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Antiviral Activity of C-2 Analogs of Enviroxime. An Exploration of the Role of Critical Functionality.

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The benzimidazole antiviral Enviroxime has potent activity against a broad range of both rhinoviruses and enteroviruses, and all attempts to develop drug resistant mutants, as measured by a significant increase in the IC_{50} have failed. Studies have shown that Enviroxime selectively inhibits plus strand viral RNA synthesis, and the development of drug sensitive mutants suggests that the mechanism of action involves the rhinovirus protein 3A. In an effort to further evaluate this class of antivirals, and to explore the role of functionality in antiviral activity, we have synthesized a series of C-2 analogs of Enviroxime. Replacing the NH_2 function of Enviroxime with H, CH_3, or OCH_3 results in a modest loss of activity suggesting the importance of a hydrogen bond donor at the C-2 position. The complete loss of activity by replacement with $NHCH_3$ infers the formation of an internal hydrogen bond with the sulfonyl group attached to N-1, thus locking the N-methyl group into an undesirable position for binding at the active site. Replacing the N-1 sulfonyl function of Enviroxime with a group that cannot form an internal hydrogen bond also results in a drop in antiviral activty, providing additional evidence that internal hydrogen bonding in Enviroxime serves to hold the remaining N-H bond of the C-2 substituent in an advantageous position to act as a hydrogen bond donor at the active site.

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Antiviral Activity, and Biological Properties of Vinylacetylene Analogs of Enviroxime

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The benzimidazole antiviral Enviroxime has many of the properties required by a drug for the treatment of the common cold. It exhibits potent and selective antiviral activity when tested in tissue culture against a broad range of both rhinoviruses and enteroviruses; and all attempts to develop resistant viral mutants, as measured by a significant increase in the IC_{50} have failed. Unfortunately when Enviroxime was studied in the clinic, it gave poor oral biavailability in man, and in addition produced emetic side effects. The unique properties of Enviroxime however have prompted continued interest in this class of compounds. Recent studies have shown that Enviroxime acts by selectively inhibiting plus strand RNA synthesis, and the development of drug sensitive mutants has suggested that the mechanism of action involves the rhinovirus 3A protein. In an attempt to improve on the properties of Enviroxime, we have recently synthesized a series of vinylacetylene analogs. These new compounds are potent inhibitors of poliovirus in tissue culture. *In Vitro* studies with hepatic microsomes show significantly reduced metabolism when compared with Enviroxime. The compounds were evaluated for oral bioavailability in Rhesus monkeys, where the role of p-fluoro substitution was found to be important. Based on these studies LY222936 was evaluated in the Coxsackie A21 mouse model, where it proved to be efficacious by oral administration.

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DIFFERENTIAL ANTIVIRAL ACTIVITY OF SEVERAL IMP DEHYDROGENASE INHIBITORS

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We investigated the antiviral activity of several inhibitors of inosine monophosphate (IMP) dehydrogenase. Ribavirin, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) and mycophenolic acid (MPA) were evaluated against a large panel of viruses. MPA was found to be a much more potent inhibitor than ribavirin of the replication of the arena viruses Junin and Tacaribe (EC_{50}: 0.002 *versus* 5 μg/ml; 1000-fold), yellow fever virus (YFV) (EC_{50}: 0.08 *versus* 28 μg/ml; 350-fold), Reovirus-1 (EC_{50}: 0.2 versus 40 μg/ml; 200-fold), Parainfluenza-3 virus (EC_{50}: 0.2 *versus* 20 μg/ml; 100-fold) and Coxsackie B4 virus (EC_{50}: 7 *versus* 70 μg/ml; 10-fold). In contrast, whereas ribavirin inhibited the replication of respiratory syncytial virus (RSV), the bunyavirus Punta Toro and influenza virus type A (H3N2) and B (EC_{50}: 2-7 μg/ml), MPA was inactive against these viruses. MPA and ribavirin had a comparable effect on the replication of vesicular stomatitis virus (EC_{50}: 10 μg/ml). Unlike MPA, but akin to ribavirin, EICAR inhibited the replication of RSV (EC_{50}: 0.2 μg/ml) and Punta Toro (EC_{50}: 0.6 μg/ml). The antiviral activity of all 3 compounds could be readily reversed by exogeneously added deoxyguanosine. The differences in the antiviral spectrum of the compounds point to differences in their mechanism of antiviral action. Ribavirin may elicit its antiviral effects through depletion of intracellular dGTP pools, 5' capping of mRNA or (in its triphosphate form) inhibition of the viral RNA polymerase. These data may help delineating molecular strategies for the inhibition of virus infections that have so far not proven amenable to antiviral chemotherapy.

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ANITI-INFLUENZA VIRUS ACTIVITY BY CIRCULAR DUMBBELL RNA/DNA CHIMERIC OLIGO-NUCLEOTIDES

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We have studied a new type of antisense oligonucleotide, with RNA-DNA base pairs (sense (RNA) and antisense (DNA)) in the double helical stem (nicked and circular dumbbell DNA/RNA chimera oligonucleotides).

The circularzation of the 40 mer DNA/RNA chimeric oligonucleotide was carried out by enzymatic ligation with T4 ligase. RNase H activity was carried out in the presence of the 45 mer HIV-1 rev RNA and circular or nicked dumbbell RNA/DNA chimeric oligonucleotide (NDRD) with E. coli RNase H within 4 h. Stability of oligonucleotides to exsonuclease digestion were obtained by treatment with nuclease S1 and SVPD. The anti-Fluv assay of the activities of test compounds in the clone 76 cells was determined by a CAT-ELISA.

The 45 mer with antisense ODN was completely cleaved by E. coli RNase H within 4 h. On the other hand, the reaction of the NDRD chimeric oligonucleotide with RNase H gave the corresponding anti-DNA together with the RNA cleavage product. The 45 mer RNA was then added to the above reaction, which produced the characteristically shortened RNA fragments. Furthermore, when the circular dumbbell RNA/DNA chimeric oligonucleotide (CDRD) was used, in place of NDRD, under the same conditions as described above, RNA template cleavage was observed. Two enzymes, SVPD and nuclease S1, were used in comparative digestion studies of NDRD and CDRD chimeric oligonucleotides. The anti-DNA was digested extensively by SVPD within 10 min, whereas NDRD and CDRD were more resistant. The S1 endonuclease activity digested the anti-DNA to mononucleotides in 30 min, whereas the NDRD and CDRD were very slowly digested.

The dumbbell RNA/DNA chimeric oligonucleotide containing theAUG initation codon targeted to the PB2 gene showed the greatest inhibition influenza RNA polumerase A of gene expression in the clone 76 cells.