting both the HIV-1 p24 and viral mRNA production from latently HIV-1-infected cell lines upon stimulation with TNF-α, PMA, or TSA. Extensive research revealed that NR-818 was unable to inhibit the NF-κB activation pathway or chromatin remodeling at the viral promoter, both known to be crucial for viral transcriptional activation. Focusing on the viral transcription process, chromatin immunoprecipitation (ChIP) experiments revealed that NR-818 was able to inhibit the Ser5 phosphorylation of the C-terminal domain (CTD) of RNA polymerase II. This step is mediated by the CDK9 subunit of *p*-TEFb, which is recruited to the viral promoter by the HIV-1 Tat protein. Since we did not find an inhibition at the level of CDK9 activity or Tat-mediated transcription in Tat-expressing cell lines transiently transfected with a LTR-GFP construct, we infer that NR-818 must interfere with the transcription process by a unique mode of action. Evidence points towards a kinase, not belonging to the CDK family, to be the target of the NAIMs, resulting in an antiviral action at the level of retroviral transcription.

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Key Mutations of the 69 Insertion Complex of Multidrug-Resistant Hiv-1 Reverse Transcriptase

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A Ser–Ser insertion at codons 69–70 together with substitutions T69S and T215Y in the reverse-transcriptase (RT)-coding region of HIV-1 are known to confer resistance to zidovudine (AZT) and stavudine (d4T). Phenotypic resistance correlates with increased ATP-dependent phosphorolytic activity on inhibitor-terminated primers. We have previously shown that an RT derived from a clinical isolate (SS RT) that contained the insertion and 10 additional mutations related to drug resistance (including T215Y) showed >10-fold increased unblocking activity on AZT- and d4T-terminated primers, when compared with an RT containing the insertion together with mutations T69S and T215Y, in an otherwise wild-type BH10 sequence. These results suggested that other mutations associated with the complex T69SSS/T215Y in clinically relevant RTs contributed to increase ATP-mediated excision activity and conferred high-level resistance to AZT and d4T in phenotypic assays.

To identify residues increasing the excision activity, we obtained recombinant enzymes bearing SS RT residues 1–135 and wild-type BH10 RT residues 136–560 (L1 RT), or residues 1–135 of the BH10 RT and 136–560 of the SS RT (L3 RT), as well as an L1 RT variant with the substitution T215Y (L2 RT) and an L3 RT derivative with T69SSS (L4 RT). Additional RTs containing mutations M41L, A62V, or K70R together with the combination T69SSS/T215Y in the BH10 background were also obtained. ATP-mediated excision activities on AZT- and d4T-terminated primers were determined and the effects of mutations were tested in phenotypic assays using recombinant HIV-1.

The L2 RT containing mutations T69SSS/T215Y and additional changes in the N-terminal region showed the highest ATP-dependent phosphorolytic activity on blocked primers, giving values similar to those reported for the SS RT. Results were consistent with phenotypic data. In contrast, L1, L3, and L4 RTs displayed low-level activity. Further experiments revealed that three amino acid changes at the N-terminal region of the polymerase (M41L, A62V and K70R) were responsible for the increased excision activity shown by RTs bearing mutations T69SSS and T215Y.

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Thiazolobenzimidazoles as Potent Inhibitors of the in vitro Replication of Coxsackie B Virus

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From a series of phenyl-substituted thiazolobenzimidazoles, several compounds were identified as selective inhibitors of Coxsackie B virus replication in Vero cells. A structure-activity relationship was established, from which the 6-trifluoromethyl substituted analogs emerged as the most potent congeners. The compounds were active against all six Coxsackie B strains tested. The in vitro antiviral activity of one of the most selective compounds, i.e. CHI-033, was assessed by (i) MTS-based cytopathic effect assays, (ii) virus yield reduction assays, (iii) real-time quantitative PCR (RT-QPCR) and (iv) by monitoring viral antigen expression. In all assays a clear concentration-response effect was obtained. The 50% effective concentration (EC50) was 0.30 ± 0.18 μg/ml, while the CC50 (50% cytotoxic concentration) of CHI-033 for Vero cells was more than 100 μg/ml, thus resulting in a selectivity index of >500. Detailed single cycle time-of-drug-addition studies (in which viral replication was monitored by means of RT-QPCR) revealed that the compound interacts with viral replication at a time that coincides with the onset of intracellular viral RNA synthesis. CHI-033-resistant virus is being generated by culturing the virus in the presence of increasing drug concentrations. Drug-resistant virus will be genotyped, which should allow us to identify the (putatively viral) molecular target of this class of compounds.

Poster Session I: Retroviruses, Respiratory Viruses, Hepatitis Viruses, Prodrugs and Drug Delivery, Virological Methods

Retroviruses

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Synthesis of 4′-Carbon-Substituted Stavudine Analogues and SAR Studies on Their Anti-HIV Activity

Hiromichi Tanaka[1], Kazuhiro Haraguchi[1], Hiroki Kumamoto[1], Takao Nitanda[2], Masanori Baba[2], Ginger E. Dutschman[3], Yung-Chi Cheng[3]

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Our recent research program on the development of synthetic methods for 4′-carbon-substituted nucleosides has led to a new strategy, ring opening of 4′,5′-epoxy-nucleosides with organoaluminum and organosilicon reagents. This enabled us to introduce alkyl, alkenyl, and alkynyl groups to the 4′-position.

As a result of this study, 4′-ethynylstavudine (4′-Ed4T) was found to be more anti-HIV active than the parent compound stavudine (d4T). This compound (4′-Ed4T) has several additional appeals as a promising anti-HIV agent: much less toxic to various cells and also to mitochondrial DNA synthesis, better substrate for human thymidine kinase than d4T, very much resistant to catabolism by thymidine phosphorylase, its activity enhances in the presence of a major mutation K103N known for NNRTI-resistant HIV.

In this conference, we present the synthesis and SAR studies of 4′-Ed4T analogues modified mainly in the sugar portion.

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Intramolecular Alicyclic Synergists for Polyanionic Antivirals

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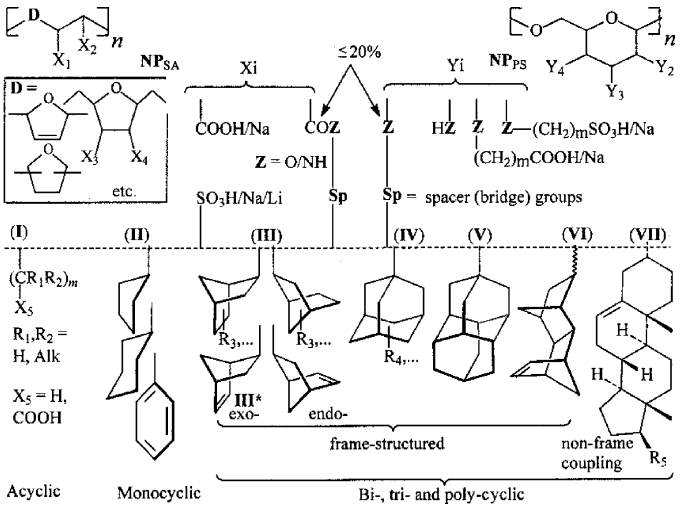
Negatively charged polymers (NP) possess a broad immunoadjuvant and antiviral activity topically useful for vaccine, drug, and microbicide development. But their efficiency is limited over a reversibility of electrostatic kind of interference with virus-specific nano-objects. To overcome this limitation the purpose-made intra-molecular modifications of NP were studied among non-toxic maleic acid co-polymers (NPSA), dextran and chitin derivatives (NPPS) within varied alicyclic modifiers application. The configurationally flexible alkyls (I), as non-alicyclic control, are ineffective synergist for NP antiviral potency. Monocycles (II) are moderate active too. On the contrary the hard-conformation frame-structured spheroids (III–VI) exhibit ability (at optimal macromolecular parameters) to be super-effective synergists for strength and diapason of NP antiviral action. Unlike small molecular III/IV-containing prototypes (amantadin, rimantadin, deitiforin, etc.), narrowly-effective inhibitors mainly of influenza A viruses, the NP-coupled modifications become effective also against many other viruses, including the drugs resistant strains [Antivir. Res. 46 (1), 44]. In focus of the anti-HIV potency the IVs provide a 10–100-fold elevation of NP activity. The more available and less toxic III species are similarly active, but III* (with spatial-optimally contactable double bond due to the exo-configuration) turns out the best synergist 20–500-fold amplifying the anti-HIV-1 selectivity up to IS∼10000. Augmentation of the frame cycles from III-IV toward V–VI results in no essential enhancement of antiviral activity, but stimulates toxicity. The recently involved in the investigation VII, cholesterol-like systems, as tools for novel Raft-targeted strategy, demonstrate capacity for at least 10-fold amplification of anti-HIV-1 potency



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Alkoxyalkyl Esters of Phosphonomethoxyethyl Purines: Synthesis and Antiviral Activity against HIV-1, in vitro

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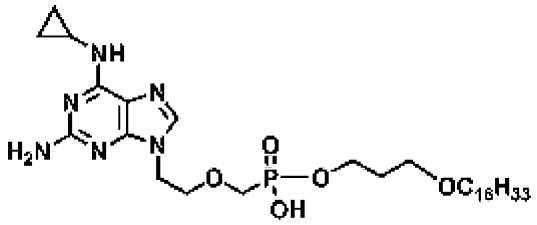
Our earlier studies showed that esterification of cidofovir (HPMPC) with alkoxyalkanols increased antiviral activity by more that two logs and promoted oral bioavailability. To evaluate this approach with purine based nucleoside phosphonates, we synthesized several alkoxyalkyl esters of acyclic purine phosphonates such as 2,6,-diamino-(9-[2-phosphonomethoxyethyl]-purine (PME-DAP) and 2-amino-6-cyclopropylamino-(9-[2-phosphonomethoxyethyl]-purine (PME-cPr-DAP) These purine phosphonates have been reported to be active against a wide range of viruses such as human immunodeficiency virus (HIV-1), other retroviruses, herpesviruses, poxviruses and hepatitis B virus. For this study several alkoxyalkyl analogs of acyclic 2,6-diaminopurine nucleoside phosphonates were synthesized and evaluated against HIV-1.



HDP PME-cPr-DAP

The alkoxyalkyl esters were more inhibitory than the unmodified compounds in p24 reduction assays in MT-2 cells infected with HIV-1. For example, hexadecyloxypropyl (HDP) and oleyloxyethyl (OLE) esters of PME-cPr-DAP were >3 logs more active than unmodified PME-cPr-DAP. In spite of increased cytotoxicity in MT-2 cells, the selectivity indexes are more than 10-fold higher then for unmodified compound. In conclusion, esterification of PME-DAP and PME-cPr-DAP with hexadecyloxypropyl- or oleyloxyethyl-residues greatly increased their antiviral activity and selectivity against HIV-1 in vitro.

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Influence of Artifical Ribonucleases Structure on Their Anti-HIV Activity

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"Chemical" ribonucleases hold promise as tools for studying the structures of RNAs and RNA-protein complexes, as reactive groups in conjugates intended for cleavage of particular RNAs, as therapeuticals inactivating virus genome RNAs or certain mRNAs, and as a promising antiviral agents. Drug design and development of new medicines directed against HIV are permanently actual tasks. The usage of modern quantitative structure-activity relationship (QSAR) methods could allow us to solve these problems more effectively.

The objective of the present work is QSAR analysis of antiviral activity of various tetrapeptides—artifical ribonucleases and consequent molecular design of new antiviral agents.

QSAR approach based on simplex representation of molecular structure (SiRMS) has been used for the solution of the formulated problem. Usage of SiRMS allows us to develop the molecular design of the new effective antiviral agents.

Thorough researches of relationship between antiviral activity (HIV-1, % of RNA P-O bond cleavage) and a structure of artifical ribonucleases have been carried out.

Statistic characteristics for PLS (partial least squares model) are quite satisfactory ($R^2 = 0.836$, $Q^2 = 0.788$). On the base of these models the molecular fragments with positive or negative influence on the explored property have been determined. Thus, for example, guanidine and triethylenediamine fragments promote antiviral action. It gives a possibility to realize based on elucidated rules molecular design of compounds with the high level of antiviral activity. The results of prognosis are verifying by the experimental investigations.

Thus, quite adequate simplex QSAR model "anti-HIV activity—artifical ribonucleases structure" was obtained and used for drug design.

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Synthesis, Anti-HIV, and CD4 Down-Modulation Activities of Novel CADA Compounds

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The cyclotriazadisulfonamide (CADA) compound specifically down-modulates the CD4 receptor expression on the surface of lymphocytes and monocytes/macrophages, the primary receptors utilized by HIV for infection of its target cells. CADA thus inhibits the entry of HIV and HHV-7 (Vermeire et al., 2002. Virology 302, 342–353). CADA chemotherapy may not be susceptible to the production of drug resistant strains of viruses, as its mechanism of action is completely different from those of any other anti-HIV drugs currently in clinical use. The CD4 down-modulating and antiviral potencies of more than 25 CADA analogs have been described (Vermeire et al., 2003. Mol. Pharmacol. 63, 203–210). Structural modifications of CADA were made to increase potency, reduce cytotoxicity, and improve physical properties. Several head group analogs were synthesized with polar groups and good leaving groups (Fig. 1). The anti-HIV and CD4 down modulation activities of these compounds are being studied. Some of these head groups may regenerate the double bond of CADA by elimination reactions, potentially producing water-soluble pro-drugs. IsoCADA (SA05), an isomer of CADA, was synthesized by cyclization of 1,5,7-triazabicyclo-[4.4.0]dec-5-ene (TBD) (Fig. 1). This structural modification may reveal a relationship between the symmetry of the molecule and its biological activity. Two new fluorine-containing analogs were also synthesized by modifying the toluenesulfonamide side arms (Fig. 1). The anti-HIV and CD4 down modulation activities of these new CADA analogs are summarized.

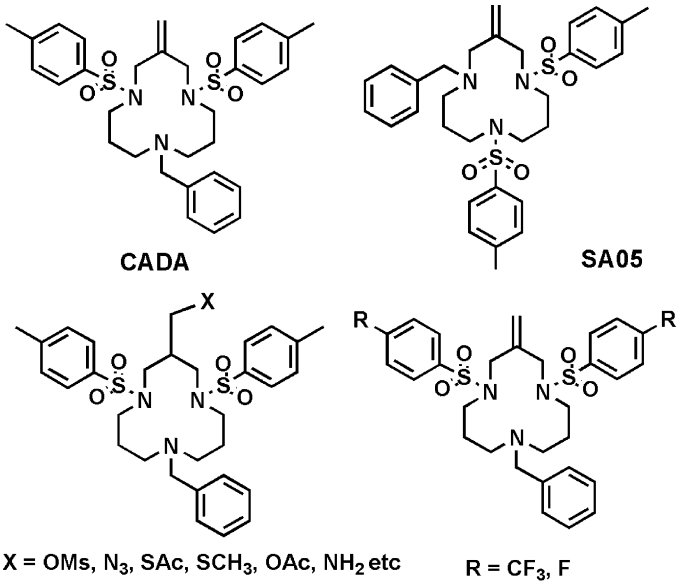

Figure 1

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Novel Inhibitors of Both the 3′-Processing and Strand Transfer Steps of HIV Integrase: Molecular Docking, Binding Poses, and Binding Affinities

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Department of Pharmaceutical and Biomedical Sciences and The Center for Drug Discovery, University of Georgia, Athens, GA 30602, USA

Drug discovery targeted at the elusive viral enzyme, HIV integrase, has not resulted in a single FDA-approved drug. In this presentation we describe our molecular modeling studies with conceptually novel inhibitors of HIV integrase that also possess potent in vitro anti-HIV activity. Docking was performed on the catalytic core of integrase represented by chain C of PDB structure code 1BL3. Building of molecules and primary modeling was done with SYBYL 7.1 on a Silicon Graphics Onyx3 (R14000) workstation. The program GOLD 3.0 (Genetic Optimization for Ligand Docking) was used extensively in evaluating the docking poses of these compounds with the active site of HIV integrase and to give information on key residues involved in the recognition and binding of these ligands. The GOLD function consists of three basic components: protein-ligand H-bonding energy, protein-ligand van der Waals energy, and ligand internal energy. Post-processing GOLD output was done with the program SILVER 1.1, a utility program supplied with GOLD for evaluating hydrogen-bonding interactions, metal coordination and Van der Waals factors. For comparison purposes, additional docking was performed using other docking protocols, notably the SYBYL module FlexX. Data obtained from these and related studies including binding poses, binding affinities, functional and conformational considerations, and GOLD function scores will be presented and explained.

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Synthesis of Novel HIV Integrase Inhibitors with Highly Potent anti-HIV Activity

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HIV integrase is essential for HIV replication and is an attractive target for drug discovery against AIDS. However, research efforts on drug discovery pertaining to HIV integrase have not resulted in a single FDA-approved drug for which mechanism of action is inhibition of HIV integrase. Recently, we have been exploring a novel class of diketo acids that are constructed on nucleobase scaffolds and that have a specific arrangement of the functional and hydrophobic group on the scaffold. These compounds are inhibitors both key steps of HIV integrase. One lead compound from this group has also been found to have remarkable in vitro anti-HIV activity. However, the syntheses of the inhibitors are quite challenging. This presentation will describe the synthetic methodologies specifically developed in our laboratory for the preparation of some representative examples of these integrase inhibitors. Purification approaches to produce highly purified compounds for biological studies will be explained. Structural, functional and conformational data obtained from extensive spectroscopic studies will be discussed. Representative anti-HIV integrase data and in vitro anti-HIV screening results will be presented.

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Potent Inhibition of Both HIV and HCV in vitro by a Ring-Expanded ("Fat") Nucleoside: Part I. Mechanistic Studies of Anti-HIV Activity

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We have recently reported the synthesis and antiviral activities of a ring-expanded ("fat") nucleoside analogue, called NZ-51, that inhibits both HCV and HIV in vitro with EC_{50} values ranging in micromolar concentrations or less, with little or low toxicity to

the host cells. In this Part I of the presentation on this subject, we report our preliminary findings on the mechanism of anti-HIV activity of this compound, along with the synthesis and antiviral activity of a few additional analogues. In view of the fact that a number of HIV patients also suffer from HCV as a major co-infection, and that a number of them ultimately die of end-stage HCV-related complications including liver cirrhosis and hepatocellular carcinoma, a drug with dual inhibitory characteristics against both viruses is highly desirable and timely.

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Effect of Different Adamantane and Norbornene Derivatives on Hiv-1 Infection in vitro

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Introduction: Amantadine is a well-known effective anti-influenza drug. It was modified to enhance its antiviral activity by chemically linkage with the water-soluble polyanionic matrix via different spacer groups. The other group of used compounds was norbornene derivatives, as norbornene is an adamantane analogue on anti-influenza activity.

Methods: The absence of cytotoxic effect was shown by MTT test for estimating cytotoxic dose (CTD50). The antiviral effect of the compounds was analyzed in lymphoblastoid MT-4 cells and in HeLa CD4+/b-galactosidase cells ("Magi" cells). The effect of the compounds was registered by immunoblotting of cell lysates and by measuring of b-galactosidase activity.

Results: The strong inhibition of HIV-1 replication was observed when the compounds were added with the virus and was expressed even when the compounds added with the virus were removed 1 h after infection.

The anti HIV-1 effect of the compounds was gradually decreased if they were added 1 and 2 h after infection, no inhibition was observed when the compounds were added 4 h after infection. The compounds did not impair the virion structure. Adamantane and norbornene derivatives were shown also to inhibit AZT resistant viral strains.

Conclusion: Adamantane and norbornene were shown to be active HIV inhibitors with the high selectivity index. The compounds are promising candidates for further investigation including preclinical studies.

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The Longer Intracellular Half-Life of Tenofovir Diphosphate Compared to Carbovir Triphosphate Correlates with Sustained Antiviral Persistence in vitro

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Introduction: The dosing interval of nucleoside reverse transcriptase inhibitors (NRTIs) is dependent on their plasma half-lives. Less is known about the effect of their intracellular half-lives on the maintenance of antiviral activity. To investigate this question, we developed a novel in vitro antiviral persistence assay. Measurement of the antiviral persistence of tenofovir (TFV) and abacavir (CBV) was coupled to measurement of the half-lives of their TFV-DP and CBV-TP anabolites.

Methods: MT-2 cells or stimulated primary CD4+ T-cells were incubated with graded concentrations of TFV or CBV for 14 h (h); then extracellular drug was removed by washing. Cells were further incubated without drug for 0–24 h and then infected with HIV-1 (IIIB or BaL). p24 was quantified on day 2; inhibition of HIV-1 replication due to intracellular drug persistence (PC50) was determined relative to a standard EC50. Decay of intracellular DP/TPs in CD4+ T-cells was measured using LC/MS/MS.

Results: In MT-2 cells, the PC50 value for TFV 12 h after drug removal remained unchanged relative to the EC50 (<3-fold shift) whereas the PC50 for CBV shifted >65-fold, indicating less persistence of CBV. In CD4+ T-cells, the PC50 value for TFV also showed a minimal shift relative to the EC50 (2.4-fold) 24 h after drug removal. CBV showed a much larger relative shift (>243-fold). Quantification by LC/MS/MS of intracellular TFV-DP and CBV-TP in CD4+ T-cells in vitro demonstrated that TFV-DP had the longest intracellular half-life of the two drugs (TFV-DP, 21 h versus CBV-TP, 5 h).

Conclusions: A novel antiviral persistence assay was developed to study the relationship between intracellular NRTI half-lives and antiviral activity. In both MT-2 cells and primary activated CD4+ T-cells, TFV had the longest persistence of antiviral activity. In CD4+ T-cells, TFV-DP also had the longest half-life of the two NRTIs. CBV-TP had a much shorter half-life than TFV-DP and showed less antiviral persistence. Although both drugs are approved for QD dosing, the half-life of intracellular TFV-DP maintains antiviral suppression in vitro over a timeframe most consistent with QD dosing.

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Development of the G-Quartet Possessing Phosphorothioate Oligonucleotide ISIS 5320 as a Potent Anti-HIV Topical Microbicide

Karen M. Watson, Tracy L. Hartman, Lu Yang, Robert W. Buckheit Jr.

ImQuest BioSciences, Inc., Frederick, MD, USA

ISIS 5320 is a phosphorothioate oligonucleotide with a molecular structure of T2G4T2. The G-quartet possessing molecule has been shown to be a potent inhibitor of HIV attachment and cell–cell fusion and acts by specifically interacting at the V3 loop of gp120. Mapping studies with monoclonal antibodies targeting epitopes in and around the V3 loop have been used to define the binding site of ISIS 5320. In vitro, ISIS 5320 inhibits all laboratory and clinical strains of HIV-1 and HIV-2 tested, including representative subtype viruses, drug resistant viruses (including MDR viruses) and viruses that utilize the CXCR4 and CCR5 chemokine receptors. Serial passage of virus in the presence of increasing concentrations of the oligonucleotide did not result in the selection of drug resistant virus strains and combination assays resulted in additive to synergistic interactions

with other approved HIV inhibitors. The antiviral and toxicity profiles of ISIS 5320 resulted in the performance of human clinical trials for the therapeutic use of the oligonucleotide to treat HIV infection. The antiviral properties and mechanism of action of ISIS 5320 suggest that it may be an excellent anti-HIV topical microbicide. ISIS 5320 was found to be highly active in a cervical explant model of HIV infection with highly significant inhibition of CCR5-tropic strains of virus. Activity was also observed in cell-free and cell-associated virus transmission assays, as well as in CD4-dependent and CD4-independent acute infection inhibition assays. In microbicidal specific combination assays, significant efficacy has been observed with ISIS 5320 used in combination with other microbicidal compounds. The results of these studies suggest that ISIS 5320 may represent a new and novel anti-HIV topical microbicide.

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Combination Topical Microbicide Therapy: Combination Efficacy in PBMCs, CEM-SS, and Virus Transmission Assays

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Though a variety of compounds are being developed as anti-HIV topical microbicides, such as polyanionic molecules, surfactants, natural products, peptides, proteins, heterocycles, and virucidal agents, clinical efficacy studies that demonstrate the ability of these agents to impact virus transmission are still in progress. It has been estimated that a microbicide that is only 60% effective would have the capacity to prevent millions of new infections each year. Thus, one of the challenges in HIV drug development is the discovery of compounds that will inhibit the sexual transmission of infectious organisms between sexual partners. The rapid mutability of HIV and the known presence of drug resistant viruses in wild type virus populations suggests that microbicide development will suffer from the same problems that exist for all HIV therapies, namely the selection of resistant virus strains that will bypass the microbicide barrier and infect target cells in the vaginal or rectal environment even in the presence of the microbicide. Thus, it is likely that HAART-like combination drug therapies will become the most effective means of inhibiting the sexual transmission of HIV. We have evaluated a wide variety of anti-HIV and anti-STI compounds in vitro alone and in combination with one another and have demonstrated that certain patterns of inhibition (additivity, synergy, antagonism) occur between the various classes of compounds. Recently, we have compared the combination anti-HIV activity of microbicide compounds in fresh human PBMCs infected with clinical isolates of HIV to the combination activity of the same test agents in CEM-SS-based cultures. In general, these two assay systems yield similar combination assay results. To provide a rationale for the combination use of the compounds in a microbicide setting, the same combination of compounds was evaluated in a microbicide-like virus transmission assay. These combination results suggest that higher levels of synergy between virus attachment and reverse transcriptase inhibitors might be expected in the microbicide environment compared to levels predicted for the systemic therapeutic environment. The results of the combination assays with various microbicides will be presented.

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Effects of Protease Inhibitors on Maturations, Production, Infectivity, and HIV-1-Induced T-cell Apoptosis of HIV-1 Following Drug Removal in Human Macrophages

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During the onset of the HIV disease, HIV RNA is continually produced in the face of treatment with HAART in circulating reservoirs and RT inhibitors are almost ineffective in the post-integration events. Among the classes of anti HIV-1 drugs, protease inhibitors (PI) are the unique to inhibit the HIV-1 production in chronically infected macrophages. In the progression of HIV infection, the role of the monocytes-derived macrophages (M/M) is further confirmed as they represent chronologically the first cytotype where the viral replication restarts as a consequence of failure or interruption of antiviral therapy. Aim of the work was to evaluate the rebound of HIV-1 production when PI have been removed in HIV-1 chronically infected M/M and, moreover, to verify the effect of this removal on virus maturation, infectivity and ability to trigger apoptosis in uninfected peripheral blood lymphocytes (PBL). A rebound of p24 Gag protein was measured starting from 12 h after drug removal yet virus infectivity remained 1 log lower than control up to 1 week. Inhibition of HIV-1 replication was still 48% and 18% upon amprenavir 20 and 4 μM, respectively. These data were confirmed by western blotting and electronic microscopy showing production and release of immature viral particles. Moreover, PI (amprenavir and indinavir) treatment dramatically reduced apoptosis of PBL co-cultured with chronically infected M/M and kept CD4/CD8 ratio above the levels of untreated controls until the 5th day of co-culture. Taken altogether, these findings suggest a wide clinical importance for amprenavir and indinavir for their relevant long-lasting antiviral effect in persistently-infected reservoirs of HIV even in case of drug interruption and/or when HIV infection can restart in districts where drugs find not sufficient concentration. Moreover, these results strengthen the evidence for an unique positive utilize of PI against ongoing and productive HIV infection.

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In vitro Selection and Characterization of HIV Mutants Resistant to the NNRTI Capravirine

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Background: Effective Inhibition of HIV reverse transcriptase (RT) currently represents a crucial objective of antiretroviral

therapy. Capravirine is a second-generation non-nucleoside RT inhibitor (NNRTI) that is capable of blocking the replication of certain NNRTI-resistant strains of HIV and was recently in clinical development. In this study, we report on the in vitro selection and characterization of viral resistance to capravirine.

Methods: Viral resistance selection experiments were performed in MT-2 cells with the HIV IIIb isolate and increasing concentrations of capravirine. Viruses were analyzed genotypically by population sequencing and by single genome sequencing (SGS). Recombinant viruses with NNRTI mutations were generated from proviral DNA clones. Phenotypic analyses were performed in MT-2 cells.

Results: Capravirine resistance selections were initiated at 1 nM (EC_{50} of 1.5 nM for capravirine). Following nine passages in the presence of increasing concentrations of capravirine, the L100I mutation emerged in RT and additional passaging led to V179D and F227C mutations at higher concentrations (100–300 nM). Further increases in capravirine concentrations led to the emergence of a L100I + V179D + F227C triple mutant, which confers >1000-fold resistance to capravirine. SGS of mixed viral populations from different passages showed that L100I, V179D and F227C were present on the same genome, with L100I as the primary mutation, and F227C and V179D were acquired sequentially at later passages. Through SGS analysis, a L100I + K166R + V179D + F227C quadruple mutation on the same genome was also observed at higher capravirine concentrations (>1000 nM). Recombinant viruses carrying these mutations were produced to assess their susceptibilities to capravirine.

Conclusions: After extensive in vitro passaging of HIV-infected cells in the presence of capravirine, neither K103N nor Y181C mutations in RT were observed. Instead, the L100I mutation was initially acquired, followed by mutations F227C and V179D. Addition of the K166R mutation to the triple mutant genome, L100I + V179D + F227C, appears to further enhance HIV resistance to capravirine.

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Lack of Knowledge about Individual's HIV Status: The Obvious Risk Factor for the Spread of AIDS/HIV Among Youth in Developing Countries

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Issue: The percentage of AIDS/HIV is increasing every year in the third worlds, and this is reinforced by the factor that majority of youth in third worlds do not know his/her HIV status.

Description: A self developed validated and reliable questionnaire [$r = 0.87$] was used to collect the data and percentage was used to analyze the data. The population of the study was made up of youth [female and male] in higher institutions, working places, market places and community streets in Nigeria, 50,000-sample size, selected through simple random sampling technique. The mean age is 25.5 years old. Relative risk [RR] calculated is 3.1, i.e. RR >1, indicating that the factor is the risk factor, and the Confidential Interval [CI] for RR at 95% Significant level is $2.61 < 3.1 < 3.90$ from the formula,

CI Lower limit $<$ RR $<$ CI Upper limit.

Lessons learned: Seventy percent of the sample population did know his/her HIV status and had had sexual intercourse in the past before, out which 20% had the unprotected intercourse once or more, 25% had protected sex while 25% were not sure of using protection means. While, 20% have knowledge about own HIV status and had had sexual intercourse before. Ten percent have no knowledge about own HIV status and had no sexual intercourse before.

Conclusion: AIDS/HIV still remains a killer disease in the third world. However, the lack of knowledge of individual's HIV status remains the only highest risk factor for the spread of the disease in the third worlds.

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The second Generation RNAi Drug Agent Which Deals with RNAi Escape HIV-1 Variants

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RNA interference (RNAi) is a potentially strong gene interference tool, which had been successfully used to silence many pathogenic viruses including HIV. However, many recent reports have shown that, in long-term assay cultures involving RNA viruses such as HIV, escape mutants breakthrough the silencing effect. In the light of this conundrum, it had been proposed that, vector designed to target multiple genes in a synergistic manner, may address the problem. Hence, we designed a chimeric RNA expression vector which express vif shRNA and decoy TAR RNA by combining vif shRNA and decoy TAR RNA with linker to which Dicer was able to recognize for cleavage, as a second generation RNAi expression vector system. The synergistic effect of these molecules enhanced the inhibition of HIV-1 replication in a long-term transduced PBMCs, H9, and Jurkat cell culture assays (9 weeks) and prevented virus breakthrough associated with siRNA-mediated escape variants. Notably, HIV-1 replication was similarly suppressed in the control cells expressing only vif shRNA for about 3 weeks, but an increase in virus replication was observed afterwards. HIV viral RNA extracted and sequenced at this point indicated escape mutants in the cells expressing the vif target in HIV. We confirmed substitution of bases in the vif shRNA target sequence. On the other hand,

the incidence of mutation was not observed in a sequence of viral RNA from the culture expressing the vif shRNA—decoy TAR RNA at the fourth week. Interestingly, virus production was inhibited for a long-term by an effect of decoy TAR RNA, through the RNA-protein interaction. Combining shRNA with decoy TAR RNA as second-generation anti-HIV shRNA may provide practical basis for applying siRNA-based gene therapy to the treatment of HIV/AIDS.

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Engineered Stem Cell Vaccine Design: The Rescue for Immune System Against AIDS

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Introduction: This is a designed efficient gene therapy against AIDS/HIV. The novelty of this AIDS Vaccine design/concept is seen in the fact that the 'pol' gene encoding for nonstructural proteins (polyproteins that generate three enzymes: reverse transcriptase, integrase and protease) is cloned in a suitable retroviral vector and adult stem cells are transfected by this and reinfused into the circulation to effectively counter HIV replication and antigenic variation.

Method: The mRNA are isolated from adult stem cells and transcribed into cDNA with Reverse Transcriptase. The cDNA are then cloned in a suitable retroviral vector (vacinia) carrying 'pol' gene that confers resistance to a strong Reverse Transcriptase inhibitor drug. The Adult stem cells are transfected by the recombinant mixture, and reinfused into the circulation of HIV infected person.

Result: The transfected stem cells are reinfused to provide renewable source of more and better empowered normal blood cell types that would disrupt and half HIV replication in the circulation. There would be efficient induction of both humeral and cellular mediated immunity with prolonged expression of antigens and protective immunologic memory generation against HIV antigenic variation.

Conclusions: This AIDS vaccine design would lead to both efficient prophylactic and therapeutic therapy against AIDS in that it would effectively take care of the problematic factor of HIV antigenic variation which has long been the main obstacle to potent AIDS Vaccine development.

Respiratory Viruses

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Increase of Amantadine Resistance Among Porzine but not Avian Influenzavirus A Strains Isolated in Germany Between 1981 and 2002

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Because of the real risk of interspecies transmission and/or reassortment between avian, swine and human influenza A strains, drug susceptibility monitoring of circulating avian and porzine virus strains appears to be warranted for effective application of antiviral drugs like amantadine.

This study was designed to gain insight into amantadine susceptibility of 6 avian and 12 porcine influenza A viruses isolated in Germany between 1981 and 2002. Virus strains were isolated in embryonated chicken eggs and passaged one time in MDCK cells. Plaque reduction assays were applied to examine virus susceptibility to amantadine. Genotyping was used to confirm drug resistance. In the result of these antiviral studies, only 3 of the 12 porzine isolates but all 6 avian isolates were shown to be amantadine-susceptible. Interestingly, the three amantadine-sensitive porzine strains were isolated between 1981 and 1987. All porzine influenza A viruses isolated later on were drug-resistant and contained the AA substitutions G16E, S31N, and R77Q in the matrix protein 2 (M2). Additionally, L27A was detected in two H1N1 strains. S31N and/or L27A are well known amino acid substitutions in M2 that confer amantadine resistance. The role of the pig as an intermediate host of avian and human influenza A viruses, the possible involvement of genetic reassortment, and the high incidence of naturally amantadine-resistant porcine influenza A viruses suggest a real risk of emergence of amantadine resistant human viruses. Therefore, further studies are ongoing now to evaluate the circulation of the resistant phenotype in pigs, birds and human.

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Antiviral Activity of Novel Isatin Derivatives Against Avian Influenza Virus (H5N1)

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Avian influenza virus infection is a fatal pathogenic caused by Influenza A (H5N1) strain from Asia. Recently much attention has been devoted to searching for effective chemotherapeutic agents and vaccines for eradication of this notorious disease. At present only chemotherapy is available to combat avian flu, for instance, Tamiflu, approved for the treatment by the US-FDA. Development of a simple, novel molecule with potential antiviral activity against is essential to treat avian flu viral infection. Isatin (2,3-dioxoindole), is a versatile lead molecule for designing of potential antiviral agents and its derivatives were reported to possess broad spectrum antiviral activity. Methisazone (*N*-methylisatin-3-thiosemicarbazone) was first clinically approved for treatment of pox viral infections, and its derivatives were documented to have anti-influenza activity. Based upon this evidence, the present work was initiated to determine the antiviral activity of novel Isatin derivatives against avian flu (H5N1) in MDCK cells. Antiviral activity was studied by virus yield assay (EC90), and cytotoxicity by neutral red uptake assay by uninfected MDCK cells. All five compounds of a series inhibited the replication of avian flu (H5N1) virus replication in MDCK cells and compounds SPIII-5H and SPIII-5Cl were most active (EC90 5.5 μg/ml, CC50 >100 μg/ml and SI > 18). Details of these studies and results of treatment of influenza-infected mice are discussed.

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Inhibition of Multiple Influenza A Subtypes in Cell Culture with Antisense Phosphorodiamidate Morpholino Oligomers

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Emergence of drug-resistant avian flu (H5N1) has stimulated development of influenza A (FLUAV) therapeutics. Arginine-rich peptide conjugated phophorodiamidate morpholino oligomers (ARP-PMO) are nuclease resistant antisense compounds that hybridize to target RNA in a sequence-specific manner resulting in disrupted RNA function. Eight ARP-PMO were designed to base-pair with various regions of A/PR/8/34 (H1N1) RNA and were then evaluated by hemagglutination and plaque assays for their ability to inhibit FLUAV production in Vero cell culture. ARP-PMO targeting the AUG translation start site of the NP or PB1 segment mRNAs, or the 3′-terminus of their respective vRNAs, were highly effective, reducing influenza virus titer by 1–3 orders of magnitude in a dose-dependent and sequence-specific manner over a period of 2 days. Two of the P-PMO, targeting the PB1 translation start site region (PB1-AUG) and the 3′ terminus of NP vRNA (NP v3′), were evaluated by endpoint dilution (TCID50) or ELISA assays against another H1N1 strain (A/WSN/33), as well as A/Memphis/8/88 (H3N2) and A/Thailand/1(KAN-1)/04 (H5N1). The PB1-AUG ARP-PMO generated over 85% specific reduction of virus level, regardless of viral subtype or methodology, at concentrations in the range of 10–20 μM. The NP v3′ P-PMO yielded similar results, with the exception of considerably lower efficacy against the H3N2 strain, with which it has two base mispairings. Studies are planned to further evaluate of at least two ARP-PMOs in animal models for H1N1 and H5N1 FLUVA subtypes.

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Antiherpetic and Anti-Influenza Activity of Aza-Crown Ethers

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Macroheterocyclic compounds containing crown fragments and nitrogen atoms show large-scale biological activity. We synthesized series of aza-crown ethers and their derivatives. We also studied anti-influenza and antiherpetic action of some of them. Anti-HSV action of studied compounds was tested using cyto-morphological method. Hep-2 cells were infected with HSV-1 strain US in dose 5 IFU/cell. The cells were incubated in Eagle's Medium that contained compounds in a dose of 10^{-4} M in experimental samples, or without them in control samples. Then cells were fixed with 96% ethanol and stained with 0.01% acridine orange solution. The amount of infected cells with DNA-containing virus inclusion bodies was counted by fluorescent microscopy. Anti-HSV activity of compounds was calculated as the difference between of the percentage of infected cells in treated cell cultures to the percentage of infected cells in untreated cell cultures. Anti-influenza activity was studied on the model of replication of A/Hong Kong/1/68 (H3N2) strain in tissue culture of chorio-allantoic membranes of chicken embryos. Compounds were used in a dose of 10^{-3} M during the study of their anti-influenza action. Diaza-18crown-6 and two of its derivatives have showmen neither anti-HSV nor anti-influenza activity. Diaza-18crown-6 derivatives that contain 2-oxyethyl- or ethoxycarbonyl-fragments decreased amount of cells infected by HSV-1 by 13 and 29%, respectively. Both of these compounds inhibited replication of influenza virus on 1.7 $\log_{10}$ TID_{50} Aza-15crown-5 did not show antiviral activity, but both its derivatives proved to be active inhibitors of HSV and influenza virus reproduction. Aza-15crown-5 derivatives that contain 2-amino-3-phenyl-propanoyl- or 5-benzyloxy-3-oxapentyl-fragments decreased amount of cells infected by HSV-1 with virus-specific intranuclear inclusions by 41 and 47%, respectively. First compound inhibited replication of influenza virus on 1.5 $\log_{10}$ TID_{50} and the second one decreased virus amount on 2.0 $\log_{10}$ TID_{50}. The results of this study show that aza-crown ethers are the perspective class of compounds for search of new antiviral agents.

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Effect of Single i.m. or i.v. Injection of Peramivir on an Influenza A (H5N1) Virus Infection in Mice

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The cyclopentane neuraminidase inhibitor, peramivir (BCX-1812, RWJ-270201) has striking inhibitory effects on a spectrum of influenza viruses in vitro, and has also demonstrated significant effects against influenza A (H1N1, H3N2) and B virus infections when administered orally to mice and ferrets. Unfortunately, clinical trials with the drug administered orally were not successful, probably due to low blood levels obtained after oral administration. Significant plasma drug levels of peramivir persist up to 6 h after intramuscular (i.m.) injection; more importantly, however, is the observation that peramivir remains tightly bound to influenza virus N9 neuraminidase for over 24 h, suggesting single i.m. or intravenous (i.v.) therapy with the drug may be highly effective against an influenza infection. Experiments now in press have indicated that single i.m. peramivir therapy administered up to 48 h after virus exposure was protective to mice infected with influenza A (H1N1) virus. In the present study, peramivir was administered i.m. or i.v. in a single injection 1 h pre-virus exposure in separate experiments to mice infected with an influenza A (H5N1) virus; efficacy was compared to similar dosages of oseltamivir and oseltamivir carboxylate run in parallel. Dosages of 20 and 10 mg/kg of peramivir administered by either route significantly prevented deaths, lessened arterial oxygen (SaO_2) decline, inhibited development of lung consolidation, and inhibited lung virus titers. The lung assays were performed at varying times after virus exposure. Oseltamivir and oseltamivir carboxylate, which do not have the same neuraminidase binding abilities seen with peramivir, were less efficacious in these experiments. Delaying the single i.v. therapy up to 72 h after virus exposure also significantly inhibited the virus infection. Peramivir appeared to be well tolerated in toxicity control animals run concomitantly with these studies. These data indicate parenterally administered peramivir may hold promise as a therapy for clinical influenza A (H5N1) virus infections.

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Inhibition of Influenza Virus A and B Production by RNA Interference

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Background: Influenza virus causes widespread infection in the human respiratory tract, but existing vaccines and drug therapy are of limited value. Recently, small interfering RNAs (siRNAs) are a powerful tool for sequence-specific, post-transcriptional gene silencing and have a potential therapeutic and prophylactic application against cancer, as well as infectious diseases. Here we show that short interfering RNAs (siRNAs) specific for conserved regions of the viral genome can potently inhibit influenza virus production in cell lines. The Influenza virus NP gene is a potential target for RNAi technology. On the other hand, the baculovirus (AcMNPV) can infect a variety of mammalian cells, facilitating its use as a virus vector for gene delivery in viral entry into cells. In this study, we describe the inhibition of influenza virus production by baculovirus-mediated shRNA expression vectors.

Methods: The pSV2neo-U6 plasmid vectors and pVL1393-based baculovirus vectors were used in this study. The influenza virus A and B NP genes were made into the target and the shRNA expression plasmid vectors were constructed under the control of the human U6 Pol III promoter. The shRNA expression plasmids or shRNA expression baculovirus vectors introduced into MDCK cells, and 24 h later the cells were infected with either A/PR8 or B/Ibaraki virus at a MOI of 0.01. At 72 h post-infection, culture supernatants were harvested and assayed to determine the virus titer by plaque assay.

Results: The influenza virus A and B NP genes when targeted with each shRNA expression plasmid vectors in MDCK cells, inhibited virus production in influenza virus infected-MDCK cells. Again, baculovirus-mediated shRNAs proved equally effective mediators of inhibition by down-regulating influenza virus replication in the influenza virus infected-MDCK cells.

Conclusion: The findings reveal that newly synthesized NP proteins are required for influenza virus replication and provide a basis for the development of shRNAs expression Plasmids as prophylaxis and therapy for influenza infection in humans.

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Antioxidant and Radical Scavenging Activity of a Plant Polyphenol-Rich Extract in the Murine Experimental Influenza Virus Infection

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A semi-standardized polyphenol-rich extract (PRE), obtained from *Geranium sanguineum* L., inhibited the reproduction of influenza viruses types A and B in vitro and in ovo and protected mice from mortality in the experimental influenza virus infection (Serkedjieva and Manolova, 1992). The selective in vitro virus-inhibitory activity of PRE was fairly modest and this was in contrast with the significant protection in vivo. Thus, the therapeutic effect of PRE needed explanation. It was presumed that it might be attributed to a combination of more than one biological activities known for natural polyphenols. We have demonstrated previously that PRE manifested strong antioxidant and radical-

scavenging activities in model systems (Sokmen et al., 2004). The current study was undertaken to investigate the effect of the plant extract on the levels of the antioxidant enzymes superoxide dismutase (SOD), catalase (KT) and peroxidase (PO) in mice lungs during influenza virus infection as well as the effect of PRE on the production of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) by alveolar macrophages in influenza virus infected mice. Mice were challenged intranasally (i.n.) with 5–10 LD_{50} of A/Aichi/2/68 (H3N2) influenza virus. PRE was administered by i.n. instillation 3 h before infection in the dose of 10 mg/kg. It was established that influenza infection induced an increase in SOD, KT and PO production and on days 6 and 9 after infection their levels reached 140–160% of placebo control. The application of PRE brought enzymes values to control levels. Influenza infection caused also a significant increase of H_2O_2, $O_2^{\bullet -}$ and NO production by alveolar macrophages; the generation of ROS and RNI peaked on day 9. PRE-treatment before viral challenge reduced this excessive production. In conclusion, the obtained results outlined the antioxidant and radical scavenging properties of the plant extract; PRE beneficially modulated the oxidative stress response in influenza virus-induced pneumonia. This alternative mechanism of action might contribute to the overall protective effect in the lethal murine experimental influenza infection.

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The Antiviral Activity of S11, a Natural Herb Extract, Against Influenza Virus Infections in Mice

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The antiviral activity of S11, one of the traditional Korean medical herb extract, against influenza virus was investigated. The 50% effective concentration (EC50) using plaque reduction assay was 31.25 ug/ml and the mean 50% cytotoxic concentration (CC50) using WST-1 assay in the MDCK cells was 334 ug/ml. Oral gavage treatment of the S11 to BALB/c mice infected with A/PR/8/34 (H1N1) influenza virus showed the therapeutic effects as delaying clinical signs, significant inhibition of death and reduction of lung virus titers. To identify the lead molecules, the S11 was subjected to further fractionation, purification, and isolation of active compounds. The antiviral activity of these natural herb compounds will be discussed. These results suggest that the S11 is a possible candidate for the development of new antiviral medicine for influenza therapy.

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Baculovirus (CpG Motifs) Induces an Innate Immune Response and Confers Protection From Lethal Influenza Virus A and B Infection in Mice

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Background: The baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV) has long been used as a biopesticide and as a tool for an efficient recombinant protein production in insect cells. In this study, we examined the immunization of a recombinant baculovirus expressing the influenza virus hemagglutinin (HA) against lethal influenza infection in mice. Protection was observed in mice immunized intranasally with not only the recombinant baculovirus but also a wild-type baculovirus. Baculovirus was also shown to induce secretion of inflammatory cytokines, such as TNF-α and IL-6, in murine RAW 264.7 macrophage cell line.

Results: A varied route of immunization with a recombinant baculovirus expressing the influenza virus hemagglutinin protein of A/PR/8/34 (H1N1) virus against lethal influenza infection was examined in mice. The recombinant baculovirus encoding the hemagglutinin gene under the control of chicken β actin promoter was inoculated twice, 2 weeks apart, at a dose of 1.1×10^8 pfu per mouse by intramuscular, intradermal, intraperitoneal, and intranasal routes. Mice intramuscularly and intraperitoneally immunized with the recombinant exhibited higher level of production of serum anti HA antibody than those immunized via the other routes, but protection was only achieved by the intranasal immunization. Surprisingly, mice immunized with a wild-type baculovirus with intranasal route were also protected from the lethal influenza virus challenge. Sufficient protection in mice was achieved by the intranasal immunizations with 10^8 pfu of either the recombinant or wild-type baculovirus, as evaluated by the reduction of virus titer, production of inflammatory cytokines, and pulmonary consolidations in the lung. These results indicate that infection with a baculovirus induces a strong innate immune response and protection of mice from lethal influenza virus infection.

Conclusion: Baculovirus (CpG motifs) induces a strong innate immune response and protection of mice from lethal influenza virus A and B infection.

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Rep 9: A Potent Broad Spectrum Aerosol Prophylaxis and Therapy Against Influenza Infection in vivo

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Potent antiviral activity of phosphorothioate oligonucleotides (PS–ONs) was observed against influenza viral infections. Antiviral activity was sequence-independent, size dependent (optimally active PS–ONs were $\geq$40 bases in length) and dependent on the presence of the phosphorothioate modification (hydrophobicity). Binding studies showed that REP 9 (a 40 mer degenerate PS–ON) interacts with both neuraminidase and hemagglutinin although the sialidase activity of neuraminidase was not affected, suggesting that the structural interactions of these proteins required for influenza activity are the target for this compound. The requirement for hydrophobicity further suggests that the alpha helical regions of hemagglutinin are one of the regions of interaction. The antiviral activity of REP 9 was conserved in many influenza A and B strains suggesting potential therapeutic activity against avian flu and other newly emerging influenza strains. REP 9 aerosol has excellent characteristics for lung deposition and aerosol treatment with REP 9 was well tolerated and highly effective against infections with influenza A both in prophylaxis and 24 h after infection. These results demonstrate the therapeutic potential of aerosolized PS–ONs against influenza infection.

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Inability to Select in vitro and in vivo of Parainfluenza Virus Variant Resistant to Novel Hemagglutinin-Neuraminidase Inhibitor BCX 2798

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BCX 2798 is a novel selective inhibitor of human parainfluenza virus infections, which design was based on the three-dimensional structure of the hemagglutinin-neuraminidase (HN) protein of Newcastle Disease virus. Compound exhibited striking activity against parainfluenza viruses in vitro and in vivo, and was efficacious in prophylaxis of lethal synergism between parainfluenza virus and *Streptococcus pneumoniae* in a mouse model. Present study was conducted to determine if BCX 2798's resistant variants of the recombinant Sendai virus whose HN gene was replaced with that of human parainfluenza virus type 1 (rSeV(hPIV-1 HN) could be selected in tissue culture and animals. For this purpose virus was serially passaged in LLC-MK_2 cells at MOI 0.1 in the presence of increasing (from 100 to 3200 μM) concentrations of compound; infected 129$\times$1/SvJ mice were treated with 10 mg/kg/day of BCX 2798 twice for five days. Treatment started 4 h before infection. Individual clones of viruses were analyzed for the presence of mutations. One mutation, E527K, on the globular head region of the HN protein was selected in tissue culture after the fifth and eleventh passages of rSeV (hPIV-1 HN). Several mutations in HN gene of rSeV (hPIV-1 HN) were selected in an animal model after the second passage of virus from mice treated with BCX 2798. Two mutations, N23S and P29Q, were located in the cytoplasmic domain of HN protein; mutations N173S and T553A were found on the globular head region of the glycoprotein. Only non-conserved amino-acid residues of HN protein were involved in substitutions. All isolated mutant viruses were stable after the five passages in LLC-MK_2 cells without drug; did not develop other substitutions in the presence of drug and displayed no resistance to BCX 2798 both in vitro and in vivo. Infectivity of all mutants was not altered to compare with the wild type of rSev (hPIV-1 HN) virus. Taking together our results indicate that prophylaxis/treatment of human parainfluenza virus infections with BCX 2798 may not lead to appearance of clinically significant variant of viruses.

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In vitro Inhibition of Maporal Hantavirus

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Hantavirus pulmonary syndrome (HPS) is an acute human respiratory disease with remarkably high case fatality rates (30–50%) for which the etiological agents are members of the Bunyaviridae family, genus *hantavirus*. Maporal (MAP) virus is a recently identified hantavirus isolated in western Venezuela, which is most similar phylogenetically to hantaviruses known to cause HPS in southern regions of South America. Despite the lack of evidence that MAP can productively infect humans and cause HPS, infection of hamsters closely resembles disease manifestations associated with human HPS. Hantaviruses, in general, are known to produce little to no cytopathic effect (CPE) in cultured cell lines. Unexpectedly, we found that MAP produces remarkable CPE in several Vero cell lines facilitating the evaluation of known antiviral agents, ribavirin and interferon alfacon-1. Both drugs were highly effective at reducing CPE, as determined by visual examination and neutral red dye uptake, associated with MAP infection. Since much of the observed CPE may be due to apoptosis of uninfected bystander cells, we also developed a quantitative (q)RT-PCR assay to detect copies of MAP genomic sequence to more directly assess the inhibition of viral replication. Data obtained using the qRT-PCR-based assay were consistent with the visual CPE reduction and neutral red-uptake cytotoxicity findings. The development of in vitro antiviral testing methods for MAP are essential to the evolution of the in vivo hamster disease model of HPS. The latter is of utmost importance considering the current need for effective antivirals for the

treatment of HPS and the lack of a suitable model that does not require biosafety level 4 containment facilities.

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Hepatitis Viruses

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Design, Synthesis, and Biological Evaluation of Novel Nucleoside Phosphoramidates as Potential Anti-HCV Agents

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Nucleoside analogues are widely used in antiviral and anticancer chemotherapy. For this class of drugs, intracellular conversion of the nucleoside analogue into the corresponding 5′-mono-, 5′-di-, and 5′-triphosphate after target cell penetration is a prerequisite for biological activity. Because of the structural differences from natural nucleosides, this conversion is often inefficient and, as a consequence, therapeutic efficacy is sometimes limited. The free phosphates, or nucleotides, have limited utility in therapy on account of their poor membrane permeability and chemical stability. One approach to improve the therapeutic potential of nucleoside analogues is the delivery of the corresponding nucleotide entities via neutral, lipophilic prodrugs, or protides.

The nucleoside aryl phosphoramidate approach, developed by McGuigan and co-workers (2004) has been successfully applied to a number of different nucleosides (AZT, d4T, ddA, d4A). The general structure of aryl phosphoramidates encompasses two masking groups, an amino acid ester and an aryl moiety bonded to the phosphate group.

In order to apply this protide technology to nucleosides with the potential for anti-hepatitis C virus (HCV) activity, we have undertaken studies designed to probe the effect of varying the natural and unnatural amino acid esters and the aryl groups used as masking groups in the target phosphoramidates. These compounds have been synthesised and evaluated using genotype 1b sub-genomic HCV replicon.

We have prepared a variety of arylphosphoramidate derivatives from a range of 4′-substituted nucleosides, including 4′-azido-cytidine, 4′-azido-uridine, and 2′,3′-protected variants. With certain nucleoside phophoramidates, we have observed dramatic enhancement (>1000-fold) of replicon activity relative to the parent nucleoside. The synthesis, biological activity and SAR of these compounds will be presented.

Reference

McGuigan, D. Cahard, Balzarini, J., 2004. Mini-review. Med. Chem. 4, 371–382.

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Design and Synthesis of Novel Anthranilic Acid Analogs as HCV Polymerase Inhibitors

Kevin Curran[1], Tom Nittoli[1], Shabana Insaf[1], Amar Prashad[1], Brawner Floyd[1], Marc Orlowski[2], Anita Howe[2], Rajiv Chropra[1], Atul Agrawal[1], Jonathan Bloom[1]

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We have identified a series of novel anthranilic acid derivatives that are potent, reversible inhibitors of Hepatitis C virus (HCV) NS5B polymerase, an essential enzyme for viral replication. The micromolar NS5B polymerase inhibitors belong to the *N*-phenoxyacetylanthranilic acid chemotype. X-ray crystallography determined that the inhibitors bound to NS5B between the thumb and palm regions adjacent to the active site. Guided by crystallography, subsequent modifications to the hydrogen bonding and lipophilic regions of the inhibitors resulted in greatly improved activity against NS5B. Further SAR studies revealed a second, more potent sub-series where the phenoxy group was replaced by an anilino group. Analogs in both subseries showed antiviral activity in a cell-based replicon model of HCV.

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Withdrawn

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Synthesis and Evaluation of Novel Potential HCV Helicase Inhibitors

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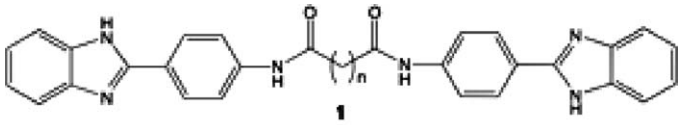
Hepatitis C is a viral infection that affects 170 million people worldwide, including 4 million in the United States and 8 million in Europe. The virus establishes a chronic infection in 55–85% of cases and 20% of affected individuals develop cirrhosis. At the moment there is neither a vaccine nor an effective antiviral therapy available and efforts to identify a specific anti-HCV inhibitor have dramatically intensified in the last few years.

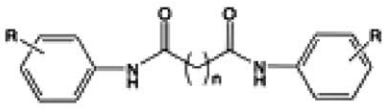
Many research groups have focused their interest on the enzymes involved in the viral replication and, among these enzyme, the viral Helicase/NTPase has proven to be a suitable target for developing novel anti-HCV compounds.

Compound **1** is a potent inhibitor of the HCV helicase and, although its mode of action is still uncertain, it has been proposed that it acts as competitive inhibitor of RNA binding. Starting from this hypothesis, we have prepared a series of novel compounds based on the structure of **1** where the benzimidazole

moiety has been replaced by different chemical groups, including the negatively charged carboxylate moiety, which should mimic the phosphate backbone of the nucleic acid.



1



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R = COOH, COOMe, SCH_3, SO_2CH_3, SO_2NH_2

The synthesis, the enzyme inhibition and the biological evaluation in replicon of these novel compounds will be presented and analyzed.

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Identification of Novel HCV Inhibitors Utilizing Virtual Screening

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Hepatitis C virus (HCV), a leading cause of liver disease, continues to be an attractive target for new drug development. Among the more favourable approaches to developing new HCV drugs is to target the RNA-dependant RNA (RdR) Polymerase (NS5B), which has been shown to be an essential enzyme for replication. There are several published non-nucleoside inhibitors of this polymerase (some in clinical development) and several published allosteric binding pockets on the protein they target.

To be successful, traditional lead identification can be time-intensive, costly and have large infrastructure requirements. Increasingly, a push towards effective computational-based screening has led to the development of virtual screening tools. Such tools allow investigation of large quantities of compounds *in silico* for particular properties without the need for compound synthesis or high throughput screening. Moreover, these techniques require only a modest infrastructure investment and are very efficient. We employed the OpenEye set of screening tools (Omega, ROCS and EON) in concert with publicly available HCV inhibitor information, and commercial databases to identify novel leads.

The inhibitor coordinates from a protein-inhibitor complex crystal structure were utilized as the target. Available compound databases (Asinex and Chembridge) were utilized as the testset of compounds. Filtered compound conformers were generated using Omega and compared with the template using ROCS with post-analysis by EON. Visual analysis to maximize particular desirable binding features while minimizing protein-inhibitor steric clash allowed the list of potential hits to be further narrowed.

Multiple classes of compound were identified from the above procedure and after sourcing a subset of the actual compounds or close analogs, they were tested for enzymatic inhibition activity and further characterized. Iteration of the process resulted in the identification of a lead compound class containing multiple active compounds, one with reasonable replicon activity. In conclusion, readily available structural and database information and virtual screening tools can be successfully utilized to identify novel inhibitors of HCV RdR Polymerase which, in turn, can serve as novel leads for developing new therapies for treating HCV.

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Synthesis, Antiviral Activity, and Cytotoxicity of Some Novel Quinazolin-4(3h)-One Derivatives

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A series of novel 6-bromo/6,8-dibromo-4-(4-oxo-2-phenyl-4H-quinazolin-3y-l)-benzenesulphonamides were synthesized by condensation of 2-substituted benzo[1,3]oxazine-4-ones and sulphonamide. Their chemical structures were assigned by means of spectral analysis (FT-IR, PMR, MS). Synthesized compounds were screened for in vitro antiviral activity against human pathogenic viruses (HIV, HCV, HSV, VV). 6-bromo-4-(4-oxo-2-phenyl-4H-quinazolin-3y-l)-benzenesulphonamide (SPS-II) and 4-(4-oxo-2-phenyl-4H-quinazolin-3y-l)-benzenesulphonamide (SPS-I) inhibits the replication of HIV-1 in acutely infected MT-4 cells at a concentration of approximately 10 μg/ml, while not being toxic to the host cell at a concentration of 60 or >125 μg/ml (selectivity index: 8 and >12), respectively. In Huh 5-2 cells SPS-I inhibited HCV RNA synthesis at EC50 of 8 μg/ml, while at CC50 for cell growth 32 μg/ml. SPS-II inhibited the virus-induced cytopathicity in Human embryonic lung (HEL) cell infection with HSV-1, HSV-2 or vaccinia (VV) at a concentration of 50 μg/ml, while not being toxic to the cells up to a concentration of 400 μg/ml. Further molecular modification in this series of compounds may help in optimising their antiviral activity.

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HCV NS5B Nonnucleoside Inhibitors Specifically Block Synthesis of Single-Stranded Viral RNA Catalyzed By HCV Replication Complexes in vitro

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HCV nonstructural protein NS5B is the catalytic subunit of the replication complexes, possessing a motif characteristic of RNA-dependent RNA polymerases. Biochemical assays using

recombinant NS5B have been used to investigate NS5B nonnucleoside inhibitors. However, the inhibitory effect of compounds often varies with the forms of recombinant NS5B and the concentrations of the template and/or primer used in the assays. In addition, it does not always correlate to that obtained with replicon-containing cells. These observations have cast concerns about the validity of these cell-free assays. In the report, we explored replication complexes, isolated as crude membrane fractions from replicon-containing cells, for their competency to synthesize viral RNA in vitro as well as their responsiveness to NS5B inhibitors. After optimizing the experimental conditions, two species of nascent viral RNA, one double-stranded and the other single-stranded, were readily detected. The addition of NS5B nucleotide inhibitor blocked synthesis of both species. The presence of nonnucleoside inhibitors, however, inhibited mostly single-stranded RNA (ssRNA) synthesis. In addition, the replication complexes isolated from the cells containing a replicon that carried a resistant mutation in NS5B to the nonnucleoside inhibitor were able to synthesize the same amount of ssRNA in vitro regardless of the presence or absence of the inhibitor, demonstrating that the phenomenon is due to the specific inhibitory effect of the compound on NS5B. Combining with kinetic studies that ssRNA synthesis was inhibited only when the nonnucleoside inhibitor was present during the pulse period, we conclude that ssRNA synthesis catalyzed by the replication complexes in vitro is likely derived from the de novo initiation.

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Potent Inhibition of Both HIV and HCV in vitro by a Ring-Expanded ("Fat") Nucleoside: Part II. Mechanistic Studies of Anti-HCV Activity

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We have recently reported the synthesis and antiviral activities of a ring-expanded ("fat") nucleoside analogue, called NZ-51, that inhibits both HCV and HIV in vitro with EC_{50} values ranging in micromolar concentrations or less, and little or low toxicity to the host cells. In this Part II of the presentation on this subject, we report our preliminary results on mechanistic studies of anti-HCV activity of this compound, along with the synthesis and antiviral activity of a few additional analogues in the series. In light of the fact that HCV is a major co-infection in patients infected with HIV, and that a number of them ultimately die of end-stage HCV-related complications including liver cirrhosis and hepatocellular carcinoma, a drug with dual inhibitory characteristics against both viruses is highly desirable and timely.

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Comparison of the Antiviral Activity of Amantadine Against Hepatitis C Virus of Different Genotypes in Cell-Based Replicon and Infectious Virus Assays

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More than 170 million people worldwide are estimated to be infected with Hepatitis C virus (HCV). In the majority of these people a chronic infection is established which can result in serious long-term liver damage including progressive fibrosis, cirrhosis and hepatocellular carcinoma. In fact, HCV is believed to cause more than 100,000 cases of liver cancer annually worldwide and accounts for at least 40% of liver transplants in the US. Current treatment options are limited and there is a high treatment failure rate. Thus, there is a real need for new treatment options. Amantadine has been evaluated as a treatment for chronic HCV infection in a number of clinical studies both as a monotherapy and in combination with other therapeutics. However, the results of these trials have been contradictory and at this time the clinical potential of amantadine as a therapy for chronic HCV infection remains unclear. Recent studies have shown that the small hydrophobic HCV p7 protein forms an amantadine sensitive ion channel providing a possible basis for antiviral activity. We examined the ability of amantadine to reduce HCV replication in both subgenomic and full-length HCV replicons of genotypes 1a strain H77C and genotype 1b strain N. In these studies amantadine failed to reduce viral RNA replication in any of the replicons tested. Further, in infectious virus assays using HCV genotype 2a strain JHF-1 10 uM amantadine failed to reduce viral RNA levels under any of the conditions tested. However, in these infectious virus studies, when the viral inoculum was treated with amantadine prior to infection of cell monolayers, or when the amantadine was added to cells 1 h after virus adsorption there was a significant reduction in the number of infectious viral foci observed after 72 h incubation ($p < 0.05$ and <0.01, respectively). These results suggest that even in the absence of a direct impact on RNA replication amantadine has antiviral activity. We are currently evaluating amantadine for activity in infectious HCV genotype 1a assays to further define its antiviral spectrum of activity. Studies to more fully define its mechanism of action in the virus life cycle are also underway.

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Celgosivir and Castanospermine are Highly Synergistic Against Bovine Viral Diarrhea Virus When Combined with Interferon Alpha 2b or with Interferon Alpha 2b and Ribavirin

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Celgosivir is an alpha glucosidase inhibitor that is being developed for the treatment of Hepatitis C virus (HCV) infections in humans. The purpose of this study was to evaluate the in vitro antiviral activity of celgosivir and its primary active metabolite, castanospermine, when combined with current approved therapies (ribavirin, interferon α-2b, or both) in a surrogate model of HCV (bovine viral diarrhea virus (BVDV)). Compounds alone or in combination were tested against BVDV in infected Madin–Darby bovine kidney (MDBK) cells. Synergies were analyzed using isobolograms and volume of synergy measurements (MacSynergy II^{TM} software). The celgosivir-interferon α2b combination was significantly more synergistic than the celgosivir-ribavirin combination (∼3-fold), or the ribavirin-interferon α2b combination (∼2-fold). Similarly, the castanospermine-interferon α2b double combination was more synergistic than the castanospermine-ribavirin combination (∼5-fold), or the ribavirin-interferon α2b combination (∼3.3-fold). The combinations of celgosivir-interferon α2b or castanospermine-interferon α2b led to significant decreases in the EC50s of celgosivir (up to >20-fold) and castanospermine (up to >50-fold). The effective EC50s of celgosivir or castanospermine were further reduced by the addition of ribavirin. The cytotoxicity of the double and triple combinations was additive or less than additive, indicating that combinations of celgosivir or castanospermine with ribavirin and/or interferon α2b were generally less toxic than expected. These results indicate that the combination of celgosivir with interferon α2b or with interferon α2b and ribavirin may be effective in the treatment of HCV.

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Antiviral Activity of the α-Glucosidase Inhibitors Celgosivir and Castanospermine Combined with NM-107, Amantadine, and NB-DNJ

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Pegylated interferon α plus ribavirin is the current standard of care for the treatment of chronic hepatitis C virus (HCV) infections. This regimen results in sustained virologic response in only about 50% of patients and is associated with significant treatment-associated toxicities. A number of approaches are being used to identify novel therapeutic combinations with better tolerability and/or efficacy. Inhibitors of endoplasmic reticulum (ER) α-glucosidase have been shown to inhibit viral replication and secretion and may have utility as part of new multi-drug treatment cocktails. The α-glucosidase inhibitor celgosivir is currently being evaluated in combination with pegylated interferon α and ribavirin in humans. The purpose of this study was to evaluate the antiviral effects of combinations of celgosivir and castanospermine, the primary active metabolite of celgosivir, with other antiviral agents having diverse mechanisms of action.

The effect of the combination of celgosivir or castanospermine with the nucleoside analogue NM-107, amantadine, and another iminosugar, *N*-butyl-deoxynojirimycin (NB-DNJ) was determined in a cytopathic assay using the HCV surrogate virus bovine viral diarrhea virus in Madin Darby bovine kidney cells. Synergies were analyzed using isobolograms and volume of synergy measurements (MacSynergy II^{TM} software). Volumes of synergy indicated that the castanospermine and NB-DNJ combination was additive, while the celgosivir and NB-DNJ combination was synergistic at high NB-DNJ concentrations (>100 μM). Celgosivir and castanospermine were synergistic with both amantadine and NM-107, with volumes of synergy between 60 and 150 μM%. Isobologram analysis confirmed these synergistic interactions.

These results indicate that celgosivir could be considered in combination regimens containing drugs that directly target viral replication like NM-107. Mechanism(s) of synergy are under investigation.

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Interference of Hepatitis C Virus Replication by Combination of Protein Kinase C-like 2 Inhibitors and Interferon-α

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Hepatitis C virus (HCV) is an enveloped virus with positive-stranded RNA genome of approximately 9.6 kilobases and a major cause of non-A and non-B hepatitis, leading to liver cirrhosis and hepatocellular carcinoma. Combination of interferon-α (IFN-α) and ribavirin is the current standard therapy for the treatment of HCV infection, but there is no specific antiviral therapy available. The HCV viral genome encodes a single polyprotein of approximately 3010 amino acids, which is proteolytically processed by a combination of host and viral proteases into at least 10 distinct structural and nonstructural proteins. The structural proteins include C, E1, E2, and p7 and the nonstructural (NS) proteins include NS2, NS3, NS4A, NS4B, NS5A, and NS5B. As new HCV specific therapies, small-molecule inhibitors against HCV enzymes including NS5B protein, the viral RNA-dependent RNA polymerase (RdRp), and NS3 protease are in clinical tests. However, rapid emerging of drug-resistant mutants has been hampering their practical clinical applications. Recently, we have shown that phosphorylation of HCV RNA polymerase by protein kinase C-like 2 (PRK2) regulates virus RNA replication. HCV RNA replication was inhibited when PRK2 expression level was down-regulated by using a PRK2-specific siRNA. In this study, we investigated the anti-HCV effect of PRK2 inhibitors in an HCV subgenomic replicon system. Treatment of the replicon cells with PRK2 inhibitors suppressing the endogenous PRK2 activity inhibited

the phosphorylation of HCV RNA polymerase and resulted in suppression of HCV RNA replication in a dose-dependent manner. Furthermore, the PRK2 inhibitor in combination with IFN-α more effectively inhibited HCV RNA replication than IFN-α alone. Because the PRK2 inhibitor did not show cytotoxicity in the cell-based drug inhibition studies and cellular proteins rarely get mutated, PRK2 can serve as a cellular target for therapeutic intervention of HCV replication. Specific inactivation of PRK2 activity will provide an opportunity to interfere with HCV RNA replication.

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Hepatitis B Virus e Antigen Production is Dependent upon Covalently Closed Circular (ccc) DNA in HepAD38 Cell and May Serve as a cccDNA Surrogate in Antiviral Screening Assays

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More than 400 million people worldwide are chronically infected with Hepatitis B Virus (HBV). The major complication of chronic hepatitis B is the development of primary hepatocellular carcinoma (HCC), which causes an estimated 500,000 deaths annually. Currently clinical treatments (α-Interferon and nucleoside analogs) of chronic Hepatitis B rarely cure the virus infection. This is due, at least in part, to their failure to eliminate viral covalently closed circular (ccc) DNA from the nuclei of infected hepatocytes. HBV cccDNA is essential to the virus life cycle by serving as the template for the transcription of the pregenomic RNA and of the subviral RNA species. Its elimination during chronic infection is considered critical to long-term therapy. However, cccDNA has not previously been targeted in high throughput screens of small molecule libraries. To screen compound libraries for antiviral drugs targeting cccDNA, we set out to develop a cell-based assay suitable for high throughput screening. Since cccDNA is time-consuming to assay, it was desirable to use a viral gene product that could serve as a reporter for intracellular cccDNA level. We predicted that the secretion of HBV e antigen (HBeAg) by HepAD38 cells, a HepG2-derived tetracycline inducible HBV expression cell line, would be cccDNA-dependent. This is because a large portion of pre-core mRNA leader sequence in the 5′ terminus of integrated viral genome was deleted, preventing HBeAg expression from transgene, but could be restored from the 3′ terminal redundancy of pre-genomic RNA during viral DNA replication and subsequent cccDNA formation. Our experimental results showed that following induction, HepAD38 produced and accumulated cccDNA, which became detectable between 7 and 8 days. HBeAg synthesis and secretion into culture fluid were dependent upon and proportional to the level of cccDNA detected. Therefore, the secretion of HBeAg by HepAD38 cells could potentially serve as a convenient reporter for the high throughput screening of novel antiviral drugs targeting HBV cccDNA.

Prodrugs and Drug Delivery

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Comparison of the Uptake and Intracellular Metabolism of Hexadecyloxypropyl Esters of (S)-HPMPA and Cidofovir

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(S)-HPMPA is a broad spectrum antiviral active against orthopoxviruses, HBV, CMV, HSV, and other herpes group viruses. We have shown that HDP-(S)-HPMPA has greatly enhanced antiviral activity against these viruses. In addition, while HPMPA itself is nearly inactive against HIV, we showed that HDP-(S)-HPMPA exhibited an EC50 >3 logs less than unmodified HPMPA in MT-2 cells by p24 reduction assay. To evaluate the metabolic basis for the increased antiviral activity, we studied and compared the cellular uptake of radiolabeled CDV, (S)-HPMPA and their HDP-esters and conversion to HPMPA-diphosphate (HPMPApp) and CDV-diphosphate (CDVpp) in MRC-5 human lung fibroblasts using HPLC Partisil SAX ion exchange chromatography. Cellular uptake of HDP-CDV and HDP-(S)-HPMPA was similar. However, when cells were exposed to the respective drugs for 6, 24 and 48 h, HPMPApp appeared much earlier than CDVpp and reached levels several fold greater than observed with HDP-CDV. Drug wash out experiments were carried out in MRC-5 cells exposed to radiolabeled HDP-CDV and HDP-(S)-HPMPA. After 24 h, the culture medium was removed and replaced with complete medium without drug and the levels of HPMPApp and CDVpp were measured by HPLC every 2 days for 0–10 days. Levels of the diphosphates declined slowly with a $T_{1/2}$ of 5–10 days. In conclusion, HDP-(S)-HPMPA is converted to its diphosphate more rapidly than HDP-CDV and reaches higher intracellular levels. This may explain, in part, its greater antiviral activity.

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Antiviral Activity and Metabolic Stability of Branched Methyl Alkoxyalkyl Esters of Cidofovir against Vaccinia, Cowpox, and Ectromelia Viruses, in vitro

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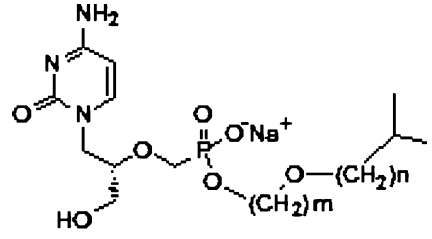
The antiviral activity and oral bioavailability of cidofovir (CDV) is enhanced when the phosphonate is esterified with various straight chain alkoxyalkyl groups. The length of this chain is an important determinant of antiviral activity and selectivity. However, in some cases, rapid metabolism to an inactive short chain metabolite was observed. To enhance the metabolic stability of

these esters, we synthesized cidofovir alkoxyalkyl esters bearing methyl groups on the penultimate carbon of the alkyl chain. Enzymatic stability of 15-Me-HDP-CDV (**1**) was tested in liver S9 fractions from various species. In mouse and human liver S9 fractions, compound **1** was completely stable for 90 min while 15–20% of the straight chain HDP-CDV was metabolized. The branched alkoxyalkyl esters were then evaluated in cells infected with vaccinia, cowpox and ectromelia viruses. The branched methyl analogs were substantially more active than CDV and equal to or slightly more active than the straight chain analogs. Compound **1** retained full activity compared to HDP-CDV and compound **2** showed greater activity against orthopoxviruses compared to its unbranched analog. We believe that the structural modification of the alkyl chain slows the formation of inactive metabolites, possibly by interfering with ω oxidation and may result in better pharmacokinetics and more potent antiviral activity against orthopoxvirus infection in vivo.



1 15-Me-HDP-CDV m=3 n=14

2 17-Me-ODE-CDV m=2 n=16

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Ethylene Glycol-Linked Amino Acid Conjugates of Cyclic Cidofovir: Synthesis and Biological Activity

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Cidofovir ([1-(S)-3-hydroxy-2-(phosphonomethoxy)propyl]-cytosine, HPMPC) is a broad spectrum antiviral agent clinically used for treatment of AIDS-related CMV retinitis. Cidofovir has limited oral bioavailability (<5%), attributed to ionization of its phosphonic diacid moiety under physiological conditions. We have shown that masking of this group by conjugation of the cyclic form of the drug (cHPMPC) via a Ser side chain P-O ester linkage with X-Ser dipeptides, where X = a hydrophobic amino acid, can result in prodrugs **1** that afford significantly improved biological availability of parent drug in an animal model. Here we describe the total synthesis of novel cyclic cidofovir prodrugs **2ab** of L-Val and L-Phe using an alternative conjugation strategy, *viz.* via an ethylene glycol link utilizing P–O and C–O ester bonds. The preparation of the HPMPC synthon from R-glycidol used our modification of the literature procedure (Brodfuehrer et al., 1994), involving reaction of tritylated (R)-glycidol directly with unprotected cytosine to achieve regiospecific opening of the epoxide ring, followed by reaction with benzoic acid anhydride to obtain the desired *N*-benzoyl intermediate needed to continue the synthesis. PyBOP was used as condensing agent in a convenient, one-pot conversion of HPMPC to cHPMPC and subsequent esterfication of the latter by the ethylene glycol-modified amino acids. The prodrugs **2** were converted to drug by cellular (Caco-2, HFF) and tissue (liver and intestinal) homogenates, but did not show enhanced oral bioavailability when evaluated in a rat model, suggesting that such compounds may be useful for understanding the effectiveness of **1** in drug delivery.

Reference

Brodfuehrer et al., 1994. Tetrahedron Lett. 35, 3243–3246.

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Stability, Transport, and Activity of Cidofovir Peptide Prodrugs

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Cidofovir (CDV) is a broad-spectrum anti-viral agent that is used to treat AIDS-related cytomegalovirus (CMV) retinitis and other CMV infections. CDV has good in vitro activity against orthopox viruses, including smallpox; however, its use is limited because of the drug's low oral bioavailability and poor transport into cells. In order to improve its oral bioavailability, our group has synthesized a series of dipeptide and amino acid prodrugs of the cyclic analog of cidofovir (cCDV). In the current project, we examined the cytotoxicity and antiviral activity of the prodrugs, showing that the compounds are not cytotoxic and have diverse activity against HCMV and orthopox viruses (vaccinia and cow pox) with 50% inhibitory concentrations ranging from 0.1 to 0.5 and 10 μM and greater, respectively for the two virus types. In vitro and in situ perfusion studies established that the permeability of the prodrugs is enhanced more than 30-fold and that the transport is mediated, at least in part, by the intestinal dipeptide transporter. We also have found that the bioavailability of the prodrugs is dependent upon the prodrug structure and that we can achieve up to an eight-fold increase in bioavailability over the parent compound in vivo. Drug stability experiments showed that in gastrointestinal and liver homogenates, the cCDV prodrugs are enzymatically hydrolyzed to the parent compound. It is clear from this work that the biologically benign dipeptide moiety, strategically linked to the drug to mask its anionic

properties, significantly enhances intestinal transport of cCDV, creating the possibility of an orally bioavailable form of cCDV with low toxicity.

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CMX052, an Orally Available Lipid Conjugate of Foscarnet for the Treatment of Drug Resistant HIV

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Foscarnet, a pyrophosphate analog approved for the treatment of CMV retinitis and acyclovir-resistant herpes infections in immunocompromised patients, is active against highly drug resistant strains of HIV-1. However, the clinical utility of foscarnet is limited because it requires controlled intravenous infusion and is associated with high risks of renal impairment and seizure caused by alterations in plasma minerals and electrolytes. Lipid conjugation has been shown to increase the in vitro activity, improve oral bioavailability, and reduce the toxicity of several antiviral drugs requiring intravenous administration because of poor bioavailability. In the case of foscarnet, conjugation to methylbatyl alcohol (CMX012) decreases the apparent EC_{50} value against HIV-1 by up to 40-fold. CMX012 was esterified to produce CMX052 in order to increase solubility and to protect against decarboxylation of the foscarnet moiety during passage through the stomach. Here we present the results of a preliminary toxicology and toxicokinetic study of the methylbatyl alcohol conjugate of foscarnet methyl ester (CMX052). Rats were given oral doses of 10, 30 and 100 mg/kg CMX052 daily for 7 days. There were no clinical signs of toxicity. Body weight and food consumption were comparable to controls and serum biochemistry, hematology, coagulation parameters and urinalysis were normal. There were no gross findings at necropsy, no effects on organ weights and no findings by histopathological examination of a wide range of tissues. Importantly, there were no changes in serum biochemistry parameters or histopathological examination that were indicative of the renal impairment or serum electrolyte changes that are associated with foscarnet. Oral dosing resulted in significant plasma exposure to CMX012 ($C_{max} > 4$ μg/mL), the biologically active deesterified form of CMX052. In conclusion, CMX052 is absorbed after oral administration, converted to CMX012, and has a good preliminary toxicity profile. These results support the development of CMX052 for the treatment of drug resistant HIV infection.

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Amino Acid Ester Prodrugs of Vidarabine: Stability, Permeability, and Activity Against Vaccinia Virus

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Vaccinia virus is a surrogate model system for study of pox virology and development of antiviral therapeutics. The potent anti vaccinia virus activity and various shortcomings of vidarabine make it a good candidate for improvement by utilizing prodrug strategy. Vidarabine is a polar nucleoside drug with low membrane permeability and rapid degradation by adonesine deaminase. 5′-Monoester prodrugs of vidarabine with various amino acids promoieties (L-valine, L-isoleucine, L-phenylalanine. L-aspartic acid, L-proline) are synthesized and evaluated for their stability, permeability and activity against vaccinia virus. Prodrugs exhibit different hydrolysis rate in Caco-2 cell homogenate (t1/2: 2–40 min). 5′-L-Isoleucyl and 5′-L-valyl monoester prodrugs exhibit comparable bio-conversion rate and hPEPT1-mediated uptake as well as Caco-2 permeability with valacyclovir, a commercially marketed oral amino acid ester prodrug. Both prodrugs have potent activity against vaccinia virus and are resistant to ADA1. Preliminary animal study shows 5′-L-isoleucyl vidarabine results in >10-fold increase in circulating vidarabine level. The results suggest that it may be possible to use amino acids prodrug strategy to improve vidarabine as anti vaccinia virus agent.

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Vidarabine Prodrugs as Anti-Pox Virus Agents

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Vidarabine [9-β-D-arabinofuranosyl)adenine or ara-A) was originally investigated as an anti-tumor agent and was later found to be active against herpes simplex virus (HSV) types 1 and 2. It was the first FDA-approved drug for treatment of systemic HSV infections. Although replaced by acyclovir and analogs for most applications, vidarabine remains an alternative therapy for acyclovir-resistant HSV and varicella-zoster virus infections. Despite its proven efficacy, vidarabine suffers some limitations including: (i) metabolism by adenosine deaminase (ADA) to its inactive hypoxanthine homolog (ara-H); (ii) low lipophilicity and membrane permeability and (iii) poor aqueous solubility, thus limiting options for parenteral and peroral delivery. Our recent interest in vidarabine was triggered by our discovery that

it was ~5-fold more active against vaccinia (VV) and cow pox (CPV) viruses than was cidofovir in plaque reduction assays. Its activity was enhanced about 10-fold by combination with 1 μM 2′-deoxycoformycin (pentostatin, a potent inhibitor of ADA) thereby providing significant superiority to cidofovir. From these results and our earlier studies on 5′-substituted vidarabine analogs (Lipper et al., 1978. Mol. Pharmacol. 14, 366–369), we determined that minimizing metabolism of vidarabine by synthesizing 5′-amino acid substituted prodrugs gave a significantly more potent anti-pox virus agent. We found that amino acid ester prodrugs of vidarabine are active against VV at non-cytotoxic concentrations. Further, using cell homogenates, purified enzyme and intact cell systems, we showed that the prodrugs are resistant to inactivation by ADA. The prodrugs also had enhanced transport potential, most likely targeting the intestinal dipeptide transporter. Finally, oral delivery of the prodrug to the small intestine resulted in a 10-fold increase in vidarabine plasma levels when compared to unsubstituted vidarabine. These properties make the prodrugs of vidarabine good candidates as orally bioavailable anti-pox virus agents that are stable in the presence of ADA.

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*cyclo*Sal-Monophosphate Prodrugs with an Optimized Mask

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The *cyclo*Sal pronucleotide system has been designed for an intracellular delivery of therapeutically active nucleoside monophosphates.

As part of recent work on the *cyclo*Sal approach, the interaction of *cyclo*Sal nucleotides with cholinesterases has been investigated. It is known that organo-phosphates may act as irreversible inhibitors of cholinesterases (*suicide mechanism*). In the case of *cyclo*Sal nucleotides, cholinesterase inhibition could lead to unwanted side effects in a possible therapeutic application. There are two types of cholinesterase found in humans, the highly specific, physiologically important acetylcholinesterase (AChE) and the much more unspecific butyrylcholinesterase (BChE) of unknown physiological importance. Fortunately, no inhibition of AChE was observed for a variety of different *cyclo*Sal nucleotides. In contrast, BChE inhibition was found in some cases.

The anti-HIV-active 3,5-bis-*tert*butyl-6-fluoro-*cyclo*Sal-d4T monophosphate is the first *cyclo*Sal derivative combining three desired properties: successful intracellular delivery of nucleotides, sufficient hydrolytic stability and strongly reduced inhibitor activity towards BChE. Because of the promising properties of this compound, we combined this mask developed for d4T with the antiviral active nucleoside analogues like d4A, ddA, AZT and Acyclovir. In this contribution we present the synthesis, hydrolysis stability, inhibition behaviour towards BChE and anti-HIV data of these new compounds.

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Synthesis and Properties of Intrinsically Fluorescent *cyclo*Sal-Pronucleotides

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*Cyclo*Sal-pronucleotides are used for the delivery of antivirally active nucleotides into cells via a pH triggered selective hydrolysis. To distinguish between intra- and extra-cellular environment enzyme-cleavable side chains were introduced in the aromatic moiety of the pronucleotide to enrich the compound inside cells. This behavior will further be described as "lock-in"-effect. Many other different *cyclo*Sal pronucleotides have been designed, all showing different hydrolysis properties and antiviral data, both originating from the nature of the *cyclo*Sal-moiety as well as of the nucleoside. To examine these differences, analytical tools of high accuracy and sensitivity were needed, being structurally as close as possible to the lead compounds. These requirements are met by intrinsically fluorescent nucleosides coupled to different *cyclo*Sal masking groups. For analysis of the purine-type nucleosides *iso*-dA with high intrinsic fluorescence properties was chosen and converted into *iso*-A, *iso*-ddA and *iso*-d4A. For the pyrimidine-type series the fluorescent nucleoside m^5K was synthesized as well as the dideoxy-compound dm^5K. These nucleosides were transformed into different *cyclo*Sal-pronucleotides and tested for their suitability for fluorescence analysis. In fact, an improvement of sensitivity by a factor of 5000 compared to UV-detection was found for some of the compounds (pmol detection). For all compounds fluorescence and absorbance spectra were recorded to determine the absorption and emission maxima. The new compounds lacked activity against HIV-1 and HIV-2 strains. However, the compounds showed low cytotoxicity, which is important for their usability as fluorescent probes in cells. Due to the analytical sensitivity, a simple model uptake study could be carried out, employing an U-tube with two aqueous phases, which were separated by an unpolar organic solvent simulating a diffusion barrier. The properties of the aqueous phases were varied and an enzyme-driven enrichment of a "lock-in"-modified intrinsically fluorescent *cyclo*Sal-pronucleotide passing the diffusion barrier could be simulated.

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Enzymatically Activated *cyclo*Sal-Pronucleotides

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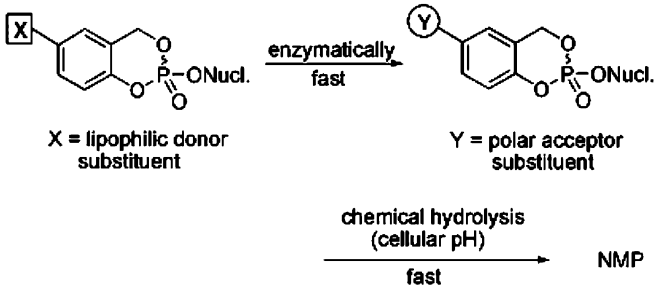
*Cyclo*Sal-pronucleotides efficiently deliver therapeutically active nucleoside monophosphates in human cells. "Lock-in"-*cyclo*Sal-pronucleotides – the so-called second generation of

*cyclo*Sal-compounds – have been designed to trap the compound by intracellular cleavage of esterase-cleavable moiety. One disadvantage of the "lock-in"-compounds is their high chemical stability, which leads to a delayed drug delivery. Therefore, conceptually different, enzymatically activated *cyclo*Sal-pronucleotides have been developed. In this concept lipophilic donor substituents attached to the aromatic ring are converted into a polar acceptor substituent by intracellular enzymatic cleavage. As a consequence the liberated acceptor group leads to a strong decrease in hydrolysis stability and a rapid formation of a charged intermediate is the result. From the phosphodiester intermediate the nucleotide is released subsequently.



The concept, synthesis, characterization and in vitro antiviral evaluation of the third generation of *cyclo*Sal-pronucleotides will be presented.

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GS9131, a Phosphoramidate Prodrug of Novel Cyclic Nucleotide Analog, Exhibits Favorable in vitro and in vivo Pharmacological Profile

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Background: N(t)RTIs are currently used as a backbone of antiretroviral combination therapy. However, their long-term benefit can be limited by adverse effects, resistance development, drug-drug interactions, and sub-optimal efficacy in treatment-experienced patients. Therefore, we searched for novel nucleotide analogs with improved pharmacological profiles.

Methods: Phosphonomethoxy-2′-fluoro-2′,3′-dideoxydidehydroadenosine (GS9148) was selected from a broad range of nucleoside phosphonate analogs. Phosphoramidate prodrug technology previously explored with tenofovir was applied to GS9148, resulting in the identification of GS9131 (ethylalaninyl phenyl ester of GS9148).

Results: GS9131 exhibits potent anti-HIV-1 activity in primary lymphocytes and T-cell lines ($EC_{50} < 150$ nM). Low cytotoxicity ($CC_{50} > 100$ μM) was observed in multiple cell types including renal cells. Diphosphate metabolite of GS9148 was shown to act as an obligatory DNA chain terminator and a competitive inhibitor of HIV-1 reverse transcriptase (RT) ($K_i = 0.8$ μM). Unlike ddI, d4T, or d4FC, neither GS9148 nor its prodrugs inhibited mitochondrial DNA replication in HepG2 cells. In a PhenoSense assay, GS9148 retained its full activity against HIV-1 variants with K65R, M184V or L74V mutations in RT. Viruses with ≥4 thymidine analog mutations showed ≤2-fold reduced susceptibility to GS9148, a shift that was smaller than that of any other tested NRTI. Following an oral dose of 3 mg/kg GS9131 in dogs, the bioavailability of prodrug exceeded 20%, resulting in high intracellular levels (9.0 ± 2.3 μM) and prolonged retention ($T_{1/2} > 24$ h) of GS9148 diphosphate in blood lymphocytes.

Conclusions: Both GS9148 and its prodrug GS9131 exhibit favorable in vitro pharmacological profiles including less resistance due to RT mutations than approved NRTIs. GS9131 possesses good in vivo pharmacokinetic properties and thus represents an attractive development candidate with potential for clinical efficacy in both treatment-naive and NRTI-experienced patients.

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Cathepsin A is the Major Hydrolase Catalyzing the Intracellular Activation of Nucleotide Phosphoramidate Prodrugs GS-7340 and GS-9131

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GS-7340 and GS-9131 are alkylalaninyl phenyl ester prodrugs of tenofovir (TFV; 9-[(2-phosphonomethoxy)propyl]adenine) and a novel nucleotide analog Fd4AP (phosphonomethoxy-2′-fluoro-2′,3′-dideoxydidehydroadenosine), respectively. Both GS-7340 and GS-9131 exhibit potent in vitro anti-HIV-1 activity, favorable resistance profile, and low cytotoxicity. Compared to tenofovir disoproxil (Viread), both prodrugs are significantly more stable in plasma and deliver >10-fold greater levels of active diphosphate metabolites into PBMCs in vitro and in vivo. The initial step in the intracellular activation of GS-7340 and GS-9131 is the hydrolysis of the alanine carboxyester by an unknown hydrolytic enzyme. The isolation and identification of this enzyme from human PBMCs is reported here.

Results: A major enzyme capable of cleaving GS-7340 and GS-9131 was purified from human PBMCs and was separable from esterases able to cleave alpha napthyl acetate (ANA). The increase in specific activity of prodrug hydrolase achieved was 3500-fold. SDS–PAGE analysis showed the presence of a prominent protein band at 29 kDa, which was identified by in-gel tryptic digestion and MS/MS sequencing of the resultant peptides as lysosomal carboxypeptidase A (cathepsin A, EC 3.4.16.5, CatA). The biochemical properties of purified prodrug hydrolase matched those of CatA. Recombinant CatA and the isolated prodrug hydrolase displayed nearly identical susceptibility to hydrolase inhibitors and substrate preference against a panel of tenofovir phosphoramidate prodrugs. Incubation of both enzymes with [^{14}C]GS-7340 and [^{3}H]difluorophosphonate resulted in the labeling of an identical 29 kDa protein (catalytic

subunit). Both labeled bands reacted with polyclonal antibodies specific for human cathepsin A. Finally, following incubation with GS-7340, approximately 6–9-fold lower intracellular concentrations of TFV metabolites were detected in fibroblasts from patients expressing non-functional Cat A (Cat A-cells) compared to normal control fibroblasts (Cat A+ cells).

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Aerosolic Poloxagel-Loaded Triphosphate Antivirals Against Influenza Infection

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Nucleoside 5′-triphosphate (NTP) is the biologically active form of many antiviral nucleoside analogs capable of efficiently blocking the production of viral nucleic acids in infected cells. We describe novel microparticulate formulations for encapsulation of NTP, drug delivery and antiviral therapy of respiratory infections. Polymer networks (poloxagels) consisted of crosslinked poloxamers and cationic polymer molecules were designed, synthesized and characterized by loading with NTP and interaction with cells. Poloxagel-NTP formulations could be obtained by simple mixing of the aqueous solution of NTP with the aqueous dispersion of poloxagel and subsequent lyophilization. Drug loading was equal up to 30% by weight. In this form, phosphates groups of NTP are complexed with amino groups of polycationic backbone of poloxagels, and the formulations could be stored at room temperature for many months without degradation of NTP. The particle size of aqueous poloxagel-NTP dispersions was low, with a hydrodynamic diameter of 0.1–0.2 μm. The rate of passive drug release in physiological solution was from 33 to 50% of loaded drug during the 24-h period. These formulations were effectively consumed by many types of cells. Significant amounts of drug and poloxagels were detected in the cellular interior after only 1–2 h of incubation. In the presence of cellular membranes drug release from poloxagel-NTP formulations was dramatically increased. We attribute this effect to the triggered release of the bound NTP as a result of competitive interaction of polycationic backbone of poloxagels with phospholipids of cellular membranes. Mucoadhesive properties of poloxamers may additionally enhance binding of poloxagels with airways/lung epithelium. 5′-Triphosphate of 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole 3-carboxamide (Ribavirin) was synthesized using phosphorylation with a tris(imidazolyl) phosphate in a convenient one-pot approach. Formulations of different poloxagels with the Ribavirin 5′-triphosphate were prepared and characterized as prospective antiviral formulations for prophylactic and therapeutic treatments of respiratory infections including influenza A virus. Aerosolic route of application of these antiviral formulations and associated problems are discussed.

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Evaluation of Interferon (IFN) Bioavailability by Relative Quantitation of MxA mRNA Expression Using TaqMan RT-PCR

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IFN-alpha and IFN-beta are currently employed in the treatment of many viral diseases, especially chronic hepatitis. IFN-beta is also employed for the treatment of multiple sclerosis (MS), a chronic and often debilitating disease of the central nervous system. However, as with other protein therapeutics, long-term IFN therapy can lead to the development of binding and neutralizing antibodies to IFNs and thus lead to deceased clinical effect of IFNs. In order to measure the bioavailability of IFNs and the level of neutralizing antibody, we have developed a realtime RT-PCR (TaqMan) assay by quantitating the expression of MxA (an IFN-induced protein) mRNA. The nucleotide sequences of MxA deposited in the Genebank were aligned, and a pair of primers and the hybridization probe were designed based on the conserved regions. A house keeping gene, GAPDH, was used as a calibrator for relative quantitation. The RNA standards were generated by in vitro transcription from cloned MxA gene in a plasmid vector. The reaction parameters were optimized. The assay was validated using PBMCs of MS patients that were treated with IFN-beta. For evaluation of the IFN bioavailability, the total RNA was extracted from PBMCs and quantitatively detected by one-step RT-PCR for both MxA and GAPDH. The results calculated by the $2^{(-\Delta\Delta Ct)}$ method showed that the difference (signal-to-noise ratio) between samples with neutralizing antibodies and samples from untreated MS patients or healthy donors were approximately 60–70-folds. This indicates that our assay is a reliable method for determination of IFN bioavailability.

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Influence of Some Permeability Enhancers on Anti-Influenza Efficacy of Transdermal Delivery System Containing Rimantadine During Experimental Infection

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Transdermal delivery (TD) of drugs is a novel method for treatment of diseases. TD is carried out by the help of transdermal therapeutic systems (TTS), which are multilayer plasters that contain active ingredients. TD have a number of advantages, such as: (1) prolongation of the drug's action; (2) drug's concentration is maintained in therapeutic range; (3) there is no trauma to patient's skin while using TD; (4) removing TTS from the skin immediately stops drug's entering to the organism; (5) first-pass effect in the liver is reduced; and (6) many highly active drugs are

irritating the gastro-intestinal tract if administered orally, others have a short half-life time—these drugs do not have downsides mentioned above if used as TTS.

In our previous research we elaborated TTS containing rimantadine (TTScr) and studied its efficacy during experimental influenza in mice. We had established that transdermal delivery of rimantadine is more effective than oral administration. The aim of this work was to increase the efficacy of TTScr. To solve this task we studied the influence of some permeability enhancers on anti-influenza efficacy of TTScr during experimental infection. Applied TTS had adhesion hydrogel matrix (polyvinyl alcohol and 1.2-propylenglycol). They consisted of a base and a plastificator, which improves the administration of active substances through the skin and does not induce irritation. TTScr (1 mg/mouse) were applied on shaven backs of experimental animals. TTS for other groups additionally contained one of such permeability enhancers as: 10 mg/mouse of DMSO or 10 mg/mouse of octanol or 1 mg/mouse of papain. TTS were applied on shaven backs of mice and stayed there from 1 day before infection to 10th day after challenge. Mice of all groups were infected intranasally with influenza virus A/PR/8/34 (H1N1), which is highly pathogenic for them. Challenge was carried out using four animals for each virus dilution within the range of 10^{-1} to 10^{-7}. Deaths of animals were recorded for 14 days. The results showed that proteolytic enzyme papain increased the anti-influenza efficacy of TTScr on 1 $\log_{10}$TID$_{50}$. DMSO and octanol did not demonstrate such activity.

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Antiviral Properties of the α-*P*-borano-2′,3′-dideoxy Nucleotide Analogues

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Nucleoside reverse transcriptase inhibitors (NRTI) are widely used in the antiviral chemotherapy. Most NRTIs require stepwise phosphorylation to the respective nucleoside triphosphates, which inhibit the viral DNA synthesis. However, the emergence of HIV-1 reverse transcriptase-dependent drug resistance limits the effectiveness of treatment by NRTIs.

The α-*P*-borano-nucleotide analogues show several unique physico-chemical and biological properties: (i) Enzymatic studies indicate that the Rp-isomer of α-*P*-borano-2′,3′-ddNDPs is a better substrate for cellular NDP kinase than the parent ddNDP; (ii) neither isomer of the α-*P*-borano-ddNDPs is a substrate for mammalian pyruvate kinase and shows very poor inhibitory properties to this enzyme; (iii) the Rp-(α-*P*-borano)-ddNTP isomers are better inhibitors of drug- and multidrug-resistant viral reverse transcriptases and are poor substrates for DNA-dependent DNA polymerises; and (iv) after incorporation into viral DNA the borano-ddNMP residues are more resistant to ATP-dependent removal from viral DNA than parent ddNTPs. To by-pass inefficient phosphorylation of the NRTIs, several prodrugs of α-*P*-borano-nucleotide analogues have been previously synthesized. A more efficient delivery system for α-*P*-borano-nucleotide analogues based on nanosized cationic polymeric gel (Nanogel) is proposed.

Selective inhibition of drug- and multi-drug resistant viral RTs, poorer inhibition of intracellular kinases and DNA polymerases by the α-*P*-borano-nucleotide analogues, and their specific delivery into infected cells in the complex with Nanogel particles suggest a new approach to the design of more powerful antiviral drugs.

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Antiviral Methods

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Development, Validation, and Optimization of a Luminescence-Based High Throughput Screen for Inhibitors of Influenza

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We have developed a high-throughput, cell-based assay to address the critical need for antiviral drugs for the treatment of influenza. In consideration of the demand to screen high volumes of compounds, we targeted the development of a 384 microtiter plate format for the assay. In this assay, the inhibition of the influenza-induced cytopathic effect (CPE) in MDCK cells was assessed using the CellTiter-Glo Luminescent Cell Viability Assay by Promega. This reagent measures the amount of ATP present in cells, which is directly proportional to the number of metabolically active cells. Validation studies were executed to establish optimal cell density, viral concentration, DMSO tolerance for compound dilution, incubation time for virus-induced CPE and effective control drug concentration. Additional parameters, such as assay variability, reagent and read stability, edge effects, and IC50 stability were also investigated during validation. We are currently initiating use of the assay to screen chemical libraries and will report our findings from library screens in addition to the aforementioned validation. We believe the approach will also provide a mechanism for discovery of new antiviral leads for influenza as well as avian flu.

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A Novel Viral Protease Assay Using a Bacteriophage Lambda-Based Genetic Screen

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We have developed bacteriophage lambda based genetic screen that can be used to isolate and characterize site-specific proteases. This genetic screen system is based on the bacteriophage lambda cI-cro regulatory circuit, in which the encoded repressor cI is specifically cleaved to initiate the lysogenic-to-lytic switch. We have adapted this simple, safe and rapid genetic screening system to predict the activities and phenotypes of human immunodeficiency virus type 1 (HIV-1) proteases in the course of viral infection and antiretroviral therapy. A specific target for the HIV-1 protease, p17–p24, was inserted into the lambda phage cI repressor. The target specificity of the cI-HIV repressor was evaluated by coexpression of this repressor with an HIV-1 protease construct. Upon infection of *Escherichia coli* cells expressing the two constructs encoding the cI-HIV-1 repressor and HIV-1 protease, lambda phage replicated up to 1000-fold more efficiently than in cells that did not express the HIV-1 protease. This assay responds appropriately to well-known HIV-1 proteases inhibitors and can be used to search for new proteases inhibitors. The high level of specificity of this system, in which modest differences in catalytic efficiency can be quantified, should be also useful for the characterization of different mutant viral proteases. We further demonstrated the broad applicability of this protease assay using other viral proteases and their cognate cleavage sites, including hepatitis c virus (HCV) NS3 protease and severe acute respiratory syndrome (SARS) coronavirus (CoV) (SCoV) 3C-like protease. Compared with other protease assay methods, this assay has the following advantages: safe, highly sensitive, highly specific, easy quantification, and rapid generation of different protease cleavage substrates using molecular cloning and expression. This system may be useful for the development of a screening method to identify viral protease inhibitors and should be also useful to characterize cellular, viral, or other infectious agent proteases with different activities and specificities.

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Development of a Virus Transmission and Rapid Resistance Selection Assay to Evaluate and Prioritize Antiviral Agents For Systemic and Microbicide Use

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A virus transmission and rapid resistance selection assay has been developed in order to quickly evaluate the biological properties of anti-infective test compounds and rationally prioritize them for further development. The transmission assay specifically evaluates the ability of test agents to suppress and clear virus replication from cultures during the serial passage of virus in the presence of fixed concentrations of the test compounds alone or in combination. The growth and expansion of virus in the infected cultures has been shown to occur through the replication of originally infected cells in the absence of virus spread, through direct virus to cell infection, and through cell to cell transmission. In order to sterilize a culture a test compound must possess the ability to specifically and potently interfere with virus replication by each of these three methods and must be able to inhibit the replication of resistant viruses which pre-exist in the viral inoculum and which rapidly grow in the presence of the fixed concentrations of the test agent. Twelve pyrimidinediones being evaluated for potential use as both anti-HIV therapeutic agents and topical microbicides were evaluated for their ability to inhibit virus transmission and to define their ability to rapidly select for resistant virus strains. These compounds were evaluated in parallel with known anti-HIV agents that inhibit virus entry (T20) and reverse transcription (Sustiva, UC781 and AZT). The results of the transmission assays suggest that significant biological differences exist between antiviral compounds and even between highly related congeners of the same class of pyrimidinediones, suggesting that the transmission and rapid resistant selection assay measures important antiviral properties of anti-HIV agents. Biological studies that evaluate the mechanisms of virus growth in the presence of high concentrations of test compounds will be described.

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Antisense Morpholino-Oligomers Directed Against Bunyavirus Genome Segments Inhibit Replication and Proliferation

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In the family *Bunyaviridae*, several members on the genus *Phlebovirus* have been reported to cause disease in humans or livestock. Among these, Rift Valley fever (RVF) virus is an important human/animal pathogen. Its widespread geographic distribution and its ability to produce severe human disease makes this virus a worldwide public health concern.

The phosphorodiamidate morpholino-oligomers (PMO) are a class of DNA-like antisense agents typically synthesized to a length of about 20 subunits and contain purine or pyrimidine bases attached to a backbone composed of six member morpholine rings joined by phosphorodiamidate intersubunit linkages. They have been shown to be effective antiviral compounds for different virus families, e.g. *Coronaviridae* and *Flaviviridae*. PMO bind to RNA preventing translation of the viral RNAs.

We used our recently developed plasmid-based minigenome rescue systems for Uukuniemi (Phlebovirus model virus) and RVF viruses to screen antiviral compounds based on the morpholino antisense oligonucleotide approach. For this the antiviral compounds were appraised on the basis of reporter gene activity (Fig. 1B). The inhibitory effects of the same compounds were also tested by measuring reduction in virus titer (Fig. 1A), by

monitoring changes in viral antigen production using an indirect immunofluorescence procedure and FACS analysis, and analysis of genome transcription/replication by RT-PCR. Indeed several PMOs could be identified with interfering effect at a low IC50 on bunyaviral minigenome rescue as well as virus proliferation. Based on these results, we plan to confirm antiviral activity of the most promising compounds in suitable animal models.

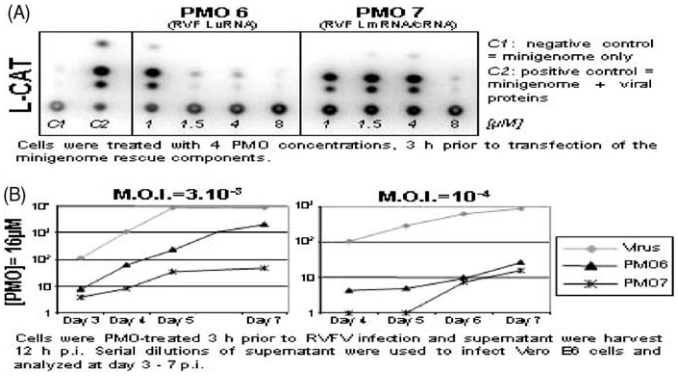

Fig. 1: Correlation between minigenome results using the RVF L segment based minigenome (A) and reduction of RVF virus titer (B) after treatment with PMO 6 and 7.

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Fractal Microscopic Description of Herpes Virus-Cell Dynamic System

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We have shown that the fractal approach to the problem of virus-cell interaction gives the unique possibility to process the data through the sequence of the direct and inverse Fourier transforms. The studies were carried on the Herpes simplex virus US-1 interacting with the Hep-2 sensitive cell culture. The object was imaged as system of bright peaks formed as a result of laser diffraction on the structural elements of the virus-cell system. The whole virus-cell interaction information is inserted into computer in a fastest parallel way. The laser intensity peaks, which form the speckle image of the system under consideration, could be transformed into the hierarchical system of the circles (or squares) according to the choice of the researcher, but conserving the same D value, which depends only on the true intermolecular interaction potential. This potential, being characteristic for every stage of virus-cell interaction, is responsible for the given structure of the dynamic virus-cell system and the unique, but the typical form of the fractal cluster corresponding both to the system itself and its image as well, was processed by computer techniques. The hierarchical fractal design of the virus-cell system, proposed here for the first time, gives the universality, needed for the quantitative description of any possible combination of the virus and corresponding sensitive cell. It should be noted, as well, that the fractal microscope use for virus-cell dynamic system imaging have all the properties, required from all other experimental tools of monitoring, including the reliability, reproducibility and preciseness. This device could be used in drug design biological test stages with the scope of time and efforts economy during the compounds libraries screening. The fractal microscope combined with the QSAR drug design technique makes the Antiherpetic drug design more competitive as compared to the regular approaches.

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Generalized Fourier Image Processing in Fractal Microscopy for Virus-Cell Interaction Imaging

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We have investigated experimentally the fractal properties of diffraction images obtained by laser irradiation of virus-cell system. It was shown that the diffraction process is mathematically equivalent to the direct Fourier transform of the said system's components modeled with simple geometrical figures (e.g. circles). Each viral family could be coded and described quantitatively with the average size of the free viral particle and the type of its symmetry. We propose here to use the inverse Fourier transform of the virus-cell system in order to get the real enlarged image of the viruses attacking the sensitive cell as well as the cell's structural transformation caused by the sequent stages of virus reproduction process. The set of bright and dark spots, which forms the virus-cell system's diffraction image, could be coded into set of numbers (matrix form of correlation vector-function) using the quantification procedure. The correlation function was used as presented in polar coordinates because the system has the axial symmetry (laser beam taken as main physical axis). The full information included into the image peaks' diameters and color index is transformed using inverse Fourier technique into set of intersecting bright and dark circles. The full in vitro dynamics of the structural changes of the virus-cell system are described by the changes of circles' diameters and the area of their intersection. It was shown, also, that the magnification of the fractal microscope could achieve 10,000× to 100,000×, depending on the laser power used. Proposed fractal microscope could be applied as well in vivo experiments until the required magnification will not make us to use projection laser with the output exceeding 25 mW. We have shown that the fractal microscope based on the inverse Fourier transform could be applied successfully in pharmaceutical antiviral drug design, laboratory and clinical trials of new antiviral preparations, especially effectively in hierarchic QSAR research.

Acknowledgement: Authors are grateful to the support under the STCU Grant #3147.

Poster Session II: Herpesviruses, Poxviruses, Other Viruses, Antiviral Targets, and Natural Products

Herpesviruses

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SAR of Alkyloxyphenyl Furano Pyrimidines: Potent and Selective Anti-VZV Agents

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We have previously reported bicyclic furano pyrimidines as potent and selective inhibitors of Varicella Zoster Virus (VZV) (McGuigan et al., 1999), with subnanomolar activity for *p*-alkylphenyl substituted analogues (McGuigan et al., 2000). These compounds however are highly lipophilic and poorly soluble in water. We then reported a series of *p*-alkyloxyphenyl compounds containing a phenolic ether aiming to enhance water solubility whilst retaining antiviral activity (McGuigan et al., 2002). We will now report the synthesis, characterisation and antiviral evaluation of a novel series of *p*-alkyloxyphenyls where there is at least one methylene spacer between the phenyl and ether group to potentially boost the pharmacokinetic profile. The alkyl chain length was fixed to retain a high ClogP value, a parameter that has previously been shown to correlate with high antiviral potency (McGuigan et al., 2000).

The target structures were prepared by the Pd-catalysed coupling of a series of para-substituted alkoxyphenyl acetylenes with 5-iodo-2′-deoxyuridine, to give intermediate 5-alkynyl nucleosides, which were subsequently cyclised in the presence of CuI to give the desired bicyclic systems.

The antiviral activity, cytotoxicity, and solubility of these compounds are to be reported.

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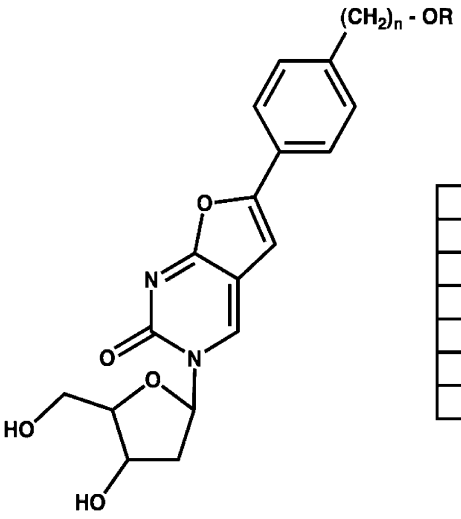

n	R
1	Hexyl
2	Pentyl
3	Butyl
4	Propyl
5	Ethyl
6	Methyl

Fig. 1: Structures of alkyloxy furnao pyrimidines as potential anti-vzv agents with enhanced water solubility.

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Withdrawn

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Some Modifications to the Bicyclic Pyrimidines: A Novel Class of Anti-Viral Nucleosides

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We have previously reported on some novel nucleoside analogues containing and unusual furano bicyclic pyrimidine base and long side chain (McGuigan et al., 1999), which were discovered to be both potent exquisitely and selective towards the varicella zoster virus. Following this discovery, three main sites for modification were identified and explored: (1) the side chain; (2) the bicyclic base; and (3) the sugar moiety. Modification to the side chain by insertion of a phenyl ring, led to the most potent anti-VZV nucleoside to date (EC50 1 nM) (McGuigan et al., 2000). The investigation into modifications at the three sites stated above has continued and we herein report further adjustments to these analogues. Replacement of the furo oxygen with sulfur on the parent nucleosides bearing an alkyl side chain has been reported to retain antiviral activity. However, those bearing a phenyl alkyl side chain are here shown to give a slight reduction in anti-VZV activity. Modifications to the phenyl ring of the side chain have included halogen substitutions, and the fluorine in particular has produced some intriguing results in that, while the ortho and meta substitutions show some anti-VZV activity, the para analogue is completely devoid of antiviral activity. We now report further studies which include the di and tri substituted phenyl analogues. Finally, we have also investigated sugar modification that has included substitutions of the 3′ hydroxyl group. Previous modifications which have replacements of the hydroxyl groups, resulted in loss of activity against VZV (McGuigan et

al., 2004). We now present some new 3′substituted analogues which have provided interesting biological results.

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SAR of Monosubstituted Phenyl Furano Pyrimidine as Potent and Selective anti Varicella-Zoster Virus (VZV) Compounds

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We have previously reported bicyclic furano pyrimidines as potent and selective inhibitors of Varicella Zoster Virus (VZV) (McGuigan et al., 1999) with subnanomolar activity for *p*-alkylphenyl substituted analogues (McGuigan et al., 2000; Srinivasan et al., 2001). The SAR is now further explored via the substitution of phenyl derivatives with electron withdrawing and electron donating groups. We now report the synthesis, characterisation, and biological evaluation of a novel series of mono substituted phenyl derivatives in order to probe the structure activity relationships in this region.

The target compounds were synthesised under Sonogashira conditions where a series of substituted phenyl acetylenes were coupled with 5-iodo-2′-deoxyuridine, to give intermediate 5-alkynyl nucleosides that were subsequently cyclised in the presence of CuI to give the desired bicyclic systems.

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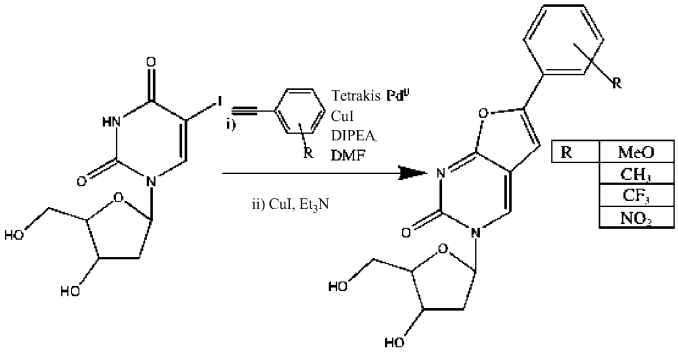

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Influence of Structure of *N*,*N*′-(bis-5-nitropyrimidyl)-dispirotripiperazine Derivatives on Their Antiherpetic Activity

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Diseases caused by herpes simplex virus (HSV) are widely distributed. Prophylaxis and treatment of these infections are important health care tasks that require also the search, design and development of new antiherpetic drugs overcome drug resistance and toxic side effects of existing drugs. Drug selection simply based on results of empirical screening is not very effective. Computer-based technologies may help to optimize the structure of antiviral compounds as well as to design and develop new drugs.

The objective of the present work is the quantitative structure-activity relationship (QSAR) analysis of antiviral activity of various *N*,*N*′-(bis-5-nitropyrimidyl)dispirotripiperazines in connection with consequent drug design.

The well-established simplex representation of molecular structure (SiRMS) QSAR approach has been used to fulfill this objective. It allows the molecular design of new effective antiviral drugs. Thorough investigation of the relationship between: (a) cytotoxic (HeLa cells and GMK cells, CC_{50}, μg/ml); (b) antiherpetic activity (HSV-1 strain Kupka, IC_{50}, μg/ml); and (c) selectivity index (ratio of CC_{50} to IC_{50}) and the structure of 48 *N*,*N*′-bis-5-nitropyrimidyl derivatives of dispirotripiperazine have been conducted.

Statistic characteristics for PLS (Partial Least Squares) models are quite satisfactory ($R^2 = 0.816–0.991$, $Q^2 = 0.637–0.868$). The results are confirmed by experimental data. Based on the obtained models, molecular fragments that promote and interfere with antiviral activity were defined. Additionally, these models provide the possibility to predict molecular fragments that will enhance antiherpetic activity and to design new well tolerated highly virus-specific drugs.

In summary, the developed simplex approach is an effective instrument for prediction and design of novel effective antiherpetic agents.

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Synthesis and Antiviral Activity of Methylene-3-Fluorocyclopropane Analogues

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Methylene-3-fluorocyclopropane nucleoside analogues **1**–**4** (B = Ade and Gua) were obtained by a highly convergent synthesis employing the key intermediate **5** for all eight compounds (Fig. 1). Analogue **1** (B = Gua) was effective against HCMV (Towne)/HFF cytotoxicity with EC_{50}/CC_{50} 2.9/>100 μM. Compound **4** (B = Ade) inhibited replication of EBV/Daudi cytotoxicity (EC_{50}/CC_{50} <0.03/>100 μM). Analogues **1**, **2** (B = Ade and Gua) and **4** (B = Ade) were active against VZV/HFF cytotoxicity (EC_{50}/CC_{50} 4.5–10/>100 μM) and **1** (B = Ade) inhibited HIV-1/MT-2 cytotoxicity with EC_{50}/CC_{50} 5.2/>10 μM. The structure-activity relationships will be discussed in context with other methylenecyclopropane analogues.

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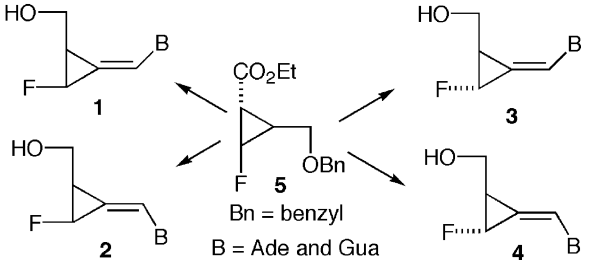

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5-Arylethynyl Derivatives of 2′-Deoxyuridine and *Arabino*-Uridine: Synthesis and Antiviral Evaluation

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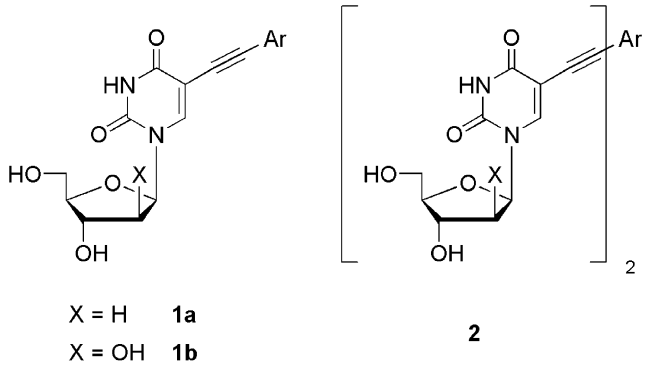
Several representatives of a series of 5-arylethynyl-2′-deoxyuridines (**1a**) bearing bulky aryl groups were recently shown to possess unexpected activity towards HSV-1. Unlike common anti-HSV drugs, these compounds retain activity towards kinase-deficient acyclovir-resistant strains. Therefore, an unusual mechanism of antiviral action is assumed. In order to investigate the mechanism and to discover more potent analogues we synthesized several novel 2′-deoxy (**1a**) and 2′-arabino (**1b**) uridine derivatives possessing different 5-arylethynyl substituents.



Dinucleosides **2** containing two uridine moieties coupled to a single polycyclic aromatic hydrocarbon (e.g. pyrene) represent another type of structural variation of nucleosides **1a**. These compounds as well as some of **1a** and **1b** possess bright fluorescence that can be used in biological evaluations.

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Inhibition of Murine Cytomegalovirus by Second Generation Ribonucleotide Reductase Inhibitors Didox and Trimidox

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Cytomegalovirus (CMV) is a wide spread opportunistic pathogen which belongs to the beta subfamily of the *Herpesviridae*. Primary infection is generally asymptomatic resulting in life long latency. However, morbidity and mortality rates post-transplantation are greatly increased following reactivation or recrudescence of CMV. Ganciclovir (GCV) and Cidofovir (CDV) have both been successful in suppressing CMV viral replication in immunocompromised patients. Although sustained use of these drugs has resulted in the emergence of multi-drug resistant strains of virus. In this study we used plaque reduction assays to determine the antiviral efficacy of two ribonucleotide reductase inhibitors, Didox (DX; 3,4-dihydroxybenzohydroxamic acid) and Trimidox (TX; 3,4,5-trihydroxybenzamidoxime) in inhibiting both wild type and drug-resistant strains of murine CMV (Smith strain). The results presented here demonstrate that both DX and TX inhibit viral plaque formation in a dose dependent manner in both wild type and the resistant strain. A 43- and 39-fold increase in drug dose was required for CDV and GCV respectfully to inhibit plaque formation by 50% in the resistant strain (CDV wt: 0.03 μM, r: 1.28 μM/GCV wt: 3.17 μM, r: 122.85 μM). This compared

to only a moderate increase in drug dose required for DX and TX to achieve 50% inhibition in the resistant strain (DX wt: 20.71 μM, r: 28.88 μM/TX wt: 7.12 μM, r: 11.59 μM), corresponding to a 1.4- and 1.6-fold increase respectfully. Further work is currently underway to determine the possible mechanism of antiviral actions and toxicity profiles of these novel virostatics.

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Inhibition of Herpesvirus Replication by a Series of Alkoxyalkyl Esters of Purine- and Pyrimidine-Based Nucleoside Phosphonates

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In patients with human immunodeficiency virus (HIV) infection, coinfection with herpesviruses continues to be a problem for patients receiving antiviral HIV therapy. Since treatment can be affected by the large number of drugs required for multiple infections, it would be useful to have antivirals that are active against both HIV and the herpesviruses. We reported previously that alkoxyalkyl ester prodrugs of cidofovir (CDV) are several logs more active against herpesvirus replication than unmodified CDV. To determine if this strategy would be effective for other acyclic nucleoside phosphonates which are active against HIV infections, hexadecyloxypropyl (HDP) esters were synthesized from 1-(phosphonomethoxyethyl)-cytosine (PME-C), 1-(phosphonomethoxyethyl)-5-bromo-cytosine (PME-5BrC), 1-(phosphonomethoxyethyl)-5-fluoro-cytosine (PME-5FC), 9-(phosphonomethoxyethyl)-2,4-diaminopurine (PME-DAP) and 9-(phosphonomethoxyethyl)-2-amino-4-cyclopropylaminopurine (PME-cPrDAP) and assayed for activity against herpesvirus replication. Overall, the HDP esters were more active than the unmodified acyclic nucleoside phosphonates, indicating that this is a useful strategy for increasing the antiviral activity of acyclic nucleoside phosphonates. One of the most active compounds was HDP-PME-cPrDAP which had EC_{50} values of 0.2, 0.3, and 0.03 μM in HFF cells infected with HSV-1, HSV-2 or HCMV, representing a 12–26-fold increase in efficacy over the parent PME-cPrDAP. Another promising compound was HDP-PME-DAP, which had EC_{50} values of 0.7, 0.2, and 0.6 uM in HFF cells infected with HSV-1, HSV-2, and HCMV, representing a 16–43-fold increase over the parent PME-DAP. The results presented here indicate that modified acyclic nucleotides with antiviral activity against HIV also inhibit the replication of some of the herpesviruses. Further evaluation of their activity against other herpesviruses that are a problem in HIV-infected patients, such as human herpesviruses type 6 and 8, is warranted and may provide new therapeutic options for patients with coinfections.

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Resistance of Human Cytomegalovirus with Single and Double Mutations in UL97 to First and Second Generation Methylenecyclopropane Purines

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We previously described first (Qiu et al., 1998. J. Med. Chem.) and second generation (Zhou et al., 2004. J. Med. Chem.) methylenecyclopropane purines that have potent and selective activity against HCMV. Strains selected separately for resistance to first-generation analogs (synadenol, synguanol) were 10–20-fold resistant to several first-generation purine analogs. Similar resistance was observed to the second-generation guanine analog cyclopropavir [IC_{50}'s in plaque assays = 0.35 and 21 μM, respectively for wild-type (wt) and synguanol-resistant (1982r) virus]. Likewise a UL97 deletion mutant (Prichard et al., 1996. J. Virol.) was resistant to both first and second-generation compounds (IC_{50}'s = 2.1 and 0.25 μM in wt; 100 and 15 μM in $UL97^{del}$, respectively for synguanol and cyclopropavir). UL97 from the HCMV strain selected for resistance to the synadenol was sequenced and two mutations were identified: M460I and C603Y. Because HCMV with either M460I or the related C607Y mutation alone was sensitive to synadenol and synguanol (Baldanti et al., 2002. Antiviral Res.), we hypothesize that two mutations are required for resistance to first- and second-generation analogs. This hypothesis was tested by construction of three strains of HCMV from HCMV AD169 BAC with one, the other, or both mutations in UL97. As expected, the two strains with the single mutations were 3- to 6-fold resistant to ganciclovir but had little resistance to the first generation compounds synadenol and synguanol (0.5- to 1-fold). Both strains were somewhat more resistant to the second-generation compound cyclopropavir (6- to 8-fold) but less so than observed in the 1982r virus with two mutations. Study of the resistance of the constructed virus with two mutations is underway. We conclude that a functional UL97 is required for activity against HCMV and that is likely that two mutations in UL97 are required for significant resistance.

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Studying of Anti Epstein-Barr Virus Activity of New Triazine-Bearing Tricyclic Bases and their *N*-Glycosidic Derivatives

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Search of new effective preparations capable to inhibit herpesviruses reproduction is stipulated by their certain resistance to different groups of chemical preparations. New triazine bearing tricyclic bases and their *N*-glycosidic derivatives structures are widely used as potential antiviral agents. The objective of the present investigation was to study the activity Triazine Bearing Tricyclic Bases Nos. 1 and 2, as well as *N*-glycosidic derivatives No. 3 against Epstein-Barr virus—lymphotropic and oncogenic virus from Herpesviridae family. As a model of EBV-infection in vitro we used the line of lymphoblastoid B-cells Raji, which infected by EBV. An inhibition of reproduction of EBV in a cell culture by No 1, No 2, and No 3 was determined by reduction of a number of genome—equivalents of EBV DNA on a cell, which were revealed by quantitative PCR with use of primers and reagents "Amply-Senc-100 R" (Russia). The first stage of investigation of substances was the analysis of their cytotoxicity for cell line Raji. They have been studied in concentrations of 1000, 500, 250, 125, 64, 32, 16, 4, 1, 0.5 and 0.1 μg/ml. The concentrations that inhibited the quantity of live cells on 50% (ID50) were equal to substances No. 1–750 μg/ml, No. 2–625 μg/ml and No. 3–125. The minimal inhibiting concentration (MIC) of Nos. 1, 2, and 3 was equal to 1 μg/ml, because the amount of genome-equivalents of DNA EBV on a cell was reduced with 28.0 up to 14. Hence, the index of selectivity (IS) was equal to 750 and 625 for triazine bearing tricyclic bases Nos. 1 and 2, for *N*-glycosidic derivatives—125.

In addition these compounds were also tested in transcription and replication model systems in vitro. Our results indicate that bases and their *N*-glycoside derivatives effect RNA and DNA synthesis in different manner.

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Antiviral Activity of a GSH Derivative of Glutathione in HSV 1-Induced Keratitis in Rabbits

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Several studies have demonstrated that different viruses induce an imbalance in the intracellular redox state through a depletion of glutathione (GSH), the main intracellular antioxidant. The imbalance in the intracellular redox state represents a key event in the development of viral infection. Indeed, our previous data showed that treatment with GSH prevents a decrease in intracellular GSH and inhibits replication of different RNA and DNA viruses in vitro and in vivo. Our recent data demonstrated that a butanoyl derivative of GSH (GSH-C4), with increased hydrophobic properties, inhibited in vitro parainfluenza-1 and HSV-1 replication more efficiently than GSH.

For this reason we evaluated the effectiveness of topical GSH-C4 administration in HSV-1-induced keratitis in rabbits. For infection, the corneal epithelium, previously scratched, was inoculated with 2×10^5 pfu/ml of HSV-1. GSH-C4, dissolved in a saline solution (150 mM, 100 ul/eye), was administered as eyedrops four times daily for ten days. A saline solution was used for the control group.

The clinical evaluation of conjunctival and corneal involvement, performed by using 0.5% fluorescein sodium eyedrops and a slit lamp fitted with a cobalt blue filter, demonstrated that GSH-C4 treatment was effective in reducing the severity and progression of keratitis and conjunctivitis. Moreover, in GSH-C4 treated animals, conjunctival HSV-1 titre, assayed by TCID50 on day 4 post-infection, was significantly reduced as compared to that of control animals (mean = 1.4×10^3 Units/ml versus 1.1×10^5 Units/ml, $n = 10$ for group). Accordingly, similar results were obtained by measuring virus titre from the corneas of GSH-C4-treated animals versus placebo animals (mean = 3.5×10^3 Units/ml versus 8.5×10^5 Units/ml, $n = 5$ per group).

Such results highlight the antiviral activity of GSH-C4 in vivo and suggest that topical GSH-C4 treatment could be considered as complementary therapy of HSV-1-induced keratitis.

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Effect of Oral Treatment with (S)-HPMPA, HDP-(S)-HPMPA, or ODE-(S)-HPMPA on Replication of Human Cytomegalovirus (HCMV) or Murine Cytomegalovirus (MCMV) in Animal Models

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Cytomegalovirus (CMV) can cause a wide variety of clinical manifestations in immunocompromised hosts or transplant recipients. We have utilized severe combined immunodeficient (SCID) mice implanted with human fetal tissue and subsequently infected with HCMV or BALB/c mice infected with MCMV to evaluate new antiviral therapies against CMV infection. In the current studies we used these two models to determine the efficacy of (S)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine ((S)-HPMPA), hexadecyloxypropyl-(S)-HPMPA (HDP-(S)-HPMPA), or octadecyloxyethyl-(S)-HPMPA (ODE-(S)-HPMPA). In the HCMV model, human fetal thymus and liver (thy/liv) tissues were implanted under the kidney capsule of mice and inoculated 16–20 weeks later with 4700 pfu of HCMV. Tissue samples were obtained at various time points for quantitation of HCMV titers by plaque assay. In general, replication of the Toledo strain of HCMV in the implant tissue increased through 21–28 days and then gradually decreased to undetectable levels by 8 weeks post-infection. To determine efficacy of these compounds, oral treatment with vehicle or 10 mg/kg of (S)-HPMPA, HDP-(S)-HPMPA or ODE-

(S)-HPMPA was initiated 24 h after infection and continued for 28 days. Cidofovir (CDV) at 20 mg/kg was administered i.p. daily as a positive control. Results indicated that (S)-HPMPA, HDP-(S)-HPMPA and ODE-(S)-HPMPA were highly effective in significantly reducing replication when compared to the vehicle control. In MCMV infected mice, HDP-(S)-HPMPA was highly effective in preventing mortality when administered orally at 30 or 10 mg/kg beginning 24 h post-viral inoculation and 30 mg/kg when treatment was delayed until 48 h post-viral inoculation. These data indicate that these compounds were highly efficacious in two animal models of CMV infection and should be evaluated for use in HCMV infections in humans.

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Potent, Antiviral Activity of REP 9 and Analogs Against Systemic MCMV Infection

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Cytomegalovirus (CMV) is a ubiquitous β-herpesvirus that asymptomatically infects immunocompetent individuals but leads to serious illness in immunocompromised individuals, such as transplant recipients, neonates and AIDS patients. Thus, the need for well-tolerated and potent antiviral compounds with activity against CMV is well recognized. In our current studies, we have evaluated the in vivo activity of REP 9, a fully degenerate 40 mer phosphorothioated oligonucleotide against murine cytomegalovirus infection (MCMV) in mice. REP 9 has potent in vitro activity against HSV-1, HSV-2, HCMV, VZV, EBV, and HSV-6 (Vaillant et al., submitted for publication). In our initial studies, infected mice were treated with REP 9 and compared to saline-treated infected control mice. Compound was administered intraperitoneally for 5 consecutive days at 10 mg/kg, starting at 2 days prior to infection. Mice were infected with 5×10^5 pfu MCMV on day 0, at 3 h post-treatment. Sera were collected at −22 h, at 36 hpi, and at 3 dpi for ELISA analysis of IFNγ production. Spleens and livers were collected at 3 dpi for determination of virus titers. At 3 dpi, virus titers in the spleens and livers were significantly reduced by REP 9 treatment as compared to control mice. Splenomegaly was observed in infected mice treated with REP 9 but not in saline treated, infected mice or in REP 9 treated, uninfected mice. IFNγ levels in mice treated with REP 9 peaked at 36 hpi compared to 72 hpi for saline-treated control mice. These data suggests that immune stimulation might contribute to the antiviral activity of PS–ONs, perhaps through IFNγ levels. A second study comparing the in vivo activity of REP 9 with two oligonucleotide analogs that do not activate TLR-9 mediated immune stimulation suggests that direct antiviral activity of REP 9 and the analogs was the predominant therapeutic mechanism in vivo. Moreover, one REP 9 analog exhibited even greater antiviral activity than REP 9 while causing no splenomegaly. Additional experiments are underway to provide insights into the mechanism of action against MCMV infection.

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Poxviruses

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Design and Synthesis of Novel Phosphonomethoxyethyl Adenine Analogs for Treatment of Orthopoxvirus Infections

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A series of novel antiviral agents was prepared based on lead compounds related to acyclic nucleoside phosphonates. These agents consist of a purine nucleus bearing a pendant phosphonic acid group. The design strategy was two-fold: (1) following the approach of the Hostetler group, to mask or partially mask the anionic phosphonate as a lipophilic ester and/or as an amino acid phosphonamidate prodrug that could enhance cellular uptake and be cleaved intracellularly; and (2) to investigate new substituents at the purine 2- and 6-positions. For proof of concept, a phosphonomethoxyethyl adenine (PMEA) scaffold was employed. Over 100 analogs of PMEA substituted at the purine 2- or at the adenine N-6 site have been synthesized and evaluated for activity against orthopoxvirus infections. Many N-6 substituents other than the previously recognized *N*-cyclopropyl have shown antiviral activity, and these structure-activity relationships are being investigated. An exciting finding has been that introduction of several novel moieties at the purine 2-position particularly the hydrazino, hydroxylamino, or the cyclopropylamino groups resulted in several compounds with excellent in vitro activity. For example, octadecyloxyethyl (ODE) 2-amino-N(6)-cyclopropyl PMEA had EC_{50} values of 0.03–0.07 μM and ODE 2-hydroxylamino-N(6)-cyclopropyl PMEA had EC_{50} values of 0.3–1.6 μM against cowpox and vaccinia viruses, respectively, using a plaque reduction assay in HFF cells. Under these conditions the parent molecule, PMEA, was completely inactive. These two compounds had CC_{50} values of 30–70 μM giving selective indices of 200–1000. These studies indicate that several modifications in the PMEA scaffold can result in good antiviral activity against orthopoxvirus infections in vitro and the most active compounds are currently being scaled up for evaluation in animal models.

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Inhibitory Activity of Isatine-Sulphonamide Derivatives Against Orthopoxvirus Replication in vitro

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Isatin (2,3-dioxoindole), a versatile lead molecule for designing of potential bioactive agents, and its derivatives have been reported to possess inhibitory activity against a variety of pathogenic viruses. Methisazone(*N*-methylisatin-3-thiosemicarbazone) was one of the first synthetic antiviral agents used clinically for the treatment of orthopox virus infections. The presence of the thiosemicarbazone, however, can result in immunosuppresion and we have attempted to replace the thiosemicarbazone with a sulphonamide in order to modify the antiviral activity. The present work was performed to evaluate the antiviral activity and cytotoxicity of some novel 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-*N*-(4,6-dimethyl-2-pyrimidiny)-benzene sulphonamides against pox viruses such as vaccinia and cowpox virus in human fibroblast cells and the activity was compared with Cidofovir(CDV). Among the compounds tested,4-[(5-methyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-*N*-(4,6-dimethyl-2-pyrimidiny)-benzene sulphonamide(SPIII-5ME), was the most active compound with an EC50 value of 18 μmol, compared with CDV, which had an EC50 of 20 μmol against vaccinia virus. All the compounds were non-toxic (>300 μlM)using a neutral red uptake assay. Substitution of a halogen atom in 5th position of ISATIN was found to abolish the antiviral activity. This compound should be evaluated in orthopox infections in animal to determine its potential for development as an effective agents for treatment of these infections.

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Anti-Orthopoxviruses Activity of the New Class of 1,2,4-Benzotriazine Derivative

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During this study, we synthesized a series of 1,2,4-benzotriazine (Fig. 1) derivatives in order to evaluate the structural features required for anti-orthopoxviruses activity. These derivatives were tested for cytotoxicity and activity against the vaccinia, cowpox, mousepox, monkeypox, and in some experiments with variola viruses in Vero and MK-2 cells.

The results from studies of structure-activity relationship revealed that only compounds containing phenyl group at C-3 and the alkoxy and fluoro substitutes in the benzene ring of benzotriazines showed anti-orthopoxviruses activity. The antiviral activity was reduced or lost after substitution with other substitutes. Thus, we find a new class of heterocyclic compounds with antiviral activity.

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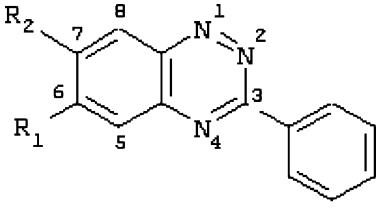

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ST-246 Inhibits Vaccinia Virus IMV Wrapping in the Intracellular Vesicles

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We have recently discovered a highly specific and potent anti-orthopoxvirus compound (ST-246) via high throughput screening (Yang et al., 2005. J. Virol. 79, 13139–13149). Marker rescue of ST-246 resistant variants localized compound resistance to the F13L gene that encodes a major orthopoxvirus envelope protein (p37), which is required for extracellular viral particle formation. p37 participates in wrapping of intracellular mature virus (IMV) in membranes derived from the *trans* Golgi or late endosomal compartment to produce intracellular enveloped virus (IEV) that are transported to the cell surface to form extracellular virus particles. To gain insight into the mechanism of action of ST-246, we examined the effects of ST-246 on the production of the extracellular viral particles in BSC40 cells infected with recombinant vaccinia virus containing a GFP-tagged p37 protein. In the presence of ST-246, IEV particle formation was dramatically reduced, plaque formation was almost completely inhibited, and IMV particles appeared to be retained in intracellular vesicles as revealed by electron microscopy. Furthermore, ST-246 prevented the intracellular localization of p37 to the late endosome compartment as measured by confocal microscopy. In contrast, ST-246 did not affect localization of p37 expressed from a ST-246 resistant virus variant. More intriguingly, the compound did not affect the intracellular localization of p37 in transfected cells. These results suggest that ST-246 inhibits an unknown virus-specific activity that requires F13L. This work underscores the

exquisite specificity of ST-246 and supports continued development of ST-246 as a potential anti-orthopoxvirus drug.

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Characterization of Virus Variants Resistant to the Antiviral Effects of ST-246

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ST-246 is a potent, orally bioavailable anti-orthopoxvirus compound that is active in vitro and in vivo. The frequency of naturally occurring ST-246 resistant variants was measured by fluctuation analysis and found to be 3.58×10^{-6}. Marker rescue of drug resistant variants localized changes associated with reduced compound susceptibility to the vaccinia virus F13L gene. The spectrum of mutations that confer ST-246 resistance was determined using an error-prone PCR procedure to increase the frequency of compound resistance by 100-fold relative to the frequency of naturally occurring resistance. Using this procedure, random point mutations were introduced into the F13L coding sequence by error-prone PCR and the mutated F13L alleles were transferred into wild-type virus genome by marker rescue. Sequence analysis of the input error-prone PCR products prior to marker rescue identified numerous nucleotide changes in the F13L coding sequence, some of which created nonsense mutations. Virus recombinants were selected that formed plaques in the presence of drug selection. This powerful selection procedure enriched for viruses that produced functional, ST-246 resistant, F13L proteins. Sequence analysis of the compound resistant F13L alleles identified numerous silent mutations scattered throughout the F13L coding sequence and 27 point mutations leading to amino acid changes that clustered around amino acid positions 258–302 within the F13L gene. Seven of these mutations resulted in single amino acid changes and could be correlated with reduced compound susceptibility. Taken together, these results suggest that: (1) mutations in at least 7 positions within F13L can confer resistance to ST-246 and (2) ST-246 resistant mutations cluster to a 58 amino acid domain in a region of the protein of unknown function.

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Activity of 5-Subsituted Pyrimidine Analogs Against Herpesvirus and Orthopoxvirus Replication in vitro

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Several 5-substituted pyrimidine analogs were identified as having antiviral activity against cowpox virus (CV) and vaccinia virus (VV) in primary human foreskin fibroblast cells. Molecules containing benzopyran, cyanovinyl, and pyrazolone moieties at this position exhibited significant antiviral activity against both these viruses. Three compounds in this series had EC_{50} values below 10 μM in a plaque reduction assay against both CV and VV. The antiviral activity of these compounds was also determined against herpes simplex virus (HSV) in a plaque assay. Two compounds with cyanovinyl derivatives at the 5 position had EC_{50} values below 15 μM against both HSV-1 and HSV-2, whereas other substituents at this position exhibited weaker activity against one or both of these viruses. Analogs containing the benzopyran substituents were the most effective against varicella zoster virus (VZV) and yielded EC_{50} values below 10 μM in a plaque reduction assay. None of the compounds were active against human cytomegalovirus. Interestingly, all of the compounds were much less effective in a thymidine kinase (TK) negative strain of CV suggesting that the activation by this enzyme was important in their mechanism of action. TK deficient strains of HSV were also comparatively resistant to some of the compounds. The TK dependence of these compounds in CV and HSV taken together with the lack of activity against cytomegalovirus replication suggests that activation by a viral TK is important in their mechanism of action. These results indicate that pyrimidine analogs with large substituents at the 5 position are substrates for the distinct TK homologs encoded by the herpesviruses and orthopoxviruses and suggest that they may be effective against infections with these viruses. Synthesis and testing of additional analogs is warranted and should help identify the most potent analogs for in vivo testing.

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In vitro and in vivo Activity of *N*-Methanocarbathymidine Against Herpesvirus and Orthopoxvirus Infections and its Mechanism of Action

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N-Methanocarbathymidine ((*N*)-MCT) is a conformationally locked nucleoside analog that is active against some herpesviruses and orthopoxviruses in vitro. This compound inhibits the replication of herpes simplex virus (HSV) with EC_{50} values below 1 μg/ml, and vaccinia virus (VV) and cowpox virus (CV) with EC_{50} values of 0.55 and 1.5 μg/ml, respectively. Assays using a thymidine kinase (TK) negative strain of CV yielded EC_{50} values 14-fold greater than a TK positive isolate. Similarly, a TK negative strain of HSV-1 was 90-fold less sensitive to the drug than wild-type strains. Thus, the antiviral activity of this molecule is dependent on the type I TK in HSV and the type II TK expressed by VV and CV viruses, suggesting that it is a substrate for these divergent forms of the enzyme. The drug is also a good inhibitor of viral DNA synthesis in both viruses and is consistent with inhibition of the viral DNA polymerase once it is activated by the viral TK homologs. It is also possible that the phosphorylated forms of the drug may inhibit other enzymes such as thymidylate synthetase and inhibit viral DNA synthesis indirectly. The interesting TK dependence of

this molecule explains the rather unusual spectrum of activity that includes orthopoxviruses, alphaherpesviruses, Epstein-Barr virus (EBV), and human herpesvirus 8 (HHV-8), since these viruses all express molecules with TK activity that could phosphorylate and thus activate the drug. Conversely, N-MCT is ineffective against the betaherpesviruses because they do not encode TK homologs. The compound is also highly effective in reducing the mortality of mice infected with CV, VV, and HSV when treatment is initiated 24 h after infection and at doses as low as 17 mg/kg. These results indicate that (*N*)-MCT is active in vitro and in vivo and its mechanism of action suggests that the molecule may be an effective and selective therapeutic for orthopoxvirus and certain herpesvirus infections and that it warrants further development.

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Drug Susceptibility Profiles of Recombinant Vaccinia Virus Harboring Mutation(s) Conferring Resistance to Cidofovir

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We have previously reported the isolation and characterization of drug-resistant mutants obtained following repeated passages of the vaccinia virus (VV, Lederle strain) in the presence of increasing concentrations of cidofovir (CDV). CDVr mutants encoded two mutations (A314T and A684V) not related to genetic polymorphism. We have now introduced these mutations in the pathogenic strain W Western Reserve and characterized the drug-susceptibility profile of the recombinant viruses and their pathogenicity in mice.

Both the A314T and the A684V recombinant viruses proved to be resistant to CDV and related compounds, such as cyclic CDV and HPMPO-DAPy {(S)-2,4-diamino-6-[3-hydroxy-2-(phosphonomethoxy)-propoxy]pyrimidine}. The virus bearing both substitutions proved to be more resistant to CDV than the single mutants. Interestingly, the A314T and the A684V mutants differed in their sensitivity to phosphonoacetic acid (PAA); the A314T and the A684V mutants being, respectively, hypersensitive and resistant to PAA. In contrast, the double mutant showed no change in sensitivity to PAA as compared to the wild-type strain. Unlike the A684V mutant that showed only a two to three-fold decrease in susceptibility towards the 3-hydroxy-2-phosphonomethoxypropyl (HPMP) purine derivatives, the A314T mutant showed cross-resistance to the HPMP purine derivatives. It should be noted that in the process of selection of CDV-resistant mutants in the presence of increasing concentrations of the compound, the A314T mutation appeared before the A684V substitution, and the latter mutation only occurred in conjunction with A314T.

When tested for virulence in a lethal intranasal infection model in mice, all CDVr recombinant viruses proved to be attenuated, suggesting that CDVr mutations are associated with reduced pathogenicity. Furthermore, we found that CDV at a dose of 50 mg/kg/day for 5 days was still able to protect mice (in terms of body weight loss) against an intranasal challenge with the A314T + A684V recombinant virus.

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Evaluating the Use of CpG DNA as an Antiviral Therapy

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At present there are no licensed antivirals against Orthopoxvirus infections such as variola or vaccinia virus (VACV). Although a stockpile of smallpox vaccine exists and has utility as a post-exposure treatment to infection, it is a live viral vaccine and as such cannot be administered to those with contraindications.

Bacterial DNA contains unmethylated motifs that, together with their flanking regions, can stimulate an innate immune response. Synthetic CpG DNA mimics the immunostimulatory activity of bacterial DNA and is recognised by intracellular toll-like receptor 9. There are four classes of CpG DNA all of which have different properties, eliciting distinct initial immune responses. Previous studies using an established lethal respiratory model of poxvirus infection demonstrated that a class B CpG DNA (7909) could provide protection from lethality against VACV in Balb/c mice when administered up to 7 days prior to challenge.

In order to evaluate efficacy Balb/c mice were challenged intra-nasally with VACV and treated with doses of 7909 ranging between 15 and 100 ug/mouse. Treatment was administered intra-nasally under light anaesthesia either on the day of challenge, 1, 2, 3, or 4 days post-challenge. Efficacy was determined by percentage body weight loss post-challenge. The optimum survival rate observed was 60% when treated with 30 ug 1 day post-challenge (70 MLD50 challenge). A survival rate of 80% was observed when treated with 50 ug 2 days post-challenge (40MLD50 challenge). The delay of treatment to either 3 or 4 days post-challenge was ineffective, indicating that the window of opportunity for delivery of 7909 is within 2 days.

Multiple doses of 7909 were used to attempt to extend this window of opportunity, delivering 7909 twice within a 3-day period. Interestingly, this had a considerable detrimental effect, actually accelerating the onset of disease and ultimately death.

Further work is required to optimise the use of CpG DNA as a potential antiviral therapy, and there is evidence to suggest that they may have immense utility as part of a co-administration therapy with other antiviral compounds, an area of work currently under investigation.

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Characterization of the Lister Strain of Vaccinia Virus Used for Vaccination Against Smallpox

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The pathogenicity and immunogenicity of the Lister (Elstree) strain of vaccinia virus, used for vaccination against smallpox, was studied in the mouse model. The virus did not reach the brain when inoculated intranasally, but when injected intracranially at a dose of 5×10^5 plaque forming units (PFU), was lethal for 50% of the mice. Lower doses of virus caused the mice to initially loose some weight but they completely recovered thereafter. A significant level of protection against a lethal dose of the WR strain was achieved in mice following immunization with the Lister strain, while higher doses and repeated vaccination procedure, were required with modified vaccinia virus Ankara (MVA).

We found that the Lister vaccine strain applied in Israel is comprised of heterogeneous virus population. We isolated and plaque-purified three virus variants differing in their plaque size in BS-C-1 cell cultures. They were named: L—large plaque, M—medium plaque and S—small plaque variants. These isolates could be neutralized by rabbit antibodies prepared against the Western Reserve strain of vaccinia virus and their one-step growth curves in BS-C-1 cells were quite similar. However, they differ in their pathogenicity to mice following intranasal inoculation of 10^7 PFU, or an intracranial injection of 5×10^4 PFU; the S variant being more virulent than the other two variants and resembles the pathogenicity of the Lister strain.

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Cell Line Dependency for Antiviral Activity of *N*-Methanocarbathymidine Against Orthopoxvirus Infections

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A novel carbocyclic thymidine analog, *N*-methanocarbathymidine [(*N*)-MCT)], was evaluated for inhibition of orthopoxvirus infections. Efficacy in vitro was assessed by plaque reduction assays against wild-type and cidofovir-resistant strains of cowpox and vaccinia viruses in nine different cell lines. Minimal differences were seen in antiviral activity against wild-type and cidofovir-resistant viruses. (*N*)-MCT's efficacy was affected by the cell line used for assay, with 50% poxvirus-inhibitory concentrations in cells as follows: mouse = 0.6–2.2 μM, rabbit = 52–90 μM, monkey = 250–>1000 μM, and human = 39–220 μM. Limited studies performed with carbocyclic thymidine indicated a similar cell-line dependency for antiviral activity. (*N*)-MCT did not inhibit actively dividing uninfected cells at 1000 μM. Activity was also determined against a thymidine kinase (TK) deficient vaccinia virus in mouse and monkey cells. The potency of (*N*)-MCT was similar to that seen with wild-type virus, suggesting that a cellular enzyme may be more important than viral TK to phosphorylate the compound. Mice were intranasally infected with cowpox and vaccinia viruses followed 24 h later by intraperitoneal treatment with (*N*)-MCT (2x/day for 7 days) or cidofovir (1x/day for 2 days). (*N*)-MCT treatment at 100 and 30 mg/kg/day resulted in 90 and 20% survival from cowpox virus infection, respectively, compared to 0% survival (placebo). Statistically significant reductions in lung virus titers on day 5 occurred in 10, 30, and 100 mg/kg/day treated mice. These doses did not spare mice from lethal vaccinia virus challenge, however, but the 30 and 100 mg/kg/day treatments significantly reduced day 5 virus titers and lung weights, and the 100 mg/kg/day treatment reduced lung consolidation. Cidofovir (100 mg/kg/day) protected all animals from death in both models. The evaluation of (*N*)-MCT may be limited to mice based upon its greatly reduced efficacy in the cells of higher animals.

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Antiviral Efficacy of ST-246 in a Ground Squirrel Model of Severe Monkeypox Virus Infection

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ST-246 is a potent small molecule inhibitor of orthopoxvirus replication that has been shown to protect mice from lethal challenge with vaccinia and ectromelia viruses. Here we report the results of preliminary trials that show efficacy of ST-246 against severe monkeypox virus infection in the ground squirrel model. Ground squirrels infected with less than 1 pfu of monkeypox virus develop a fulminant disease resembling human hemorrhagic smallpox: the most severe and lethal form of the disease. Oral administration of ST-246 at 100 mg/kg once per day for 14 days protected ground squirrels from a lethal challenge with 100 and 1000 pfu of monkeypox virus. Compound treated animals showed no weight loss or evidence of disease, and blood chemistry values were similar to uninfected animals. In contrast, placebo-treated animals showed elevated liver enzyme (ALT and AST) levels and all animals died by day 12 post-infection. When treatment with ST-246 was delayed 24, 48, 72, and 96 h, 100% protection was observed in the 24, 48, and 72 h groups, and 66% protection in the 96 h group. Severe pathologic changes were observed in the organs of the animals receiving placebo, especially in the lungs, liver, and spleen. In contrast, the organs of the animals receiving ST-246 at 0, 24, 48, and 72 h post-infection appeared grossly and microscopically normal. Thus, ST-246 appears to be a promising candidate for continued development as a therapeutic agent for severe orthopoxvirus infection.

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ΔF13L-Vac (p37-Deleted Vaccinia Virus) is Attenuated in Mice and Protects Against Infection with Wild-Type Virus

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ST-246 is a potent inhibitor of the replication of various orthopoxviruses. Resistance of cowpoxvirus to ST-246 maps to a mutation in V061, which is homologous to vaccinia virus F13L (Yang et al., 2005. J. Virol.). The latter encodes the envelope protein p37 required for production of extracellular virus. Deleting F13L resulted in a virus (ΔF13L-Vac) that is replication-competent in cell culture but that produces smaller plaques than the wild-type WR-Vac. Whereas intravenous (i.v.) inoculation of NMRI mice with 2×10^5 PFU of WR-Vac resulted in 59 ± 11 pox tail lesions per mouse, the same inoculum of ΔF13L-Vac caused no lesions ($p < 0.001$). Athymic nude (nu/nu) or SCID mice inoculated iv with 2×10^5 PFU ΔF13L-Vac did not develop tail lesions. The mean day of death in nu/nu mice inoculated with ΔF13L-Vac was 22 ± 1 days as compared to 9 ± 1 days for WR-Vac-infected mice ($p < 0.001$); SCID mice survived the infection. We next studied whether ΔF13L-Vac is able to protect mice against a subsequent infection with WR-Vac. To mimic the human vaccination protocol, NMRI mice were infected intracutaneously (i.c.) by means of scarification at the lumbosacral area with 5×10^5 PFU ΔF13L-Vac or placebo. None of the infected mice developed lesions at the inoculation site. One week later, animals were infected ic with 5×10^5 PFU of WR-Vac. All placebo animals, but none of the ΔF13L-Vac animals developed poxvirus lesions. In a second set of experiments, mice were again inoculated ic with placebo or ΔF13L-Vac and were infected one week later with 2×10^4 PFU of WR-Vac by the iv route. Placebo animals developed an average of 14 ± 4 pox tail lesions; no lesions developed in the ΔF13L-Vac animals ($p < 0.001$). In a third set of experiments, NMRI mice were inoculated iv with either 2×10^4 PFU of ΔF13L-Vac or placebo, and none of the mice developed lesions. One week later, animals were inoculated iv with 2×10^4 PFU WR-Vac. The placebo group developed an average of 11 ± 8 lesions as compared to 1.2 ± 0.7 lesions in the ΔF13L-Vac mice ($p < 0.05$). ΔF13L-Vac may thus be considered as a severely attenuated virus that may have potential for use as a smallpox vaccine.

Coxsackie, West Nile, Flavi, Arena, and Other Viruses

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Inhibition of Coxsackievirus B3 Replication in HeLa Cells and Cardiomyocytes by Peptide-Conjugated Phosphorodiamidate Morpholino Oligomers

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Background: Coxsackievirus B3 (CVB3) is the most common cause of viral myocarditis, but existing drug therapy is of limited value. Antisense oligonucleotides (ASONs) designed to pair with viral RNA could inhibit viral replication. However, the effectiveness of traditional ASONs is limited due to poor cellular uptake and degradation by nucleases. Phosphorodiamidate morpholino oligomers (PMOs) contain backbone modifications, which make PMOs more resistant to nucleases. In addition, an arginine rich peptide (P007) is conjugated to the 5′ end of the oligomer to improve its delivery into cells. These features make P007-conjugated PMOs (P-PMOs) promising candidates for the inhibition of CVB3 infection.

Methods: Total 8 P-PMOs targeting distinct regions of viral genome and one scrambled sequence were designed and chemically synthesized. FITC labeled P-PMOs were used to observe their distribution of by confocal microscopy. Viral protein VP1, viral titre, and cell viability were measured by Western-blot, plaque assay, and MTS assay, respectively.

Results: P-PMOs showed increased cellular uptake compared to non-conjugated PMOs. Among the 8 P-PMOs, P-PMO-6, targeting the internal ribosomal entry site in the 5′ UTR, showed the most potent anti-CVB3 ability in a dose-dependent manner. Both infected HeLa and cardiomyocytes HL-1 cells treated with P-PMO-6 showed drastically reduced VP1 production and 3.0 log decreases in viral titres as compared to the controls. Cell viability assay revealed that 83 and 89% of treated HeLa and HL-1 cells were still alive as compared to 11 and 10% of control-treated cells and 47% antiviral activity still existed after 5 days treatment. In addition, cells treated post-infection showed similar inhibition of viral replication. Furthermore, the specificity of the P-PMOs was demonstrated by their inability to inhibit RSV infection in HeLa cells.

Conclusion: We have showed that P-PMOs can effectively inhibit viral replication in vitro, providing a new possibility for antiviral intervention.

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QSAR Studies Demonstrate the Influence of Structure of [(Biphenyloxy)Propyl]Isoxazole Derivatives on Inhibition of Coxsackievirus B3 (CVB3) Replication

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Picornaviruses are responsible for various human viral diseases including common cold, encephalitis, meningitis, myocarditis, etc. Up to now, there is no specific antiviral therapy to treat or prevent such viral disease. The usage of modern computer technologies may help to solve this problem more effectively.

The objective of the present study was the quantitative structure-activity relationship (QSAR) analysis of antiviral activity of a set of [(biphenyloxy)propyl]isoxazole derivatives that inhibit CVB3 replication in HeLa cells. Based on results from QSAR, the structure of new potential antiviral agents should be predicted by using consequent molecular design.

The QSAR approach applied is based on simplex representation of molecular structure (SiRMS). The relationship between: (a) antiviral activity against the pleconaril-sensitive clinical CVB3 isolate 97–927 (IC_{50}, μg/ml); (b) cytotoxicity in HeLa cells (CC_{50}, μg/ml); and (c) selectivity index (SI = ratio of CC_{50} to IC_{50}), and structure of 21 [(biphenyloxy)propyl]isoxazole derivatives has been studied systematically.

Quite adequate QSAR models ($R^2 = 0.831$–0.931, $Q^2 = 0.674$–0.896) have been obtained using PLS (partial least squares) method for all parameters studied. The models are in close correlation with experimental data. Structural fragments with positive or negative influence on cytotoxicity as well as antiviral activity have been determined on the base of these models. For example, QSAR analysis of antiviral activity of [(biphenyloxy)propyl]isoxazole derivatives revealed that the presence of *m*-nitrophenyl or *p*-trifluorophenyl fragment has distinctly negative influence on antiviral action. Compounds with strong antiviral activity have to contain an oxadiazole fragment. Moreover, our data allow the virtual screening and molecular design of new well-tolerated compounds with strong anti-CVB3 activity.

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Molecular Genetic Study of the Disoxaril Mutants of Coxsackievirus B1

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Analysis of the RNA sequence of the disoxaril-resistant mutants of the Coxsackievirus B1 was carried out. The wild-type disoxaril-sensitive strain (Connecticut 5) and two disoxaril-resistant mutants (one of them produced in FL cells and the other one isolated from brains of newborn mice) infected with Coxsackievirus B1 and treated with disoxaril and a disoxaril-dependent mutant strain obtained from the resistant strain by 9 passages in cell culture were included in the present study.

A RT-PCR assay with primer sets selected from a region of the Coxsackievirus B1 genome coding for the capsid protein VP1 was carried out. A parallel comparative analysis of the sequences of resulting fragments from the disoxaril mutants studied and the GenBank sequence of origin of the VP1 gene of Coxsackievirus B1 was performed with the BLAST alignment tool.

Distinct alterations in the VP1 locus of the disoxaril-resistant and the disoxaril-dependent mutants compared to the sequence of origin from the GenBank (namely, a deletion of UUG at ntt. 2749–2751 and an insertion of UUU at nt.2769) were observed. High-degree similarity (97%) between the resistant mutant produced in cell cultures and the dependent strain was observed, while the similarity to the wild strain was only 91%. The resistant mutant obtained in mice was found to be very similar to the strain, developed in cell cultures. A putative 3-D model of the spatial folding of the target protein in disoxaril mutants is proposed.

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Characterization of Triple Combinations of Enteroviral Replication Inhibitors Effective Against Experimental Neurotropic Coxsackievirus B1 Infection in Newborn Mice

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In previous study of ours we presented a new approach to combined application of antivirals—consecutive administration of the partners. This schedule could be considered especially suitable for treatment of enteroviral infections, in which the development of resistance is very rapid due to the extremely high viral mutation rate. This approach aims to restrict the resistance development in experiments in vivo, using antivirals with proved high efficiency in experiments in cell cultures.

The screening of various double, triple, and quadruple combinations that we carried out showed that two of the triple combinations, namely disoxaril (WIN compound)/oxoglaucin (a

new antiviral drug, developed in our laboratory)/PTU-23 (a classic enteroviral inhibitor) and disoxaril/oxoglaucin/guanidine-hydrochloride (a classic enteroviral inhibitor) manifest significant effect of protection in newborn mice with neurotropic coxsackievirus B1 infection.

In the current study the role of the chronology of arrangement of the antivirals included in the combinations was investigated. In the experiments carried out with the triple combination disoxaril/oxoglaucin/guanidine-hydrochloride, it was found that the optimal treatment course should start with disoxaril. The treatment course is quite successful when disoxaril is followed by guanidine-hydrochloride. The effect of the triple combination starting with oxoglaucine, followed by guanidine-hydrochloride was moderate. The course starting with guanidine-hydrochloride proved to be ineffective.

Furthermore, we studied the virus sensitivity to the inhibitors-partners (IC50 values) and some other phenotypic characteristics of the brain isolates, e.g. the size of the plaques and the pathogenicity for mice.

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Isoxazolecarbonitriles: A Novel Class of AntiCoxsackievirus Compounds Blocking Virus Adsorption

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Recently our contribution to the development of new antipicornavirus agents has led to the discovery of 5-methylthio-3-aryl-isoxazole-4-carbonitrile derivatives whose in vitro anti-coxsackievirus B1 activity were dependent on the nature of the substituents on the para position of the phenyl ring. Particularly, compounds 5-methylthio-3-[4-(3-phenyl-1-propoxy)phenyl]isoxazol-4-carbonitrile (**ON-7**) and 5-methylthio-3-[4-(4-phenoxy-1-buthoxy)phenyl]isoxazol-4-carbonitrile (**ON-10**) exhibited an interesting antiviral activity with high selectivity indexes.

In the present study, we investigated on the mechanism of action of these compounds. Studies on time of addition experiments suggested that these compounds exert a different interference with an early step of the viral replicative cycle. In fact, compound **ON-7** was effective when added within 1 h after the end of the adsorption period and no reduction was observed if it was added during the adsorption period. Whereas a reduction of virus titer was observed for **ON-10** when was added during the adsorption period, while no reduction was observed if the compound was added after this period (time 0). The influence of the compounds on virus adsorption step, studied by the infective center assays, indicated that **ON-10** primarily interferes with Coxsackie B1 cellular attachment. At a concentration 100 times the ID_{50}, inhibition of adsorption of Coxsackievirus by **ON-10** was complete, while similar concentration of **ON-7** had no effect.

Our experiments on neutralization of viral infectivity and on thermal stabilization demonstrated that the compounds were able to directly inactivate Coxsackievirus, and the infectious titer was restored to the original value after extraction of the compound with chloroform. However, the compounds did not protect the viral infectivity against heat inactivation at the different concentrations used.

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Transforming Growth Factor-Beta 1 Improves Blood-Brain Barrier Properties in Mice Infected with West Nile Virus

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The blood-brain barrier (BBB) fulfills a vital protective function by limiting entry of potential pathogens, toxins, and inflammatory cells into the central nervous system (CNS). Disruption of the BBB is a common component of many CNS diseases, including viral diseases such as that caused by West Nile Virus (WNV). Transforming Growth Factor-β1 (TGF-β1) has previously been shown to improve the function of an in vitro model of the BBB. We evaluated the role of the BBB in WNV infection in mice by determining the ability of intraperitoneally (i.p.) administered sodium fluorescein to move from the circulating blood to the central nervous system. To demonstrate BBB permeability a mean and normal range of permeability values was determined in 30 non-infected C57/BL6 mice. In subsequent experiments, any animal expressing a permeability value greater than 2 SD above the mean was considered abnormally high. We determined that elevations in BBB permeability can be detected in mice 8 days after subcutaneous inoculation with WNV. WNV inoculated animals were treated with doses of 3000, 1000, or 300 ng/kg/day of TGF-β1 or with drug vehicle once daily via the i.p. route on 7 and 8 days post-virus inoculation (dpi), and then assayed for BBB permeability on 8 dpi. Sixty-two percent (8/13) of placebo-treated animals had abnormally high permeability values, while animals treated with 3000 and 1000 ng/kg/day of TGF-β1 had 29% (2/7) and 57% (4/7) of animals with abnormally high permeability values, respectively. In contrast, none of the animals treated with 300 ng/kg of TGF-β1 (0/8) expressed abnormally high permeability values, which was significantly lower ($p < 0.01$) than placebo-treated animals. These results suggest that TGF-β1 may improve the function of the blood-brain barrier in WNV infected mice.

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Treatment of West Nile Disease with Neuroprotective Agents

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People infected with West Nile virus (WNV) usually see their physicians after showing symptoms suggestive of neurological infection. WNV infects the central nervous system (CNS) of rodents 3–5 days after s.c. viral challenge. Yet, most published animal studies begin therapeutic treatments before or soon after viral challenge. The question addressed in this study is if neuroprotective agents can be efficacious when administered early before brain infection or later after the virus is demonstrated to be in the brain. The drugs evaluated in WNV-infected rodents were NMDA and AMPA receptor antagonists, modulators of nitric oxide synthase (NOS) and nitric oxide production, and riluzole for reducing glutamate excitotoxicity. Serial doses of diethyldithiocarbamate (DDTC) and N(G)-monomethyl-L-arginine (L-NMMA), an inducer or inhibitor of NOS, respectively, administered i.p. daily for 10 days beginning 4 h before viral challenge slightly improved survival of mice, but the difference was not statistically significant. Tolerated doses of two NMDA-receptor antagonists, flupertine (7 mg/kg) and MK-801 (1 mg/kg), and one AMPA-receptor antagonist, GYKI 52466 (10 mg/kg), were administered twice daily (b.i.d.) on 4 though 9 days post-virus inoculation (dpi). GYKI 52466 slightly improved mouse survival and weight gain, but the difference was not statistically significant. Talampanel, an AMPA-receptor antagonist and a derivative of GYKI 52466, slightly improved hamster survival ($p \leq 0.05$) when treatment began on 5 dpi, but repeated experiments using different doses and slightly different protocols gave mixed results. Riluzole, the only drug shown to improved survival of amyotrophic lateral sclerosis (ALS), presumably by reducing glutamate excitotoxicity, was not effective against WNV disease when administered b.i.d. beginning 5 dpi. Overall, neuroprotective agents did not consistently improve WNV disease, although slight improvements in animal survival might be relevant to improvement of neurological sequelae in WNV-patients.

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Identification of West Nile Virus-Infected Cells in the Central Nervous System of Rodents Early in Infection: Implications for Treatment.

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Hamster and mouse models for West Nile virus (WNV) disease were used in this study to identify infected cells of the central nervous system (CNS) early in the course of infection. This information may be relevant to therapeutic strategies since most WNV-infected people visit their physicians after showing symptoms suggestive of neurological infection. We subcutaneously infected adult mice and hamsters using 105.3 tissue culture infectious doses of WNV. Tissues of infected and control animals from 3 to 11 days post-viral injection (dpi) were fixed by cardiac perfusion using 4% paraformaldehyde. We localized WNV, neuronal and astroglial markers in the paraffin embedded tissue sections by immunofluorescence. The images were captured using the confocal microscope (Bio-Rad, MRC 1020). We observed the presence of WNV antigen in CNS tissues of mice and hamsters as early as 3 and 5 dpi, respectively. A strong WNV-specific immunofluorescence staining was observed in the cytoplasm of neurons from the spinal cord, cerebellum, cerebral cortex, and midbrain of these rodents. The WNV-specific staining co-localized with neuron-specific markers; however, astroglial markers were not co-localized with WNV antigen in brain sections. The lack of tropism by WNV for astrocytes was also confirmed in primary murine astrocyte cultures. Interestingly, infected neurons in the midbrain of 7-day infected hamsters co-localized with calbindin, which is a calcium-binding protein and mostly expressed in the interneurons of the CNS. Therapies were evaluated in hamsters or mice at a time-point when WNV-stained neurons were identified in the CNS.

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Antiviral Mode of Action of Carrageenans Against Dengue Virus in Vero and HepG2 Cells

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Dengue virus (DENV) is an arthropod-borne flavivirus that has re-emerged in recent years as an increasingly important public health threat with nearly 50 million infections occurring each year. At present neither specific antiviral therapy nor vaccine exists for the treatment and prevention of DENV infections. Carrageenans are sulfated galactans that can be extracted from red seaweeds and comprise diverse structures with a wide range of biological properties useful in biomedicine. In a previous

study we have demonstrated the antiviral activity of commercial λ- and ι-carrageenans against DENV type 2 and 3 in Vero (monkey kidney cells) and HepG2 (human hepatoma cells), showing inhibitory concentration 50% (IC_{50}) values in the range 0.14–1.1 μg/ml and selectivity indexes (CC_{50}/IC_{50}) in the range 1000–10000. In the present work we studied the mode of action of these polysulfates against DENV-2 in Vero and HepG2 cells, first analyzing the influence of time of addition of compounds on anti-DENV activity by an infectious centre assay. The highest inhibitory effect was observed when the compounds were added during adsorption or at 1 h p.i., being ineffective at later times. Then, the effect of compounds on virus adsorption and internalization was studied separately by a virus yield inhibition assay. Significant antiviral efficacy was attained if compounds were present either only during DENV-2 adsorption or internalization. The possible effect of carrageenans on viral protein synthesis, the subsequent stage of the virus cycle occurring during the first hour of infection, was analyzed by pulse-labeling with (^{35}S)-methionine. No alterations in DENV protein synthesis in carrageenan-treated cells were observed. When cells were transfected with purified DENV-2 RNA in the presence of λ-carrageenan no inhibition in fluorescent cell focus formation and virus production was detected. Besides, no significant direct virucidal effect on DENV-2 was shown by the compounds. These results indicate that both DENV adsorption and internalization seem to be the main target for these compounds, lacking effect on the steps that occur once the viral genome is inside the cell during in vitro infection of human and monkey cells.

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Antiviral Activity of Iminosugar Compounds with Modified Alkyl Side Chains

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Multiple members of the Flavivirus genus of the family Flaviviridae cause lethal hemorrhagic fever or encephalitis. The public health significance of the hemorrhagic fever and encephalitis caused by such flaviviruses is enormous and global and there is a tremendous need for antivirals. Imino sugar glucosidase inhibitors have been shown to have selective antiviral activity against viruses such as bovine viral diarrhea virus (BVDV) and West Nile virus (WNV) that have common requirements for their glycoprotein processing during virus production. We are developing imino sugar deoxynojirycin (DNJ) derivatives through chemical synthesis of compounds with various alkyl side chains and antiviral testing against BVDV and WNV as well as in WNV subgenomic replicon assays. Briefly, using a single step growth (virus yield reduction) assay for BVDV and WNV, a series of DNJ derivatives containing various conformational locking side chains were shown to have antiviral activity. Preliminary structure-activity relationships (SAR) were obtained for further modification of the alkyl side chain and improvement of these DNJ derivatives. The activity and mechanisms of action of these compounds will be presented.

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A Nucleoside Inhibitor of Hepatitis C Virus Replication Efficiently Inhibits the Replication of Flaviviruses in vitro

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Several flaviviruses cause life-threatening diseases in man. Currently, there is no therapy available for these infections. In recent years, several highly selective inhibitors of the replication of hepatitis C virus (HCV) were designed. Most small molecule inhibitors of HCV that are in preclinical or clinical development are either protease or polymerase inhibitors. Most of these compounds are highly selective for HCV and are unlikely to exhibit activity against flaviviruses. Nucleoside polymerase inhibitors, however, may have the potential to inhibit the replication of flaviviruses as well. We evaluated in vitro whether the active component of the anti-HCV compound Valopicitabine, i.e. 2′-C-methylcytidine inhibits the replication of flaviviruses in cell culture. The compound was found to exhibit specific antiviral activity against yellow fever virus 17D (EC_{50} = 3.1 μg/ml in CPE reduction assays and >98% reduction at 5 μg/ml as assessed by qPCR) and dengue fever virus type 2 (EC_{50} = 16.5 μg/ml in CPE reduction assays). The compound also efficiently inhibited West Nile virus replication (>98% at 5 μg/ml as assessed by qPCR and >98% by plaque reduction neutralization test at 50 μg/ml). In the absence of any drugs for the treatment of flavivirus infections, it may be envisaged that nucleoside polymerase inhibitors, when marketed for the treatment of HCV infections, could be used off-label for the treatment of life-threatening flavivirus infections. Even if such drug would not be able to completely inhibit flavivirus replication, a partial reduction of the viremia during the acute phase of the infection may be sufficient to prevent the development of a fulminate disease and thus protect against virus-induced mortality.

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Cage Compounds as Inhibitors of Arenaviruses Reproduction

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Arenaviruses are one of the most dangerous tools of bioterrorism in the view of pathogenicity and epidemiological threat. For the purpose of searching new remedies for treatment are-

naviruses infections the synthesis of new derivatives of cage compounds has been carried out. The prepared compounds are bridgehead derivatives of cage compounds bearing different functional groups such as hydroxy, acylamino, alkoxycarbonylamino, alkylthiocarbonylamino groups as well as iminoalkyl adamantane derivatives, some adamantylated heterocycles and compounds containing two adamantane moieties in a molecule.

Fig. 1 R1, R2, R3 = H, Alk, Ar; X = OH, NHCOOR, NHCOR, C(R) = NR, Hetaryl A = –NHCOCONH–

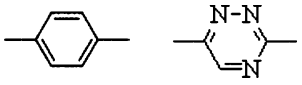

Fig. 2:

The antiviral activity of the cage compounds has been studied in respect to arenaviruses Lassa (Sierra-Leone strain) and Pichinde (AN-3739 strain) on the Vero cells culture.

Different level of antiviral activity was shown by 15 compounds. The most active compounds are monosubstituted adamantane derivatives having sufur and nitrogen-containing substituent in the bridgehead position. The data on the activity confirm the availability of searching inhibitors of arenaviruses reproduction in the cage compounds series.

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Ribavirin and Consensus Interferon-Alpha Combination Therapy of Acute Arenaviral Disease

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Several arenaviruses endemic to the South American (Junin, Machupo, and Guanarito) and African (Lassa) continents are known to cause frequently fatal hemorrhagic fever. With the exception of ribavirin, which has demonstrated efficacy in cases of Lassa fever, there are no other effective therapeutics for the treatment of arenaviral hemorrhagic fever. The outcome of treatment is ultimately dependent upon early diagnosis and the tolerability of ribavirin by patients at the high doses required for effective antiviral activity. We have recently demonstrated that consensus interferon-alpha (IFN-alfacon 1) can protect hamsters from lethal Pichinde virus (PCV) infection (Gowen et al., 2005. Antimicrob. Agents Chemother.), which serves as a model for acute arenaviral disease in humans. Here we demonstrate highly effective therapy through the combined use of ribavirin and IFN alfacon-1 for the treatment of PCV infection in hamsters. Ribavirin was given orally, twice per day for 7 days, and IFN alfacon-1 was administered intraperitoneally, once per day for 10 days. Treatments were initiated 1–5 days post-infection with various dose combinations, many which were less than optimal when the drugs were given independently. Combining suboptimal doses of ribavirin (5–10 mg/kg/day) with IFN alfacon-1 (5–10 mg/kg/day), we were able to show increased protection from mortality, reduced viral burden and liver disease, and greatly extended survival times as compared to treatments where drugs were administered alone. Our data indicate that synergistic activity resulted from combination therapy and that this activity may slow down the progression of disease and decrease fatality rates seen with severe arenaviral infections. Further, combination therapy reduces the effective dosage of ribavirin, which would serve to limit its toxicity.

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REP 9, a Degenerate Phosphorothioate Oligonucleotide that Inhibits Rift Valley Fever Viral Infection in vivo

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Rift Valley fever virus (RVFV; genus Phlebovirus, family *Bunyaviridae*) is an arbovirus transmitted by many species of mosquitoes. This virus is a major public health concern in sub-Saharan Africa and Egypt, which spread to Yemen and Saudi Arabia. In this area, RVFV is responsible for dramatic epidemics/epizootics underlining the need for efficient antiviral/prophylactic measures.

REP 9 is a 40 mer phosphorothioate oligonucleotide, which has previously been shown to have broad-spectrum activity in several viruses (Vaillant et al., submitted for publication). We used a vaccine strain (MP12) as well as the wild-type RVFV (ZH501), to test the ability of REP 9 to inhibit bunyavirus proliferation.

In vitro data showed reduction of virus titer for both strains using REP 9 at nanomolar concentrations. Moreover, the absence of the phosphorothioate modification in a stabilized REP 9 analog resulted in a loss of antiviral activity, suggesting that as in other viruses, the increased hydrophobicity of REP 9 is essential for its antiviral activity. Based on the inhibitory activity observed in vitro, we started with in vivo efficacy studies by utilizing a validated mouse model used in our laboratory. More animal experiments are ongoing to confirm the in vitro results and to evaluate the antiviral effect of the REP 9.

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Antipoliovirus Activity and Mechanism of Action of 3-methylthio-5-phenyl-4-isothiazolecarbonitrile

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Our previous studies described the synthesis and the antiviral activity of 3,4,5-trisubstituted isothiazole derivatives that were found to be particularly effective against picornaviruses. Compound 3-methylthio-5-phenyl-4-isothiazolecarbonitrile (**IS-2**) exhibited an interesting anti-poliovirus activity with high selectivity index.

In the present study, we investigated on the mechanism of action of this compound.

Studies on the time of **IS-2** addition to poliovirus type 1 infected cells suggested that the compound may inhibit some early processes of viral replication. In order to determine its mechanism of action, we evaluated the rate of attachment and internalization of purified [^{3}H]uridine-labeled poliovirus to Hep-2 cells in the presence or absence of **IS-2**. No effect on poliovirus adsorption and internalization to host cells was detected. We also investigated the influence of the compound on virus uncoating using labeled poliovirus and measuring the radioactivity of oligoribonucleotides formed from viral RNA susceptible to ribonuclease. These experiments demonstrated that poliovirus uncoating is influenced by **IS-2** action.

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Efficacy of Exogenous Interferon Treatment of Venezuelan and Western Equine Encephalitis Viruses in vivo

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Alpha Togaviruses are medically important arboviruses, with clinical cases occurring each year in North, South, and Central America. The recent increase in the threat of the use of these viruses as bio-terrorism agents has led to increased efforts to develop therapeutic agents for treatment of these viruses. Venezuelan (VEE) and western equine encephalitis (WEE) viruses have been listed as Category B Priority Pathogens by the National Institute of Allergy and Infectious Disease (NIAID). The goal of these studies was to characterize animal models for VEE and WEE for use in evaluation of antiviral therapies. C3H/HeN mice were infected through the intranasal (i.n.) route with a vaccine strain of VEE, TC-83. Virus was detected in the brain 2 days post-viral injection (dpi). Brain titers increased to a peak titer of $10^{9.5}$ 50% cell culture infectious doses per gram tissue ($CCID_{50}$/g) on 4 dpi, maintained a titer of 10^9 $CCID_{50}$/g through 7 dpi, and dropped slightly to $10^{8.6}$ $CCID_{50}$/g by 8 dpi. Virus was also detected in spleen, liver, and kidney. Treatment of VEE-infected mice with interferon alpha B/D, a human consensus interferon, resulted in 100% survival, whereas all placebo-treated animals died by 9 dpi. Syrian Golden hamsters were infected with 10^3 $CCID_{50}$ WEE through intraperitoneal (i.p.) injection. Morbidity, including hind limb paralysis, tremors, nasal bleeding, and hunching, and some mortality were seen as soon as 4 dpi. The majority of deaths occurred on 5 dpi. Virus was detected in all organs assayed (brain, liver, and spleen) with peak titers occurring 4 dpi. Interferon alfacon 1 (IFN alfacon), a human consensus interferon, active in hamsters, was effective in significantly reducing mortality ($p < 0.001$ as compared to placebo). There was a trend for reduction of brain titers in IFN alfacon-treated animals (mean titer $10^{4.8}$ $CCID_{50}$/g) as compared with placebo (mean titer $10^{9.5}$ $CCID_{50}$/g), although this difference was not statistically significant. These models will be useful in screening potential antiviral agents for efficacy against VEE and WEE.

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Comparison of the Inhibitory Effects of Ribavirin and Interferon Alfacon 1 on a Yellow Fever Virus Infection in Syrian Golden Hamsters

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Yellow fever virus (YFV) has caused significant morbidity and mortality for centuries. Primates were the only animal models for visceral YFV. Recently, hamsters were found to have morbidity and mortality when injected with a hamster-adapted Jimenez strain of YFV (Tesh et al., J. Infect. Dis. 183, 1431–1436). The objective of this study was to characterize this model of YFV viscerotropic disease for the study of effects of antiviral compounds and to test compounds with known efficacy for use as a positive control. Animals were challenged with a 10^{-4} dilution (a dilution previously shown to cause high mortality) of a liver homogenate made from livers taken 3 days post-viral injection (dpi) from hamsters challenged with the Jimenez strain. There was 66% mortality in animals challenged with the virus up to 9 dpi. Virus titers in the liver peaked 6 dpi as determined by QRT-PCR. A significant increase in serum levels of ALT (6 dpi), alkaline phosphotase (6 dpi) and bilirubin (6 dpi), and a significant decrease in amylase (8 dpi), albumin (6 dpi), and glucose (6 dpi) were observed. Hepatic icterus was observed in hamsters that exhibited disease signs at the time of necropsy. Hamsters were treated with ribavirin or interferon (IFN) alfacon 1, a consensus interferon. Ribavirin and IFN alfacon 1 both significantly ($p < 0.001$) reduced mortality as compared with placebo-treatment. There was also significant reduction in weight loss with ribavirin ($p < 0.05$) and IFN alfacon 1 ($p < 0.01$) treatment as compared with placebo. Disease signs, such as lethargy and lying prostrate, were also reduced with treatment

of ribavirin and IFN alfacon 1. Viral liver titers from treated animals were not significantly different from titers in placebo-treated animals. The hamster model of YFV disease will serve as a suitable model for the evaluation of antiviral compounds for efficacy against the virus.

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Activity of 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines Against Polyomaviruses

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Polyomaviruses are small DNA tumor viruses that depend on the host cellular DNA polymerase for their replication. Three polyomaviruses have been associated with tumor formation in humans: JC virus (JCV), BK virus (BKV) and simian vacuolating virus (SV40). In addition, some of them have been associated with viral diseases. JCV can cause progressive multifocal leukoencephalopathy in immunosuppressed patients, while BKV is considered to be the causative agent of polyomavirus-associated nephropathy, which leads to kidney transplant failure. SV40 has not been associated with a well-defined disease, but viral DNA sequences and protein expression have been detected mostly in central nervous system (CNS) tumors which strengthens the evidence for the association of this virus with human cancer.

The activity of various acyclic nucleoside phosphonates (ANPs) such as cidofovir and adefovir against murine polyomavirus and primate SV40 in vitro has already been demonstrated (Andrei et al., 1998. Antimicrob. Agents Chemother. 41, 587–593). Here, the activity of a new class of ANP's, namely 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines, against polyomaviruses was assessed. Confluent UC1-B cells were infected with either of the four murine polyomavirus strains MN/RDE Toronto, PTA, 2PTA2 or LID-1, while BSC-1 cells were infected with either the primate SV40 strain A2895, the SV40 PML-1 strain EK or the SV40 PML-2 strain DAR. After removal of the residual virus, serial dilutions of the test compounds were added. The viral cytopathic effect was recorded microscopically after 4–6 days (murine polyoma virus) or 5–7 days (SV40). HPMPO-DAPy (2,4-diamino-6-(R)-[3-hydroxy-2-(phosphonomethoxy)propoxy]pyrimidine) and PMEO-DAPy (2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine) were less active/selective than cidofovir and adefovir against the three SV40 strains tested. HPMPO-DAPy and PMEO-DAPy proved to be equally active as cidofovir and adefovir against the murine polyomaviruses.

Antiviral Targets

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7-Deaza Carbocyclic N-3 Isonucleosides as SAHase Inhibitors for Antiviral Chemotherapeutics

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Inhibition of biologically significant enzymes critical to nucleotide metabolism and viral replication is a well-established chemotherapeutic approach to the treatment of many diseases. Transcriptional 5′-capping of viral mRNA has been implicated as an "elongation checkpoint" critical to the replication cycle of many viruses. This capping process is accomplished by various methyltransferases, therefore disruption of methylation becomes an attractive target for therapy. This can be accomplished in several ways; in particular, by direct inhibition of methyltransferases (MeTase) and/or indirect inhibition of S-adenosyl-L-homocysteine hydrolase (SAHase), both established cellular targets for antiviral, antiparasitic and anticancer agents. Modified nucleosides, in particular carbocyclic nucleosides, have exhibited potent inhibitory activity against both SAHase and MeTase. Inspection of the recent literature has revealed a close correlation between SAHase inhibition and potent biological activity against negative stranded (−)-RNA viruses (i.e. Arenaviridae, Paramyxoviridae, Rhabdoviridae), double stranded (a)-RNA viruses (Reoviridae), poxviridae, as well as HIV-1, thus supporting the importance of SAHase as a viable chemotherapeutic target. Herein we report the design, synthesis, and preliminary biological activity of a new class of structurally novel carbocyclic nucleosides.

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Phosphorylation of α-*P*-Borano Substituted Nucleoside Diphosphates

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Most nucleoside antiviral agents require stepwise phosphorylation to their respective triphosphates in order to be activated in the cell. α-*P*-borano substituted nucleoside triphosphates are of interest because they have proven to be good substrates for HIV-1 reverse transcriptase (RT) and may therefore be useful antiviral agents. Studies in our laboratory have indicated that the α-*P*-borano substitution of 2′3′-dideoxycytidine triphosphate (ddCTP) resulted in a 28-fold increase in efficiency of incorporation by MMLV RT compared to non-substituted ddCTP. However, the potency of these α-*P*-borano substituted nucleoside analogs as anti-viral drugs highly depends on their ability to be activated to nucleoside triphosphate (NTP).

The phosphorylation of nucleoside analog diphosphates to their respective triphosphates has remained largely unexplored.

Here, the roles of several phosphorylating enzymes are examined. In our laboratory, nucleoside diphosphate kinase, creatine kinase, and pyruvate kinase are being evaluated for their specificity towards nucleoside analog diphosphates. The effects of nucleobase, ribose, α-phosphate substitution and stereochemistry of the boranophosphate group are of interest.

The binding affinities of the substrates for creatine kinase (CK) and pyruvate kinase (PK) were determined using a fluorescence-quenching assay, which allowed us to investigate the substrate affinity in the pre-steady state. Rabbit muscle CK and PK were titrated with a wide range of NDPs and NTPs by monitoring a decrease in enzyme intrinsic fluorescence. The affinities of these substrates were determined to establish a structure-activity relationship for CK and PK and to evaluate the effect of a substrate α-*P*-borano modification. CK showed stereospecificity towards the Sp isomer of ADPαB whereas PK showed stereospecificity towards the Rp isomer of ADPαB. Negative cooperativity was observed for all studied substrates. Steady-state experiments are also being performed directly following the product formation using UV-visible spectroscopy and high performance liquid chromatography (HPLC). These kinases were investigated because they may serve as a means for activation of antiviral α-*P*-borano substituted NDPs.

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Design of DNA Binding Agents that Target the Origin of Replication (ori) of HPV31

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Traditional antiviral targets encoded by the small human papillomavirus (HPV) genome are lacking. For this reason, we chose to target DNA sequences within the HPV genome in an effort to identify compounds that would block viral DNA replication in cells. We chose compounds known as polyamides, which are related to distamycin and other natural products, as our DNA binding agents. Unlike many literature studies where polyamides were designed to block formation of the transcription complex for a particular gene, we chose to target sequences within the origin of replication (ori). Thus, pyrrole-imidazole polyamides, with some containing fluorescent probes to aid in cell localization studies, were designed to recognize the HPV31 ori. The principles used to design these compounds will be described. We used "traditional" hairpin polyamides and some more unusual structures related to very recent literature reports. From the focused library that we prepared, two highly active molecules were identified. The rest of the molecules had minimal or zero activity. No cellular toxicity was observed, either in this project or in a related program where polyamides were used to affect COX-2 transcription (and subsequent expression) in rheumatoid synovial fibroblasts. Of particular interest is the difference between the active molecules and two closely related compounds that were inactive: the active species bind and recognize two more HPV DNA base pairs than do the related but inactive structures. This presentation will provide detailed chemistry background and structural information to complement our cell work that is also being presented at the meeting.

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2-(4-Phenyl-4-Piperidinyl)Ethyl Amine-Based CCR5 Antagonists: Derivatizations at the N-terminal of the Piperidine Ring

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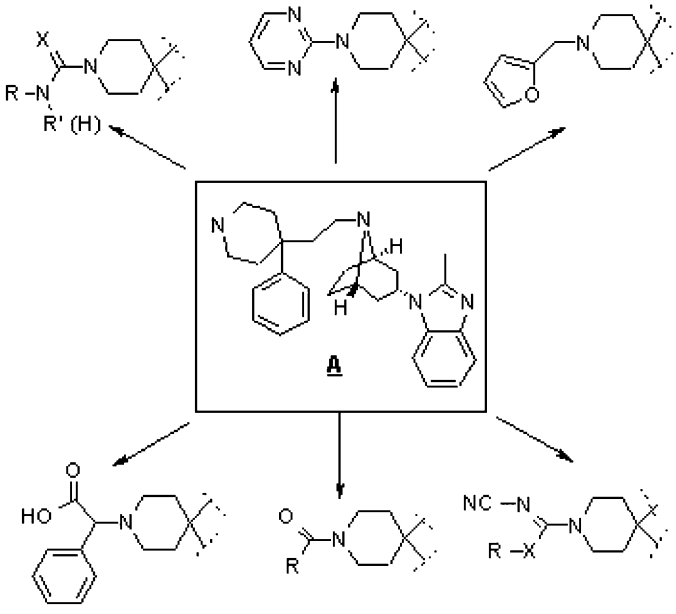
Discovery of the chemokine receptor CCR5 as a co-receptor for HIV-1 infections revealed a novel approach to HIV-1 treatments and preventions. CCR5, a member from the family of 7TM G-protein coupled receptors, thus became an attractive target pursued in the pharmaceutical industry. With the recent successful developments of several small molecules in clinic, these CCR5 antagonists hold great promise to be the next generation of anti-HIV medicines. This poster will describe our efforts at the N-terminal piperidine ring of template A to improve pharmacological properties of derived molecules.



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Two Forms of HIV-1 Matrix Protein as Targets for Antiviral Compounds

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According to current models, proteolytic processing of HIV-1 Gag precursor occurs within the virions which detach from infected cells. Meanwhile, the viral protease is activated much earlier, and Gag p55 cleavage initiates in infected cells. We followed the fate of matrix protein cleaved in infected cells (cMA) in comparison with MA cleaved in the virions (vMA) and showed that both forms differ in their localization in the infected cells and in the virions, both forms are involved into virus pathogenesis and represent the targets for antiviral compounds. MT-4 cells were labeled with [3H]-leucine or myristic acid, and 2 h after labeling protease inhibitor was added to separate the cleavage of cMA from vMA. cMA was found in the nuclear and membrane fractions of infected cells while cCA resided in cytoplasm. 18–20 h after labeling cMA was found in the virions localizing in the cores. vMA was located under lipoprotein envelope of the virions. New membranotropic antiviral compounds based on adamantane- and norbornene-related derivatives not toxic for the host cells were added to MT-4 cells before infection or 1–2 h later and at concentration 2–6 ug/ml blocked reverse transcription, the transport of cMA into the nuclei, and the production of infectious virus. The compounds inhibiting very early step of virus life cycle are optimal candidates for microbicides. To enhance their antiviral activity, we plan to associate polyanionic matrix with MA imitating peptides and cholesterol-like fragments.

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The Candidate Microbicide CADA, an Entry Inhibitor that Specifically Targets the Cellular CD4 Receptor, Prevents HIV and SIV Infection of Human and Simian Cells

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The cyclotriazadisulfonamide (CADA) compounds were shown to be potent inhibitors of HIV replication in human T-cell lines, PHA-stimulated PBMCs, and monocytes/macrophages (EC_{50}: 0.3–3.2 μM). The prototype compound, CADA, had consistent activity against laboratory adapted and primary clinical isolates of HIV-1, irrespective of chemokine receptor preference (R5, X4, R5/X4). CADA acted synergistically when evaluated in combination with various other HIV drugs, such as reverse transcriptase (RT), protease, and virus entry inhibitors. Flow cytometric analysis revealed a significant decrease in the cell surface and intracellular expression of the CD4 receptor in human cells after CADA-treatment. Moreover, the anti-HIV activity of CADA correlated with its ability to down-modulate the CD4 receptor in human T-cells. Here, we report the consistent antiviral activity of CADA against: (i) drug-resistant viruses (i.e. viruses resistant to RT inhibitors, protease inhibitors, and enfuvirtide); (ii) different HIV-1 subtypes (A, B, C, D, A/E, F, H, O); and (iii) various HIV-2 strains examined. In addition, CADA potently inhibited SIVmac251 infection of PBMCs isolated from macaques (EC_{50}: 1.6 μM). Comparable results were obtained in human cells infected with SIVmac251. Flow cytometric analysis also demonstrated a significant and dose-dependent down-regulation of the CD4 receptor expression at the cell surface of simian PBMCs after treatment with CADA. The combination of CADA with cellulose acetate 1,2-benzenedicarboxylate (CAP), an enteric coating polymer for capsules and tablets, resulted in a synergistic antiviral activity. In summary, our data indicate that CADA may qualify as a potential anti-HIV microbicide drug candidate for the prevention of the sexual transmission of HIV. The preparation of gel formulations of CADA (as single drug and in combination with CAP) for vaginal administration in non-human primates is currently under investigation.

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Varicella Zoster Virus is Sensitive to Compounds that Target Host Cell Cycle Functions

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Varicella zoster virus (VZV, Human Herpesvirus 3) infection causes chicken pox, latency is established in neurons, and reactivation leads to shingles. Acyclovir and its derivatives are the treatment of choice for both manifestations of VZV. New therapeutics are needed because acyclovir-resistant strains exist, and treatment must begin within 48 h. We have studied the anti-VZV properties of roscovitine, a cyclin dependent kinase (cdk) inhibitor. Here, we tested more compounds that block the cell cycle and determined that VZV is acutely sensitive to them. Their effects on VZV replication were tested in human foreskin fibroblasts (HFFs) because these primary cultures should have a normal cell cycle (unlike tumor cell lines). The cytotoxicity of the drugs was determined by neutral red dye uptake assays. HFFs were inoculated with a low MOI (0.01) of VZV-infected cells, which remains entirely cell-associated, and then treated with drugs or diluent for 48 h. VZV spread and replication were measured by infectious focus assay and quantitative Real Time PCR. All of the drugs tested (acyclovir [ACV], phosphonoacetic acid [PAA], aphidicolin, aloisine A, purvalanol A, roscovitine, R-roscovitine, S-roscovitine, indole-3-carbanol [I3C], L-mimosine, dichloro-β-D-ribofurano-sylbenzimidazole [DRB]) had some anti-VZV activity. The selective indices of aphidicolin (833), purvalanol A (570), and I3C (1000) were greater than the positive controls ACV (250) and PAA (60).

Aphidicolin inhibits mammalian DNA polymerase and is in clinical use for cancer; purvalanol A, a 2,6,9-trisubstituted purine, primarily inhibits cdk1; and I3C is derived from cruciferous vegetables and inhibits cell proliferation. The concentrations of these compounds that inhibited VZV replication were much less than those needed to cause cell cycle arrest, suggesting that VZV depends on the enzyme activities targeted by these compounds and not on cell proliferation per se. These three drugs will be studied next in skin organ culture and in the SCID-hu mouse model of VZV pathogenesis. The results presented here demonstrate that targeting cell functions can inhibit VZV replication and help us better understand virus–host interactions.

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Nano-Responsible Multifunctional Antivirals

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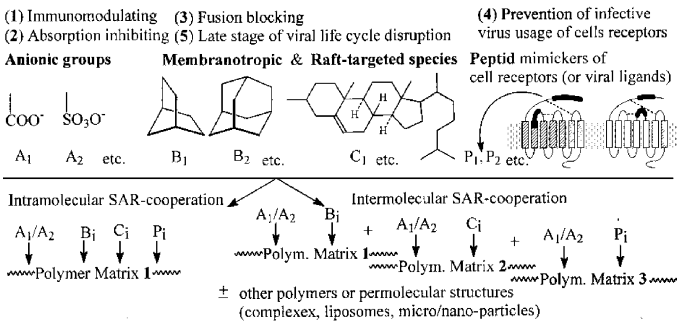
The viruses could be identified as supra-biopolymeric nanoscale complexes, parasitic intervention in cells of which occurs on an inter-polymeric reactions level. So the antiviral safety can not be fully provided without adequate nano-responsible antivirals (NAV). Here we discuss a strategy and methodology for the multi-functional NAV development by rational macromolecular SAR-cooperation of: (1) polyelectrolyte-specific interferon induction and immunomodulation; (2) electrostatic-selective prevention of viruses absorption on plasma membranes; (3) membrane-targeted blocking of post-absorption steps (fusion); (4) macromolecular prevention of structure-specific interactions of viral and cellular receptors; as well as (5) polymeric-associated disruption of the latest stage of viral replication (virions assembly and maturation). A cooperation of the (1) and (2) functions was explored by synthesis and SAR-optimization of succinate and carbohydrate polymeric derivatives modified with controllable combinations of anionic (A1/A2) groups. The immune-mediated protectors against tick-born, rabies, and other viral infections in vivo, and HIV-1 absorption inhibitors in vitro, were developed. This pre-NAV generation was used as a macromolecular platform to step-by-step targeted design and synthesis toward high effective multi-functional NAV where virus-responsible nano-selectivity was achieved by rational intra- or inter-molecular cooperation of virus-specific membranotropic vectors (Bi), Raft-targeted anchors (Ci), and peptide-kind mimickers of virus usable receptors of human cells (Pi), particularly CCR5/CXCR4. As a result, the novel nano-sensitive systems possessed unique wide multi-synergistic antiviral potency on a high level selectivity up to SI = 20,000 (against HIV-1 strains) were created and purposed for advancement of antiviral vaccines, drugs, and microbicides.



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Antiviral Activity of [(Z)- and (E)-9-[3-(phosphonomethoxy)prop-1-en-1-purines] in Cell Cultures and Evaluation of their Diphosphates in Cell-Free System

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Novel acyclic purine phosphonate derivatives bearing a double bond conjugated with the nucleic base, namely, (Z)- and (E)-9-[3-(phosphonomethoxy)prop-1-en-1-yl]purines, were synthesized, and their efficacies against HIV-1 and HSV-1 were evaluated in cell cultures. The activity of (Z)-isomer was higher against HIV than that of the reference 9-[2-(phosphonomethoxy)ethyl]adenine (adefovir) and comparable in respect to the activity of adefovir against HSV. The (E)-isomer showed low antiviral activity against both viruses. The compounds were less toxic towards cell cultures if compared to adefovir. The diphosphates (Z)- and (E)-9-[3-(phosphonomethoxy)prop-1-en-1-yl]purines were evaluated as substrates towards HIV-1 reverse transcriptase and HSV DNA polymerase. (Z)-Isomer was shown to be a more efficient substrate for both enzymes than the (E)-isomer. Human DNA polymerase alpha could incorporate neither of the diphosphates into the 3′-end of the growing DNA chain.

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Inhibition of Japanese Encephalitis Virus RNA Replication by the Peptide Nucleic Acids Targeted to the *Cis*-Acting Element of the Viral Genomic RNA

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Japanese encephalitis virus (JEV) is a member of *Flaviviridae* family and a cause of viral encephalitis. Despite the major clinical impact of JEV, no specific and effective antiviral drugs are

available to this virus. JEV genome is an approximately 11-kb single-stranded positive-sense RNA that has a cap structure at its 5′ terminus but lacks a poly(A) tail at its 3′-terminus. The coding region of the genome is flanked by 5′- and 3′-untranslated region (UTR). The 3′-UTRs on both plus- and minus-strand JEV genome contain important *cis*-acting elements required for the replication of the viral RNA genome. Peptide nucleic acid (PNA) is a synthetic oligonucleotide, in which the phosphodiester backbone of DNA/RNA is replaced with a polyamine-(2-aminoethyl) glycine skeleton. PNA offers a potentially powerful approach for recognition of RNA and silencing of gene expression. In this study, we investigated the antiviral effect of the PNAs targeted to the 3′-UTR region of JEV genome. To evaluate the PNA-mediated inhibitory effect on RNA synthesis in vitro, the RNA-dependent RNA polymerase (RdRp) of JEV, NS5 protein, which plays a major role in replication of the viral genomic RNA, was expressed in *Escherichia coli* and purified to near homogeneity by sequential column chromatographies. The recombinant JEV NS5 protein exhibited a primer-dependent RdRp activity in vitro on a homopolymeric template, poly(A). In addition, it was able to accept both plus- and minus-strand 3′-UTRs as templates for RNA synthesis in the absence of an exogenous primer. It could utilize the 3′-end 83-nt of JEV genome as a minimal template. In vitro RdRp assays using this functional recombinant JEV RdRp in the presence of the PNAs targeted to the JEV 3′-UTR 83-nt showed a dose-dependent RNA synthesis inhibition. Delivery of the inhibitory PNAs to the JEV-infected cells suppressed JEV replication, as determined by Western-blot analyses and plaque assays. Our results showed a sequence specific inhibition of JEV replication by antisense PNAs, suggesting the possible application of PNA as a novel anti-JEV agent.

Natural Products

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Evaluation of Some Pharmacological Activities of Selected Bulgarian and Turkish Medicinal Plants

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Natural products can be an important source of new pharmaceuticals. Research on antivirals of natural origin is mainly focused on plants, since, among other reasons, they can be selected on the basis of their ethnobotanical use. Plant extracts and natural plant products exhibit also a variety of biological activities with pharmacophoric utility. The population of the Balkan peninsula, like people from all continents, has long applied poultices and imbibed infusions of hundreds of indigenous plants. The present report summarizes the antiviral screening study of 134 plant products, obtained from 67 Bulgarian and Turkish medicinal plants. They were tested for inhibitory effect on the reproduction of selected influenza virus (flu) strains in MDCK cells and herpes simplex virus (HSV) strains in MDBK cells. The reduction of virus-induced CPE and infectious virus yields were used as measures of viral inhibition. Fifteen samples (11.2%) inhibited flu reproduction, and twelve samples (7.5%) were active against HSV. The anti-flu activity was confirmed in vivo for all tested samples. The most effective products were tested further for their antiproteolytic, antioxidant and immunogenic capacities and for potential antibacterial and antifungal effects. The following correlations among the variety of biological and pharmacological activities of the plant products were observed: the anti-flu effect was associated with anti-HSV effect and vice-versa in 55.5%; the antiviral effect was connected with antioxidant activity in 100%; the anti-flu effect was associated with immunogenic properties in 100%; there was found no correlation between the antiviral effect and the antiproteolytic capacity, the anti-viral properties and bacterial or fungal inhibition, the anti-viral activity and the polyphenol contents.

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Antiviral Properties of Proteolysis Inhibitors and of Compounds Containing their Fragments

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Our previous investigations have revealed antiviral activity of some proteolysis inhibitors such as E-aminocaproic acid (E-ACA) and para-aminomethylbenzoic acid (PAMBA) in vitro, in vivo and in clinic. Construction of QSAR computer-assisted hierarchical system for the effective anti-herpetic (anti-HSV) and anti-influenza (anti-Flu) agents' selection as well as the elaboration of new methods of their synthesis are permanently the object of keen interest of our team. The objective of this study was to investigate the efficacy 2,6-di-substituted pyridines and their analogs combined with the fragments of proteolysis inhibitors in the framework of the QSAR approach. Molecules of new compounds consisted of "nucleus" (Py or Ar) and two symmetrical fragments: E-ACA-carbonyl or PAMBA-carbonyl taken from the inhibitors' molecules. Anti-Flu activity in dose 10-3 M was studied in vitro on the model of A/Hong Kong/1/68 (H3N2) reproduction in tissue cultures of chorioallantoic membranes of 12 days old chick embryos. Anti-HSV activity in doses 10-4 M was studied on models of reproduction of HSV-1 on cell culture Hep-2. Compounds with Py-nucleus, contained PAMBA-carbonyl or E-ACA-carbonyl fragments, demonstrated sufficient anti-HSV activity (39.4 and 61% of reduction of intra-nucleus virus-specific inclusions on infected cells account accordingly). 3,5-Dihydrazine-carbonyl-2,6-dimethylpyridine showed high anti-HSV (52%) activity. The

efficacy of the designed antiherpetic compounds obtained with the combined efforts of QSAR computer-assisted design, properties prediction, synthesis, and biological testing as well as the correction introduced after the iteration circle passsage have proven to be the efficient modern way of drug design.

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Oxoglaucine—A Broad-Spectrum Anti-Enteroviral Inhibitor

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A series of aporphinoid alkaloids isolated from *Glaucinum flavum* L. or obtained synthetically, were tested in vitro for antiviral activity against viruses belonging to picorna-, orthomyxo-, paramyxo- and herpesviruses. One of them, oxoglaucine, manifested a well-pronounced inhibitory effect on poliovirus 1 replication in FL cells measured by the semi-quantitative agar-diffusion plaque-inhibition test. In virucidal activity testing the compound did not show direct virucidal effect on the extracellular virus. Oxoglaucine's 50% inhibitory concentration for poliovirus 1 (Mahoney) was found to be 0.188 μg/ml in the CPE-inhibition test and 0.041 μg/ml in the classical plaque-inhibition test. Similar values were obtained for the vaccinal poliovirus type 1 strain, LSc-2ab. The antiviral effect of oxoglaucine on the replication of viruses belonging to another enterovirus species was tested, i.e. coxsackie and echoviruses (HEV-B). CVA-9, the six coxsackie B viruses and 6 echoviruses were tested for their sensitivity against the antiviral effect of oxoglaucine by the end-point dilution method in the multi-cycle CPE inhibition set-up in FL cells. Oxoglaucine revealed a marked inhibitory effect on all tested enteroviruses. The concentrations that reduced virus titer by 1 lg ranged from 0.01 to 1.0 μg/ml. Selectivity index was greater than 100 and even greater than 1000 for some of the viruses tested. Time-of-addition study by the one-step virus growth cycle set-up showed strong virus inhibition during the early periods of virus replication.

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Effect of Plant Polyphenols Quercetin and Rutin on Oxidative Stress and CYP Dependent Monooxygenases in Liver of Influenza Virus-Infected Mice

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In this study an investigation and comparison of the effects of plant flavonoid polyphenols quercetin and its sugar-containing homologue (rutinoside) rutin on the "oxidative stress" in liver, isolated from influenza virus A/Aichi/2/68 (H3N2) (2.0 of LD50) inoculated mice, is carried out. It was found that experimental influenza virus infection is accompanied with graduated oxidative disturbances in the liver of mice, despite the absence of virus and inflammation in this tissue. It was found that experimental influenza virus infection is accompanied with a significant increase of lipid peroxidation products, a decrease of natural antioxidants (vitamin E, glutathione) and CYP, an inhibition of cytochrome c-reductase and liver monooxygenases (analgin-*N*-demethylase and amidopyrine-*N*-demethylase) as compared to control (non-infected) animals. The preliminary (5 days) supplementation of mice with rutin, quercetin or its combination, and their subsequent virus inoculation influence significantly all analyzed parameters as compared to controls. The protective effect of rutin against influenza virus-induced lipid peroxidation and activities of CYP and liver monooxygenases in liver was better expressed than the effect of quercetin may be due to containing of rutinoside part or difference of its metabolism.

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Antiviral Activity of S22, Natural Herb Extract, Against Influenza A Virus Infection in vitro and in vivo

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One of the traditional Korean medical herb extract named S22 was investigated to determine the anti-influenza virus activity in vitro and in vivo. The S22 showed potent antiviral activities against A/PR/8/34 (H1N1) influenza virus with the 50% effective concentration (EC50) values of 62.5 μg/ml and the 50% cytotoxic concentration (CC50) values of 673.86 μg/ml in MDCK cells. Treatment with the S22 appeared capable of significantly ameliorating the influenza virus infection in mice by oral gavage treatment.

The S22 treated mice showed significantly higher survival rate and lower pathogenic index as well as lung virus titers than untreated control mice. Further, the S22 was extracted with CHCl3, EtOAc and *n*-BuOH for isolation of active compounds. The anti-influenza effects of these active compounds will be discussed.

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Antiviral Effect of Some Nigerian Herbal Extracts on Newcastle Disease Virus

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The antiviral effect of aqeous and ethanol extracts of Ocimum gratissimum (OG), Terminalia catappa (TC), Gynostemma pentaphyllum (GP), Newbouldia laevis (NL), Aspilia africana (AA) and Phyllantus amarus (PA) leaves was examined by cultivation of virus and extracts in embryonated chicken eggs. Extracts were

inoculated immediately after virus (zero time) or 2 h after virus inoculation. Virus replication was compared with those of controls by haemagglutination assay. At zero time, aqeous extracts of OG, TC, PA, and GP inhibited virus growth by 50, 61, 72, and 94%, respectively whereas those of NL and AA did not. Ethanol extracts of OG, TC, PA and GP at same time inhibited by 25, 100, 72, and 100%, respectively whereas NL and AA did not. At two h after virus inoculation aqeous extracts of OG, TC, PA and GP inhibited virus growth by 92, 6, 67, and 100%, respectively whereas NL and AA had no effect. Ethanol extracts of TC, PA and GP inhibited the virus by 94, 78, and 94%, respectively whereas those of OG, NL, and AA did not. The herbs were studied because they were being used by some herbalists in the treatment of human infectious diseases.

First Author Index—Abstract Number

Full Author Index—Abstract Number

(Bold number signifies first author).

Invitation to the Twentieth International Conference on Antiviral Research
Palm Springs, CA, USA
April 29–May 3, 2007

The 20th International Conference on Antiviral Research will be held in the Palm Springs, California area. The conference will begin on Sunday, April 29, 2007 and will end on Thursday afternoon, May 3, 2007. All scientific sessions will be held at the Westin Mission Hills Resort, Rancho Mirage, CA.

The purpose of the International Conference on Antiviral Research is to provide an interdisciplinary forum at which investigators involved in basic, applied, and clinical research worldwide can meet to review recent developments in all areas of antiviral research. Specific topics to be covered in the program include synthesis and chemistry, biochemistry and mechanism of action, molecular biology and drug targeting, in vitro evaluation, animal models, pharmacokinetics, toxicology, and clinical trials. Within these areas of interest, there will be invited overview speakers, oral presentations, and poster presentations.

Palm Springs lies on the western edge of the Coachella Valley in central Riverside County approximately 100 miles east of Los Angeles. It is an excellent location for the Conference because it is a year-round premier vacation destination with something for everyone. More than 2000 years ago, the valley was home to the Cahuilla Indians. With the advent of the transcontinental railroad in 1877 it became accessible to the general population. After World War II there was rapid growth in new housing and businesses. It soon became a trendy playground for Hollywood stars. The Westin Mission Hills Resort & Spa is just a short drive from downtown Palm Springs and the equally charming Palm Desert. It is situated on a prime 360-acre setting in sunny Rancho Mirage, surrounded by enchanting landscaped courtyards, extensive waterways, and stunning views of the mountains. The hotel's Spanish-Moorish architecture and landscaping reflect the natural beauty of the desert. The extensive recreational facilities include everything from biking and tennis to golf on two world-class courses.

The climate is ideal with 354 days of sunshine and an average temperature of a dry 90 degrees Fahrenheit during the day and 60 degrees Fahrenheit at night in May. Among many local attractions are the must-see Indian Canyons with undisturbed natural beauty, Joshua Tree National Park tour with rock gardens of the San Andreas Fault, the tallest cactus garden in the state and 1000-year-old Joshua trees. Many other attractions are close by or within easy driving distance such as Knott's Oasis Water Park, the Children's Discovery Museum, McCallum Theatre for the Performing Arts, The Living Desert and Desert Museum, Palm Springs Air Museum, Palm Springs Aerial Tramway to the mountains, Moorten Botanical Garden, and the Wineries of Temecula Valley.

The famous El Paseo Shopping District of Palm Desert and downtown Palm Springs offer not only a large variety of galleries, boutiques and shops too numerous to mention, but there are restaurants for virtually every palate. Whether your tastes run to burgers, sushi, pizza, escargot, steak, Mexican or Continental you will find it here with a California flourish in every price range.

We hope you will take advantage of this opportunity to combine an important learning experience with a magnificent travel experience and join us in Palm Springs, California for the 20th International Conference on Antiviral Research.

ISAR Conference Committee

Future Conferences

2007: April 29–May 3, Palm Springs, CA, USA

2008: April 13–17, Montreal, QC., Canada