post-infection, to ducks inoculated with 5×10^8 DHBV genomes. The antiviral effect of REP 9AC treatment was assessed by monitoring serum viremia by quantitative PCR and serum and liver DHB sAg by ELISA or immunohistochemistry. Short-term daily (14 days) REP 9AC treatment starting 1 day prior to infection resulted in undetectable DHBV infection in 80% of ducks 53 days after cessation of treatment. The same duration of daily REP 9AC treatment starting 4 days after DHBV infection, when 2–6% of hepatocytes are typically infected led to undetectable levels of DHBV DNA in 100% of ducks within 10 days after the initiation of REP 9AC treatment and remained undetectable in 75% of ducks for 49 days after treatment. REP 9AC treatment in fully infected ducks was started 14 days after DHBV infection. Ducks received 7 daily, followed by 7 weekly doses. This treatment resulted in rapid decreases (>3 log) in serum DHBV DNA at the end of the daily treatment in all ducks. However, only 20% of the ducks had a sustained virological response 49 days after cessation of treatment. These data show that REP 9AC treatment can control post-infection spread of DHBV in Pekin ducks and can result in sustained virologic responses after treatment is halted. Thus, REP 9AC might be a valuable monotherapy or an adjunct to nucleoside analogue therapy for HBV infection. An expanded therapeutic study with longer treatment duration and followup is in progress.

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50

Characterization of the Mechanism of Action of PG-301029: A Novel Late Stage Inhibitor of HCV Replicon Replication

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PG-301029 is a small molecule that was identified as an effective inhibitor (EC_{50} value of 2.8 μM) of bovine viral diarrhea virus (BVDV). Subsequent research suggested a novel mechanism of antiviral action that resulted in a highly significant reduction of viral RNA synthesis that was not related to inhibition of virus entry, IRES translation initiation, inhibition of the NS2/3 or NS3/4A viral proteinase activities, or the NS5B viral RNA-dependent RNA polymerase. PG-301029 was 100- to 200-fold less toxic than ribavirin to cells infected with BVDV, and facilitated a reduction in the toxicity of ribavirin in combination anti-BVDV assays. PG-301029 was also shown to be nontoxic to fresh human hepatocytes at the highest concentration tested (1.33 mM). These data suggest the possibility of using the compound as a monotherapy or in combination with ribavirin, or ribavirin plus interferon for treatment of HCV. In addition, serial passage of BVDV-infected cells in the presence of escalating doses of PG-301029 failed to select for resistant virus, suggesting a high genetic barrier for resistance and that the antiviral activity may be mediated through a cellular target. Subsequent research demonstrated that PG-301029 was efficacious in an HCV replicon system, yielding an EC_{50} value of 1.5 μM and a TC_{50} greater than 42 mM. Attempts to select PG-301029-resistant HCV replicons containing a neomycin selectable marker have demonstrated that culture of the replicon-producing cells in the presence of G418 and 7.5 μM PG-301029 resulted in replicons with three potential resistance engendering mutations—one in NS3 and two in NS5A. Based on these data, we hypothesize that PG-301029 inhibits HCV replication through a novel late stage mechanism of action. Hypothesis driven research is currently focused on the identification of the mechanism of action of PG-301029 using the HCV replicon system. Results will be presented detailing characterization of HCV replicons containing the putative resistance engendering mutations, the effect of PG-301029 on HCV RNA synthesis and stability, and gene expression examining the differential expression of host cell genes in PG-301029 treated Huh-7 cells and Huh-7 cells transfected with HCV replicons.

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51

Predictive Factors to Response to Interferon Therapies for Treatment-naive Patients Infected with Hepatitis C Virus in Islamabad and Vicinity Areas: A Study from Pakistan

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Viral load determinations can give an idea of disease severity. The initial viral load before any treatment is needed to check the response of any interferon therapy. Hepatitis C is a silent, systemic disease that has many extrahepatic manifestations in addition to hepatic inflammation and fibrosis, some of which may result in a poor health-related quality of life. Fatigue perhaps the most frequent and disabling extrahepatic symptom of hepatitis C virus (HCV) reported in almost one-half of all chronically infected individuals. This large study (4324 patients) was conducted at Nuclear Medicine Oncology and Radiotherapy Institute on the HCV patients referred from major hospitals of Islamabad and its vicinity during the year 2006–2007. Based on their personal history data, a questionnaire was filled by all the patients visiting the hospital stating their demographic data, treatment information, biochemical tests results (ALT, bilirubin and ALK), symptoms/complaints, any family history of hepatitis, any past history of drug abuse, dialysis, blood transfusion, surgery, multipricks, dental surgery, barber and stomach problem. Interestingly almost 82% (3546) patients reported as they have the fatigue, general body ache, and leg pains. Significant number of patients 75% (3244/4324) belong to families where any of their family member was suffering from HCV. 118 patients were found to have their counterpart HCV positive which may be the case of sexually transmitted disease but it was not clear who caught the first. Naïve viral load was quantified on Corbett Research Real Time PCR system using aj Roboscreen kit. It was found on average 8×10^6 IU/ml HCV incidence is increasing in Pakistan and it is very important to have baseline data for effective treatment. This will be the first study from Pakistan stating the prevalence of treatment-naïve patient viral load.

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52

Design, Molecular Modelling Studies of Specific Targeted Candidate Inhibitors of HCV NS5B RNA Polymerase

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Hepatitis C virus (HCV) a member of the *Flavivirus* genus, is a negative strand RNA virus that is estimated to have infected 170 million people globally. Ribavirin and interferon combined chemotherapy is now available to treat HCV viral infection. Due to high rate of viral drug resistance, we need new targetted therapy to combat HCV. Structure-based drug design methods utilize knowledge of three-dimensional structure of an enzyme to develop some novel inhibitors of HCV. NS5B protein encoded RNA depen-

dent RNA polymerase activity has critically important role in HCV RNA replication and so considered as attractive therapeutic target for designing newer classes of compounds. Present work is to investigate the molecular modelling studies of quinoline derivatives such as Chloroquine, Hydroxychloroquine and Primaquine with HCV RNA polymerase. Results of the modelling studies indicate that the quinoline derivatives strongly interacts with the residues in the primer grip site of the polymerase. Quinoline derivatives were also subjected to HCV RNA subgenomic replicon assay and the results are; HCV RNA synthesis inhibited by Chloroquine at 10.75 mM and Hydroxychloroquine at 6.6 mM. Details of modelling studies will be presented.

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53

Identification and Characterization of Pyrimidinediones as Potent Non-nucleoside Reverse Transcriptase Inhibitors of Hepatitis B virus

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Based on extensive data demonstrating that pyrimidinediones are potent non-nucleoside inhibitors of HIV-1 reverse transcriptase (NNRTI), we hypothesized that these compounds may inhibit hepatitis B virus (HBV) reverse transcription. We have thus assessed the ability of 68 pyrimidinedione congeners to inhibit HBV replication using a HepG2.2.15 cell culture system and a quantitative PCR assay for the detection of HBV viral DNA in treated cell culture supernatants. Initial screening using a 3-dose concentration scheme resulted in the identification of twelve pyrimidinedione molecules (SJ26, SJ44, SJ46, SJ49, SJ50, SJ53, SJ56, SJ59, SJ68, SJ59, SJ80, and SJ86) which displayed submicromolar EC_{50} values and TC_{50} values greater than 10 mM. Expanded analysis of the anti-HBV activity of these twelve compounds using 9-dose concentration points with half logarithmic dilutions of the compounds demonstrated that 3 of the 12 compounds (SJ59, SJ68, and SJ80) had EC_{50} values less than 1 mM in the expanded full dose response evaluations and two additional compounds (SJ26 and SJ49) had EC_{50} values less than 2.5 mM. Anti-viral activity against a broad range of RNA and DNA viruses indicated that these compounds specifically inhibited the replication of HIV and HBV, supporting the hypothesis that they inhibit reverse transcriptase and providing a rationale for development of a first-in-class NNRTI of HBV and/or HIV–HBV co-infection. Each of these SJ compounds inhibit HIV-1 RT at nanomolar concentration levels. Based on these data, compounds SJ26, SJ49, SJ59, SJ68, and SJ80 are being subjected to further characterization and development as therapeutic agents. Results will be presented detailing the in vitro characterization of these compounds for anti-viral efficacy and toxicity in combination with approved HBV therapeutics (interferon, lamuvidine, and tenofovir), analysis of anti-viral efficacy against lamuvidine-resistant virus, accumulation of intracellular cccDNA and rcDNA, inhibition of HBV reverse transcriptase activity, and toxicity against primary hepatocytes.

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54

Prophylactic Efficacy of Intranasally Administered HSP Nanoparticles for Treating a Lethal SARS-CoV Infection in BALB/c Mice

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HSP nanoparticle (sHsp-PCN) is a small heat shock protein cage nanoparticle that elicits the formation of inducible bronchoalveolar lymphatic tissue (iBALT) in the lungs. iBALT is a transient tertiary lymphatic tissue that acts like conventional secondary lymphatic tissues in that it generates local primary immune responses—B and T cell protective responses is induced by antigen presentation by APCs. Thus, it might be useful in priming the local immune environment of the lung to prophylactically treat infectious lung disease. A series of experiments were done to determine how to treat a lethal SARS-CoV infection in BALB/c mice with sHsp-PCN. In each experiment, 15 mice were treated intranasally (I.N.) with PSS and10 mice were treated I.N. with sHsp-PCN or with Poly IC:LC at 1 mg/kg, a known inhibitor of death in the lethal SARS-CoV mouse model. When mice were pretreated with sHsp-PCN one time at days −17, −13, −9, −5, −2, all mice survived the infection as did mice treated I.N. with Poly IC:LC (1 mg/kg, qd X +4, +24) with all untreated, infected mice dying. sHsp-PCN was well tolerated in sham infected and infected mice; all mice gained weight. When sHsp-PCN was administered less often prophylactically (qd X1, days −17, −13; qd X1, days −9, −5, −2; qd X1, days −5, −2; qd X1, day −2; or qd X1, day −2, +8 h), the percentage of survivors decreased to 45%, 50%, 50%, 30%, 10%, respectively. As expected, when sHsp-PCN was administered therapeutically (bid X1 at −4 h, +8 h; qd X1 on day 1; qd X2 on days 1, 2; or −4 h, +8 h, days 1, 2), it did not significantly prevent death. In a moderately lethal infection, sHsp-PCN pretreatment appeared to be dose responsive, with 80% survivors at a 5-mg/kg dose, 60% survival with a 2-mg/kg dose, and 40% survivors in mice 0.1 mg/kg; 40% of mice survived receiving PSS. Thus, sHsp-PCN appears to be an effective prophylactic treatment for lethal infections in BALB/c mice induced by mouse-adapted SARS-CoV. The data suggest that this technology might represent a novel way of ameliorating if not preventing pulmonary virus infections in general and should be further pursued.

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55

The Activity of New Cage Compounds Against Avian Influenza Virus (H5N1)

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High pathogenic strains of influenza type A (H5N1) virus have been the cause of large-scale death in poultry and death of over 200 humans. Functional derivatives of cage compounds are known as one of perspective classes of organic compounds for search of antiviral agents. During our investigation we have synthesized series of functional derivatives of adamantane: amides, hydrazones, amino, hydroxy, carboxy, carbamoylamino derivatives and wide range of adamantyl substituted oxygen, sulfur and nitrogen containing heterocycles. Antiviral activity of synthesized compounds