

## Abstract

### BACKGROUND:

HIV-1 integrase (IN) has recently been validated clinically as an important target for the treatment of HIV/AIDS. A number of IN inhibitors are in late stages of clinical trials. We report here novel pyrazolopyridine compounds as a new class of IN inhibitors.

### METHODS:

The DNA competition and cross-competition kinetic studies were performed using the HIV-1 IN strand transfer assay. In the single-cycle infection assay, MT-4 cells were infected with a defective (env-), luciferase-bearing NL-4.3 virus pseudotyped with HIV-1 env (HXB-2). In the multi-cycle infection assay, the antiviral activity of the compounds was determined by a cytoprotection assay (MTT) using NL-4.3 in MT-4 cells. The cytotoxicity of the compounds was tested in parallel using the same assay but without adding virus to the cells.

### RESULTS:

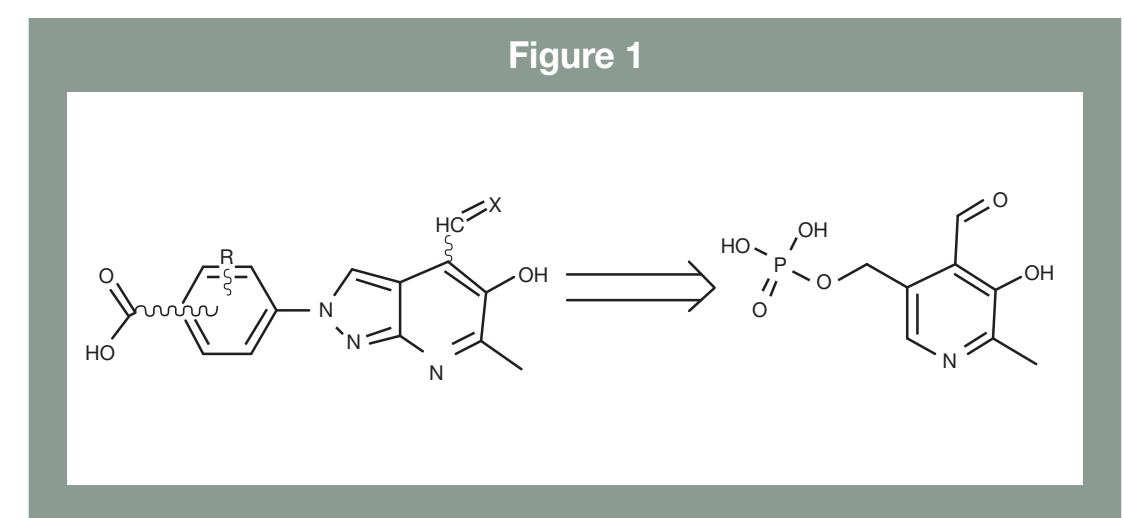
Pyridoxal-5'-phosphate derivatives were initially developed as non-cytotoxic IN inhibitors with submicromolar activities against IN enzyme and wild-type HIV-1 viruses in cellular assays. The lead optimization of physicochemical properties and potency has led to the identification of a novel pyrazolopyridine series. A number of pyrazolopyridine compounds demonstrated potent inhibition ( $IC_{50}$  = 80-900 nM) of IN strand transfer activity but did not exhibit significant inhibition of IN 3'-processing activity. Preliminary kinetic analyses suggested that these compounds inhibited the strand transfer in a non-competitive manner with respect to the DNA substrate. Furthermore, preliminary inhibitor cross-competition studies showed that the pyrazolopyridine compounds did not compete kinetically with known competitive strand transfer inhibitors such as diketo acid compounds, suggesting a different mechanism of action or a different binding site. Finally, a number of pyrazolopyridine compounds showed high nanomolar activity against wild-type viruses in cellular assays without cytotoxicity ( $IC_{50}$  > 100  $\mu$ M).

### CONCLUSIONS:

A novel series of pyrazolopyridine IN inhibitors has been developed. These compounds demonstrated potent inhibition against HIV-1 IN strand transfer activity. More importantly, pyrazolopyridine compounds showed a potentially distinct mechanism of strand transfer inhibition, which may lead to a different resistance profile from known competitive strand transfer inhibitors currently under the clinical development.

## Background

Pyridoxal-5'-phosphate (vitamin B6) is a ubiquitous enzymatic cofactor in many biochemical processes. It is also a common molecular probe for labeling putative nucleotide binding sites in many enzymes and receptors. 6-Arylazo pyridoxal phosphate derivatives were initially developed as potent, non-cytotoxic inhibitors with sub-micromolar activities against HIV-1 IN enzyme and wild-type HIV-1 viruses in cellular assays. Although stable in powder form, typically these compounds showed poor prolonged stability in solution and are suspected to be light sensitive. Several new families of molecules based on the structure activity relation of 6-Arylazo pyridoxal phosphate derivatives were developed, which led to the development of pyrazolo[3,4-b]pyridine derivatives with improved properties.



## Results

### Inhibition of HIV-1 integrase and wild type virus

Screening of these novel compounds using the Bioveris HIV Integrase strand transfer assay showed a similar structure activity relation to the parent 6-azopyridoxal phosphate, with activities ranging from 0.1 to 100  $\mu$ M  $IC_{50}$ . The anti-viral properties of these molecules followed a similar pattern (Table 1). The pattern of  $IC_{50}$  obtained from the strand-transfer assay, when matched to the  $EC_{50}$  obtained from the cytoprotection assay, are unusually close in value (Figure 2). The cytotoxicities of the compounds are usually greater than 100  $\mu$ M, which provides an attractive therapeutic window.

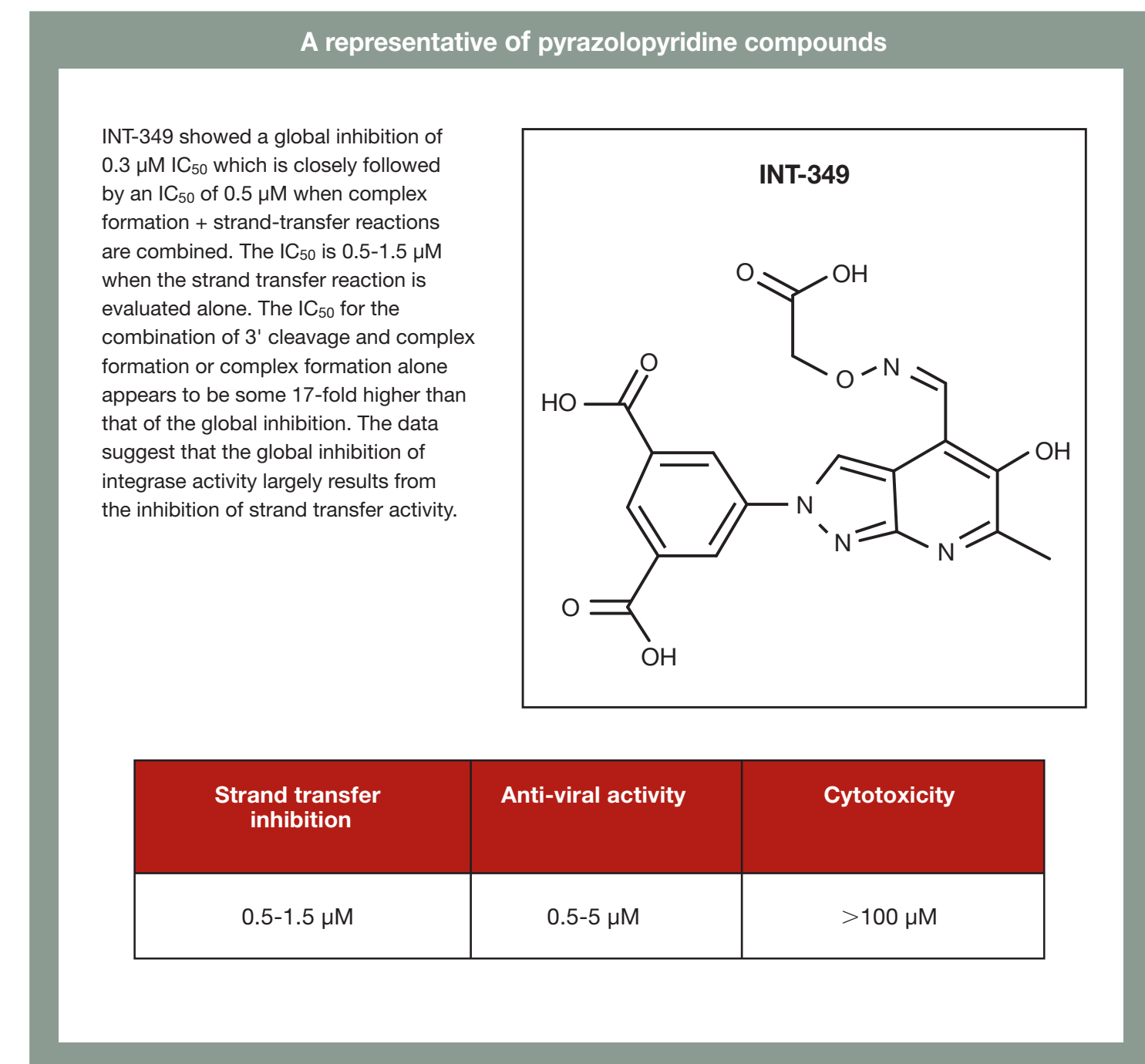
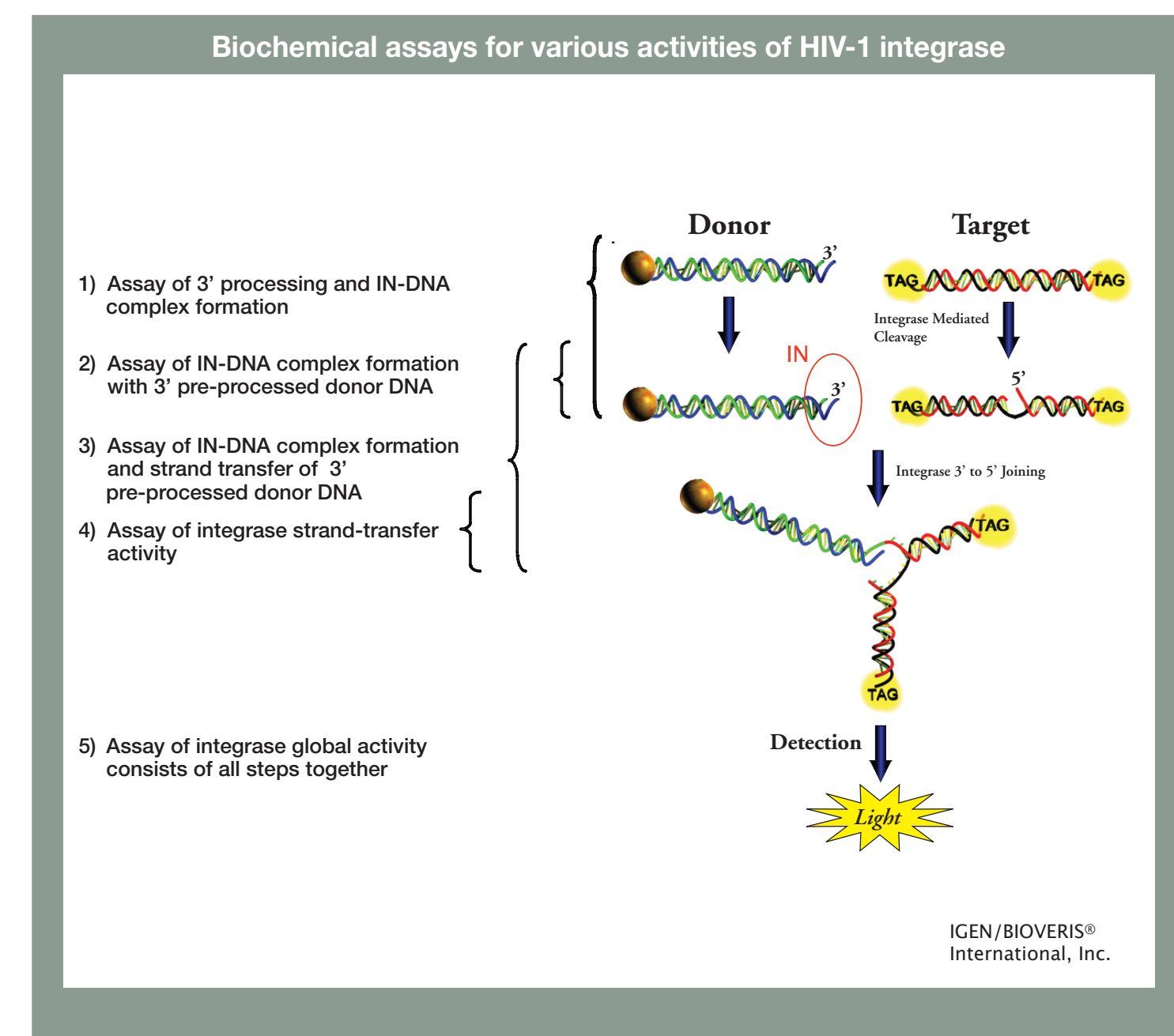
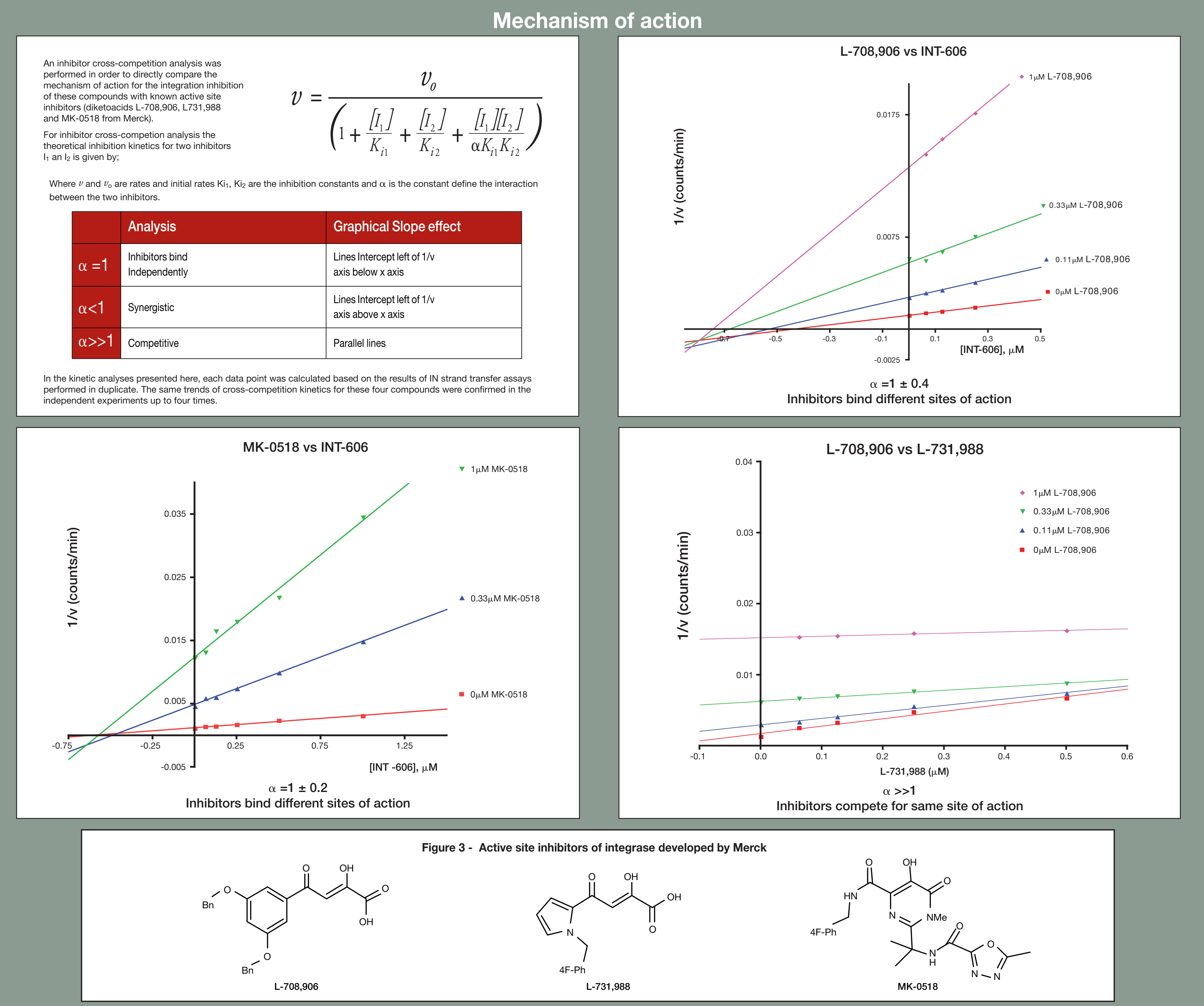
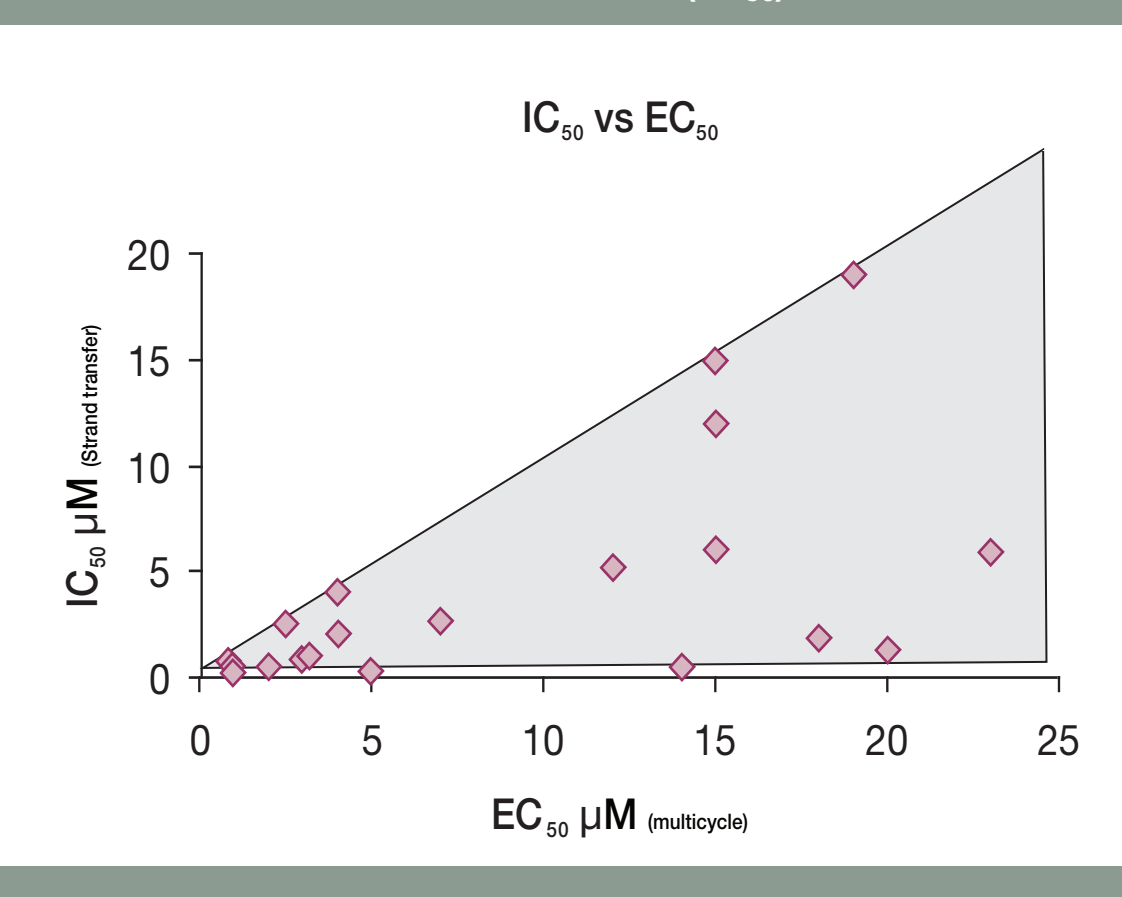


Table 1 - Anti-integrase, cytoprotection and cytotoxicity data

Compound	Enzyme assay $IC_{50}$ ( $\mu$ M)	Cytoprotection assay $EC_{50}$ ( $\mu$ M) <sup>a</sup>	Cytotoxicity $CC_{50}$ ( $\mu$ M) <sup>b</sup>
INT-351	12	15	>100
INT-355	19	19	>100
INT-356	15	15	>100
INT-368	0.5	1	50
INT-369	0.5	1	>100
INT-370	0.3	1	50
INT-371	0.2	0.2	8
INT-373	1.9	18	>100
INT-374	9	32	>100
INT-375	2.7	7	>100
INT-379	0.5	2	>100
INT-380	2.5	>50	>100
INT-382	2.5	2.5	>100
INT-383	4.0	4.0	>100
INT-388	2	4.0	30
INT-390	1.2	20	>100
INT-391	0.5	14	>100
INT-402	6	nd	>100
INT-423	7	15	>100
INT-424	9	17	>100
INT-425	6	15	>100
INT-426	5.9	23	>100
INT-427	5.2	12	>100
INT-519	0.2	5	>100

<sup>a</sup> The antiviral activity was determined based on inhibition of the cytoprotective effects induced by wild-type NL4.3 virus in MT-4 cells cultured for 6 days. Quantification was effected using a MTT colorimetric assay.  
<sup>b</sup> Cytotoxicity was determined in MT-4 cell lines in presence of integrase inhibitors, cultured over a period of six days and quantified using an MTT assay.

Figure 2 - Correlation between IN strand Transfer Inhibition ( $IC_{50}$ ) and Anti-Viral effect ( $EC_{50}$ )



## Conclusions

We report for the first time a novel series of pyrazolopyridine based IN inhibitors. These compounds demonstrated potent inhibition against HIV-1 IN strand transfer activity and anti-viral activity. More importantly, pyrazolopyridine compounds showed a potentially distinct mechanism of strand transfer inhibition.

Kinetic analysis suggests these inhibitors bind a different site of the integrase enzyme from that of known active site inhibitors. This effect may lead to a different resistance profile from known competitive strand transfer inhibitors currently under the clinical development.

