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D-*carba*-dT as a Promising New Antiviral Compounds?

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Carbocyclic nucleosides are interesting potential antiviral agents. Two examples of bioactive and clinically approved carbocyclic nucleosides are the anti-HIV active nucleoside abacavir and the anti-HBV active compound entecavir. Despite the high activity of used antiretroviral drugs today, there is an urgent need to develop new compounds due to the emergence of resistant virus strains. In this context we are developing new synthetic pathways for the efficient synthesis of carbocyclic nucleosides. Recently, we have prepared D-*carba*-dT by a stereoselective approach using a new chemoenzymatic synthesis. In the key reaction a so far unknown enzymatically catalyzed resolution of racemic mixture was achieved leading to an enantiomerically pure starting material. Interestingly, the remaining enantiomer can be easily converted into the other enantiomer, so that finally yields of up to 80% of the desired stereochemically pure enantiomer leading to the D-series of carbocyclic nucleosides were obtained. At the same time, this new approach offers also an access to L-*carba*-nucleoside analogues. Enantiomerically pure D-*carba*-dT proved to be anti-HIV active against HIV-1 and HIV-2 in the same activity range of activity than the clinically used d4T. Moreover, D-*carba*-dT showed very interesting antiviral properties, e.g. against resistant virus strains. D-*carba*-dT was also converted into its *cyclo*Sal-pronucleotide to study if the phosphorylation into the monophosphate can be improved by direct delivery. Further mechanism of action studies using the triphosphate of D-*carba*-dT were carried out using a primer extension assay using RNA- as well as DNA-templates and reverse transcriptase. These studies revealed a delayed chain termination of the RT-catalyzed DNA synthesis instead of the commonly observed immediate chain termination caused by known nucleoside analogues like d4T or AZT. Due to the unusual properties D-*carba*-dT may be an interesting compound that deserves further investigations.

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QSAR Analysis of Poliovirus Inhibition By Dual Combinations of Antivirals

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We have applied Hierarchical QSAR Technology (HiT QSAR) approach employing simplex representation of molecular structure to the analysis of in vitro inhibitory effects of dual combinations of picornavirus replication inhibitors against poliovirus 1 (Mahoney). The possible dual combinations of arildone, disoxaril, enviroxime, guanidine, HBB, PTU-23, ribavirin and S-7 were investigated. Antiviral activity was expressed in $lgIC_{50}$ (μM). Both molecules in the binary combinations were represented their simplexes. These representation allowed analyzing the synergy, antagonism or additivity in the combination's interaction with the biological target. We dubbed this approach as "double 2D QSAR". Predictive QSAR models were obtained using PLS approach and validated using 8-fold external cross-validation ($R^2_{ext} = 0.67–0.93$). Adequate models ($R^2_{ext} = 0.53–0.97$) were obtained in the same way for IC_{30}, IC_{40}, IC_{60} and IC_{70} inhibitory concentrations. The usage of predicted values of IC_{30}, IC_{40}, IC_{60} and IC_{70} in the framework of the feature net approach allowed to increase the quality of our QSAR model ($R^2_{ext} = 0.71–0.94$). Resulting QSAR models were analyzed, structural fragments and parts of combination promoting the antiviral activity were determined, e.g. oxypropylisoxasole. The developed model was then used to predict novel potent combinations of drugs. Oxoglaucine and pleconaril (which was very similar to disoxaril) were predicted as novel potent components of an inhibitory combination. HiT QSAR proved itself as an adequate tool for QSAR analysis of combinations and, although the method used is suitable only to binary combinations, it can be easily extended for more complex tasks.

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Anti-human Cytomegalovirus Activity by 4′,5,7-Trihydroxy-3′,5′-dimethoxyflavone (TRICIN)

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Aim: Effective new anti-human cytomegalovirus (HCMV) agents and regimens need to be developed. We examined the anti-HCMV effects of crude extract (TWEBS) and five compounds, p-coumarid acid, 3-hydroxy-4- methoxybenzaldehyde, p-hydroxybenzaldehyde, 3-hydroxypyridine, and 4′,5,7-trihydroxy-3′,5′-dimethoxyflavone (tricin), isolated from Sasa albo-marginata, a bamboo known in Japan.

Method: Among TWEBS and five compounds screened in a plaque reduction assay, for showed anti-HCMV activity in the human embryonic fibroblast cell line, MRC-5. The anti-HCMV mechanisms of the there were examined by western blot analysis using primary antibody specific for an immediate early (IE) antigen of HCMV, specific for a structural late antigen of HCMV.

Results: Treatment of cells with at least 0.001% of TWEBS inhibited the observable cytopathic effects of HCMV on infected cells. Next, we examined the anti-HCMV properties of five compounds isolated from TWEBS. In a viral plaque-reduction assay, tricin was found to be the most effective among the five compounds. Tricin showed dose-dependent inhibitory properties with a 50% effective concentration of 0.17 μg/ml (selective index = 1206) but had no virucidal effect on cell-free HCMV. Western blot analysis demonstrated that tricin decreased the expression of IE antigen and late antigen of HCMV in the infected cells.

Conclusion: Tricin isolated from Sasa albo-marginata, has anti-HCMV activity in MRC-5 cells. Tricin is a novel and unique compound with potential anti-HCMV activity. Future studies should evaluate these findings in vivo.

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