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Studies of HIV Integrase Inhibitory Activity of Novel Isatine Derivatives

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Background: The development of antiviral drugs has provided crucial new means to relieve the debilitating effects of many viral pathogens. A rich source for the discovery of new HIV infection inhibitors has been and continues to be, the 'mining' of the large diversity of compounds already available in nature and specifically those from new chemical entities. Isatine (2,3-dioxoindole) is a versatile lead molecule for designing potential antiviral agents and is also reported to possess wide spectrum of antiviral activities. HIV Integrase is a crucial enzyme for HIV replication and an attractive therapeutic target for designing novel anti-HIV agents. To understand the molecular mechanism for the antiviral efficacy of isatine we investigated its inhibitory activity against HIV-1 integrase.

Methods: Novel isatine-sulphadimidine derivatives have been studied against HIV-1 integrase enzymatic activity. All compounds were investigated for both 3′-processing (3′-P) and strand transfer (ST) activity of HIV-1 integrase enzyme.

Results: All compounds exhibited significant inhibitory activity against HIV-1 integrase (3′-P: 5.7–32.5 μM and ST: 3.55–28 μM). The 5-chloro-N-acetyl derivatives (SPIII-5Cl-AC) displayed inhibitory activity against both 3′-P and ST In enzymatic activity (3′-P IC_{50}: 6.8 ± 0.6 μM and ST IC_{50}: 3.55 ± 0.02 μM).

Conclusions: From these studies Isatine derivatives are inhibitors of HIV integrase enzymatic activity. This is the first report showing the anti-HIV-1 IN activity of isatine derivatives. HIV integrase inhibitory activity of isatine derivatives.

Compounds	IC_{50} 3′-P, μM	IC_{50} ST, μM
SPIII-5H	7.4 ± 1.6	6.18 ± 1.3
SPIII-5Cl	5.7 ± 1.0	4.5 ± 1.3
SPIII-5Br	32.5 ± 2.4	28.0 ± 1.2
SPIII-5Me	16.5 ± 2.5	10.6 ± 2.0
SPIII-NA	16.3 ± 1.6	13.6 ± 1.0
SPIII-5Cl-AC	6.8 ± 0.6	3.55 ± 0.02

The results are IC_{50} ± S.D., $n = 4$ for HIV-1 IN inhibitory activity.

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Combined Anti-influenza Virus Effect of Natural and Synthetic Viral Inhibitors

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The data from the combined application of viral inhibitors suggest that this could be a promising approach in the control of viral infections. Our research group has a substantial experience in this field of research. The aim of the present work was to investigate the combined anti-influenza virus effect of a protease inhibitor (PI), produced by *Streptomyces chromofuscus* 34-1 (SS 34-1) with a number of alternative antiviral agents. Earlier research proved that SS 34-1 inhibited significantly the replication of influenza viruses *in vitro* and *in vivo* (Angelova et al., 2006). The PI was a hydrophobic and a termostable protein, had a molecular mass of 11.2 kDa, isoelectric point of 7.5 and a high content of hydrophobic amino acids and proline. The N-terminal sequence demonstrated its homology to the *Streptomyces* subtilisin inhibitors family. The combined virus-inhibitory effects of SS 34-1 with either Rim – rimantadine hydrochloride, PC – a plant polyphenol-rich extract or a number of protease inhibitors (ACA – ε-amino caproic acid, SS 225 – a microbial PI, STI – soya trypsin inhibitor, Apr – aprotonin and Leu – leupeptin) was tested on the reproduction of influenza virus A/Germany/34, strain Rostock (H7N1) in MDCK cell cultures. The reduction of the virus-induced CPE and the decrease of HA and infectious virus production were used as measures of viral inhibition. The simultaneous use of SS 34-1 + Rim, a selective anti-influenza drug, in doses, which by themselves do not suppress significantly viral replication, resulted in an additive increase of inhibition. The combination SS 34-1 + PC, a plant preparation with established anti-influenza-virus activity, lead to a synergistic limitation of viral replication. The combined effect of SS 34-1 and the protease inhibitors Apr, Leu and STI was indifferent. Only the combinations of SS 34-1 + ACA were of the additive to synergistic type. Our results show that in order to achieve a synergistic virus-inhibitory effect of the combinations it is essential to carefully choose the individual components and the precisely select their doses.

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A Catalytic 3D Model Development of HIV-Integrase and Drug Resistance Understanding by Molecular Dynamics Simulation

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Even though 25 years of an anti-HIV drug discovery, resistance and cross resistance possess major threat. The approved drug Raltegravir is currently available to target virally encoded enzyme "HIV Integrase". The slow drug development against integrase may attribute due to poor 3D structural and catalytic information of this enzyme. The recent reports explain the molecular basis of catalysis by integrase and their inhibitors. However, a complete model giving a detailed account of various biochemical reactions controlled by integrase, its inhibition and drug resistance is still to be understood. Therefore, we report a creation of integrase model using homology followed by global minimization, validated by experimental facts. This 3D model explains the possible mode of action during strand transfer. The studies were further extended to understand the molecular basis of drug resistance by molecular dynamics simulation. The dual mutant G140S-Q148H, having negligible impact on virus replication, however dynamics result demonstrate geometrical constrain for the binding of either Raltegravir or Elvitegravir and causing high resistance. Furthermore, the flexible catalytic loop has been studied for the compensatory role of mutant S140, which favors stabilization by initiating a polar interaction within the loop by its associative residue N117.

The present computational studies demonstrate the two metal-ion mechanism of potential molecules as INSTIs and present a valid 3D catalytic model. This model further provides insight for new drug discovery through the graphical and empirical drug response in comparison to biochemical evidence against WT and mutant strain (Q148H, N155H, G140S, G140S/Q148H) of HIV Integrase.