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

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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

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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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