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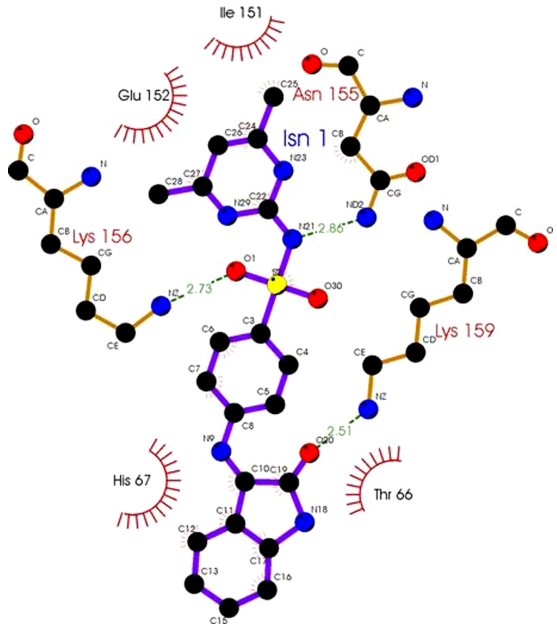
Design, Molecular Modelling Studies on Isatin Analogues as Novel Inhibitors of HIV Integrase

P. Selvam[1,*], M. Chandramohan[2], N. Saravanan Prabhu[3], M. Suresh Kumar[3]

[1] *Devaki Amma Memorial College of Pharmacy, Chelembra, Malapuram, Kerala, India;* [2] *Bharat Ratna Kamarajar Liver Hospital and Research Centre, Madurai 625020, India;* [3] *Centre for Bioinformatics, School of Life Sciences, Pondicherry University, Puducherry 605014, India*

HIV is a member of lentivirus genus, which belongs to retrovirus family and it is single-stranded, positive-sense, enveloped RNA virus leads to the most dreadful disease AIDS. Reverse transcriptase, integrase and protease are the three enzymes which are used as a drug target for the HIV treatment. Among these targets, HIV integrase is the enzyme responsible for integration of viral DNA into the host genome, which is the essential step for viral genome replication. We have synthesized a series of five novel isatin derivatives, which possesses anti-HIV activity identified by the in vitro enzyme inhibition study on HIV integrase. Among them, SPIII-5H possesses highest activity with least IC50 value of 3 μM. In this work, we have predicted the binding modes of isatin derivatives onto the active site of HIV integrase using Glide 5. This docking study shows that critical viral DNA substrate binding residues such as Lys156, Lys159 and Asn155 donates hydrogen bond to compound the active site residues such as Glu 152, Thr66, Ile 151 form hydrophobic interactions. These interactions contribute inhibition activity to the compounds and they share common pharmacophoric features with each other and with available inhibitor 5CITEP. Therefore in this work we propose the pharmacophoric features and bioactive conformation of isatin derivatives and can be used as drug lead for drug designing purpose in the future.



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Activity of Isatine-sulphadimidine Derivatives Against 2009 Pandemic H1N1 Influenza Virus in Cell Culture

Periyasamy Selvam[1,*], Markandavel Chandramohan[2], Brett L. Hurst[3], Donald F. Smee[3]

[1] *Devaki Amma Memorial College of Pharmacy, Malapuram, India;* [2] *Bharat Ratna Kamarajar Liver Hospital and Research Centre, Madurai, India;* [3] *Institute for Antiviral Research, Utah State University, Utah, USA*

Background: The development of antiviral drugs has provided crucial new means to mitigate or relieve the debilitating effects of many viral pathogens. New classes of inhibitors are essential to combat swine influenza viral infection.

Methods: A series of isatine-sulphadimidine derivatives were screened for antiviral activity against swine influenza A/California/07/2009 (H1N1) virus in MDCK cell culture. Cytotoxicity of the synthesized compounds was also tested in uninfected MDCK cells.

Results: All the compounds inhibit the influenza A (H1N1) in MDCK cells. The most active compounds, SPIII-5Br and SPIII-5H, inhibited virus-induced cytopathology by 50% at 27 and 30 μM, respectively, with 50% cytotoxicity occurring at a much higher dose (975–1000 μM). The positive control compound ribavirin inhibits the replication of the virus at 18 μM and cytotoxic concentration was found to be >1000 μM.

Conclusions: SPIII-5Br and SPIII-5H exhibited potency in the same range as ribavirin, and are suitable candidate molecules for further investigation.

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Studies of HIV Integrase Inhibitory Activity of *Morinda citrifolia* L Noni Fruit Extracts

Periyasamy Selvam[1,*], Kasthuraiah Maddali[2], Christophe Marchand[2], Yves Pommier[2]

[1] *Devaki Amma Memorial College of Pharmacy, Malapuram 676364, India;* [2] *Laboratory of Molecular Pharmacology, National Cancer Institute, Bethesda, MD 20892, USA*

Background: The development of antiviral drugs has provided crucial new means to mitigate or 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 botanical extracts. *Morinda citrifolia* is used in the Indian system of medicine for the treatment of variety of diseases including HIV/AIDS and enriched with flavinoids, anthroquinone and glycoside, but activity against HIV integrase not yet been studied, based on this fact present work is to study HIV integrase inhibitory activity of different extracts of *Morinda citrifolia*.

Method: *Morinda citrifolia* (MC) fruit extracts have been studied against inhibition of HIV-1 integrase enzymatic activity. All extracts of *Morinda citrifolia* were investigated for both 3′ processing and strand transfer process of HIV-1 integrase enzymatic activity.

Results: All extracts exhibited significant inhibitory activity against HIV-1 integrase enzyme (3′P 0.031–24 μg/ml. and ST 0.02–18 μg/ml). The acetone extract (AWT) displayed potent

Extracts	IC_{50} 3′P, mg/ml	IC_{50} ST, mg/ml
AMC-F	0.031 ± 0.006	0.02 ± 0.002
MMC- F	0.40 ± 0.02	0.28 ± 0.02
CMC-F	11.7 ± 1.4	7.45 ± 1.6
EAMC-F	24.35 ± 3.0	18.8 ± 2.0
HMC-F	3.6 ± 0.5	1.47 ± 0.08
ETMC-F	0.13 ± 0.02	0.041 ± 0.003

The results are IC50 ± S.D., $n = 4$ for HIV-1 IN inhibitory activity.

inhibitory activity against both step of HIV IN enzymatic activity (3′P IC_{50}: 0.031 ± 0.006 μg/ml and ST IC_{50}: 0.02 ± 0.002 μg/ml).

Conclusion: Anthroquine, flavinoid and alkaloids are the principle active constituents of *Morinda citrifolia*, which may responsible for HIV integrase inhibitory activity. This result presented herein substantiated the basis for combined usage of medicinal plants in AIDS treatment by Indian traditional practitioners.

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Synergistic Inhibition of Influenza a Virus Replication by a Bacterial Protease Inhibitor and a Plant Preparation

Julia Serkedjieva [1,*], Michele Dalgalarrondo [2], Iskra Ivanova [3]

[1] *Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria;* [2] *Institut National de la Recherche Agronomique-BIA-FIPL, Nantes, France;* [3] *Department of Microbiology, Sofia University, Sofia, Bulgaria*

While current anti-influenza drugs target viral components, cellular proteins are emerging as potential targets for new anti-viral drugs. The principal idea is to affect the mechanisms underlying virus–cell interactions and favoring viral replication in virus-infected cells. As the cleavage of influenza virus haemagglutinin precursor by trypsin-like proteases of the host is essential for infectivity, it would be reasonable to use protease inhibitors (PI) to impede viral replication. Earlier research proved that a proteinaceous PI, produced by *Streptomyces* sp. 34-1 (SS 34-1) inhibited significantly the replication of influenza viruses in cell cultures and protected mice from mortality in the experimental influenza virus infection (Angelova et al., 2006). The isolated PI was purified by anion-exchange chromatography and reversed phase-HPLC analysis. The N-terminal sequence demonstrated its homology to the *Streptomyces* subtilisin inhibitors family. Here we present the results on the collective virus-inhibitory effects of SS 34-1 and a plant polyphenol extract (PC) on the reproduction of influenza virus A/Aichi in MDCK cell cultures. The application of SS 34-1 and PC in doses, which by themselves do not suppress significantly viral replication, led to additive to synergistic enhancement of the inhibitory effect. The antiviral activity was determined by the difference in the infectious titers of control and treated viruses and the combined effect was defined on the base of infectious viral yields. As EC_{50}'s of the individual components in the effective combinations were reduced 4–8-fold. Preliminary experiments in mice confirmed the synergistic enhancement of the individual protective effects. Analysis by differential scanning calorimetry showed that at pH 7.5 the denaturation t^0 was 75 °C and the denaturation of PI was irreversible. Analysis by circular dichroism in the UV region 190–250 nm showed that at pH 7.5 the spectrum of the PI presented 2 minima at 208 and 222 nm, typical for an α-helical structure. At pH 2.5 and after heating the spectrum corresponded to an unordered structure.

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Optimization of Novel Broad Spectrum Anti-influenza Therapeutics

William Severson [1,*], Xi Chen [1,3], Colleen Jonsson [1,2,5], Clinton Maddox [1,2], Joseph Maddry [1,3], James Noah [1], Stephen Ontiveros [1], William Parker [1], Donald Smee [4]

[1] *Department of Biochemistry, Birmingham, AL, USA;* [2] *SRMLSC, Birmingham, AL, USA;* [3] *Department of Chemistry Southern Research, Birmingham, AL, USA;* [4] *Institute for Antiviral Research, USU, Logan, UT, USA;* [5] *Predictive Medicine for Biodefense and Emerging Infectious Diseases, University of Louisville, Louisville, KY, USA*

We screened the NIH MLSCN 100,000 compound library and discovered a novel scaffold that shows sub-micromolar activity against H5N1and H1N1 influenza viruses *in vitro*. Cheminformatics and medicinal chemistry analyses were performed of the hit compounds and SAR led to the synthesis of several second-generation compounds with potent nanomolar activity and increased polarity for hit-to-lead optimization. Of the second-generation compounds, several met our activity criteria for identification of lead compounds: an efficacy EC_{50} value of <1 μM and toxicity to efficacy ratio SI_{50} of >10 in secondary assays. We screened several lead compounds against 15 influenza A and B viruses in cell culture. They were active against H1N1 and H5N1 viruses, but not against H3N2 and B viruses. Interestingly, neuraminidase assays reveal that this scaffold did not inhibit viral neuraminidase. Real-time q-RT-PCR results revealed that these chemotypes significantly reduced RNA levels as compared to the no drug influenza infected MDCK cells. This suggests that these compounds target an early event in the viral life cycle in agreement with time-of-addition experiments whereby a significant reduction in virus-specific protein synthesis occurred 6 h post-infection in the presence of compound. Indirect immunofluorescence studies suggest these compounds affect the nuclear export of the N proteins from H1N1 virus. To supplement our *in vitro* studies, we have developed an HPLC procedure to measure the concentration of lead agents in mouse plasma after IP injection. Preliminary pharmacokinetic results indicate that significant plasma levels (μM) were achieved with five of the eight compounds that have been tested. These compounds were retained in the plasma for over 1 h. Because of the nM potency of these compounds, these results suggest that these five compounds are candidates for evaluation of efficacy in animal models. We are in the process of determining the maximally tolerated dose of these five agents. One or more of these will be selected for initial *in vivo* evaluation and the data will be presented.

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