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A refined pharmacophore model for HIV-1 integrase inhibitors: Optimization of potency in the 1H-benzylindole series

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Abstract—We report herein the development of a new three-dimensional pharmacophore model for HIV-1 integrase inhibitors which led to the discovery of some 4-[1-(4-fluorobenzyl)-1*H*-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acids that are able to specifically inhibit the strand transfer step of integration at nanomolar concentration. The synthesis of the new designed molecules is also described.

Integrase (IN), one of the three enzymes expressed by HIV, catalyses the insertion of viral DNA into the genome of the host cell through a multistep process. In the '3′-processing' step, IN removes a dinucleotide from each 3′-end of viral cDNA, while in the 'strand transfer' reaction, the two newly processed 3′-viral DNA ends are inserted into the host cell DNA.[1] Moreover, it has been suggested that the integration mechanism involves divalent metal ions (Mg^{2+} or Mn^{2+}) in the enzyme catalytic site. The HIV-1 IN crystal structures available to date show only a single binding site for Mg^{2+} or Mn^{2+}, whereas both biochemical and structural studies offer a plausible two-metal model for the catalytic centre of IN.[2] Integrase strand transfer inhibitors (INSTIs) represent the major leads in the development of anti-HIV-1 IN drugs.[3] These molecules act by sequestering the metal ions bound in the active site of the enzyme.[2,4] In particular, β-diketo acids[3] (DKAs) and their derivatives[5] were the first INSTIs to display potent antiviral activity via inhibition of IN activity. We previously generated a three-dimensional (3D) pharmacophore model for DKA-like derivatives acting as INSTIs, which enabled us to rationally design and synthesize new potential DKA IN inhibitors characterized by the presence of a 1*H*-benzylindole skeleton.[6] The biological results showed that all derivatives selectively inhibited the ST step and highlighted the validity of our molecular modelling approach. Recent clinical trials have shown two new INSTIs, **1** (GS-9137) and **2** (MK-0518) (Chart 1), to be highly effective, and both compounds appeared well tolerated with respect to toxicity compared with placebo controls.[7] More recently, the FDA approved MK-0518 (raltegravir) as the first drug (Isentress) that is active against IN enzyme. [8]

The aim of this work was to create a new pharmacophore model using structural and biological data of compounds **1** and **2** in order to explain their putative binding requirements and to use the information obtained to optimize our INIs.

Our previous 3D pharmacophore model for IN inhibitor DKA-like derivatives, developed using the Catalyst HypoGen module, contained four features: one hydrophobic aromatic region (Y′), two hydrogen-bond acceptors (A1′–A2′), and one hydrogen-bond donor (D′).[6] The superimposition of the new compounds **1** and **2** on our HypoGen pharmacophore model showed that these compounds fitted with all the chemical features of our hypothesis, thus confirming the reliability of the developed model (Fig. 1).

Keywords: Integrase; HIV; DKAs; Pharmacophore model; Synthesis of indole derivatives.
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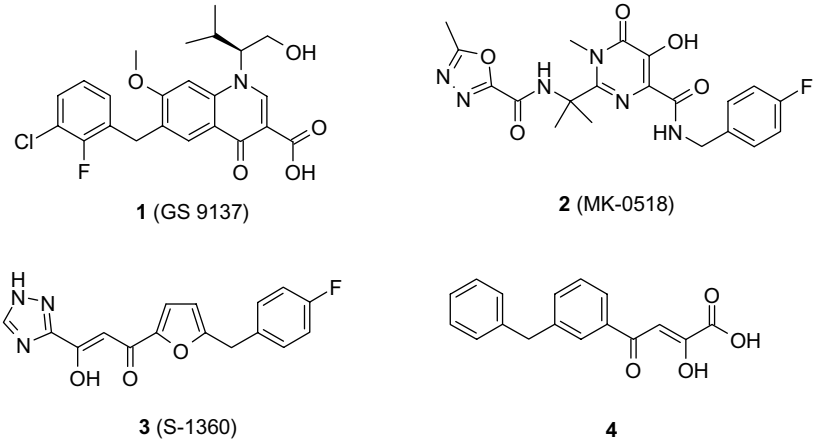


Chart 1. Chemical structures of integrase strand transfer inhibitors.

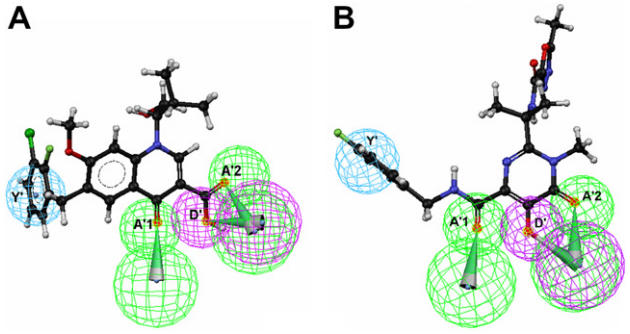


Figure 1. INSTIs 1 (A) and 2 (B) mapped into HypoGen pharmacophore model (A′1–A′2, hydrogen-bond acceptor, green; Y′, hydrophobic aromatic, cyan; D′, hydrogen-bond donor, magenta).

However, the superimposition of compounds **1** and **2** with respect to other first-generation INSTIs such as **3** (S-1360),[9] and **4**[2] (Chart 1) highlighted some additional structural characteristics which were not explored by our pharmacophore (Fig. 2) implying an explanation for their higher activity.

On this basis we used derivatives **1** and **2** as templates to generate a new 3D pharmacophore model.

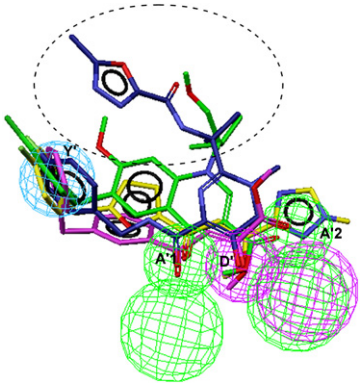


Figure 2. HypoGen pharmacophore model aligned to compounds 1 (green), 2 (blue), 3 (yellow) and 4 (magenta) (A′1–A′2, hydrogen-bond acceptor, green; Y′, hydrophobic aromatic, cyan; D′, hydrogen-bond donor, magenta). Part not explored by pharmacophore is circled.

Ten hypotheses, with a ranking score ranging from 37.6397 to 35.8411, were developed using the algorithm Catalyst/HipHop.[10] The results are presented in Table 1.

The first three ranked pharmacophore hypotheses (Hypo1–3) had the same ranking score and contained the same chemical features in a similar spatial arrangement: four hydrogen-bond acceptors (A1–A4), two hydrophobic aliphatic regions (Z1 and Z2) and one aromatic feature (Y) (Fig. 3) highlighting important structural similarities between compounds **1** and **2**. Since Hypo1–3 had therefore identical statistical relevance and composition, Hypo1 was selected for further studies (see Fig. 3).

The superimposition of this automated common-features pharmacophore model and our previously developed hypothesis showed the same three-dimensional orientation of the four features Y and A1–A3 compared with features Y′ and A′1–D′–A′2 (Fig. S1 in supplementary data). In particular, both features A1–A3 and A′1–D′–A′2 seem able to coordinate the two magnesium ions required for IN activity. The other three features (A4,

Table 1. Summary of the common feature hypothesis run

Number[a]	Composition[b]	Ranking score[c]
Hypo1	YZZAAAA	37.6397
Hypo2	YZZAAAA	37.6397
Hypo3	YZZAAAA	37.4411
Hypo4	ZZHAAAA	36.0397
Hypo5	YZHAAAA	36.0397
Hypo6	YZHAAAA	36.0397
Hypo7	YZHAAAA	36.0397
Hypo8	YZHAAAA	36.0397
Hypo9	ZZHAAAA	36.0397
Hypo10	ZZHAAAA	35.8411

[a] Numbers for the hypothesis are consistent with the numeration obtained by the hypothesis generation.
[b] Y, hydrophobic aromatic; Z, hydrophobic aliphatic; A, hydrogen bond acceptor; H, hydrophobic group.
[c] The higher the ranking score, the less likely it is that the molecules in the TS fit the hypothesis by a chance correlation. Best hypotheses have the highest ranks.

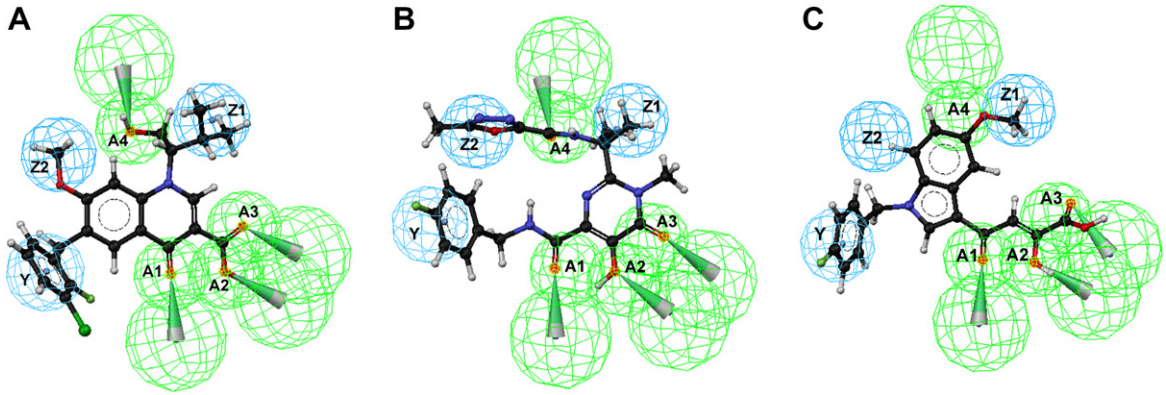


Figure 3. The top scoring HipHop pharmacophore Hypo1 is mapped to IN inhibitors **1** (A), **2** (B) **31** (C) (A1–A4, hydrogen-bond acceptor, green; Z1–Z2, hydrophobic aliphatic, blue; Y, hydrophobic aromatic, cyan).

Z1 and Z2) of the new model represent a novelty for DKA derivatives and could explain the higher anti-IN activity of compounds **1** and **2** which could be particularly useful for the optimization of already known INSTIs. In order to confirm the reliability of this pharmacophore model, we used some INSTIs reported by Sato et al., in which some structural modifications allowed the transformation of quinolone antibiotics into potent IN inhibitors such as compound **1**.[11] Indeed, on matching these compounds in the HipHop model, a good correlation was observed between activity data and BestFit values (supplementary data, Table S1). These observations were interpreted by us as an indication that substituents able to occupy the new features could be exploited to modify the intrinsic potency of our 1*H*-benzylindole derivatives. This idea was supported by biological results obtained for some of our very potent INSTIs.

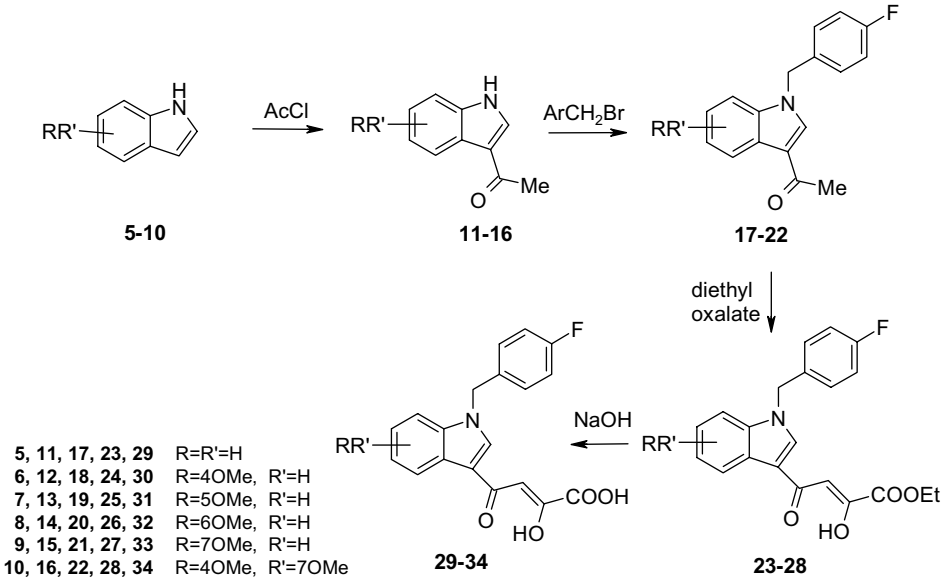
In fact, compound **31** (Scheme 1), which is characterized by the presence of a methoxy group on the indole system, proved to be more potent (IC_{50} against ST = 0.27 μM) than its demethoxy analogue, **29** (IC_{50} against ST = 2.33 μM).

Interestingly, the overlapping of compound **31** onto our new hypothesis (Fig. 3C) showed that the methoxy group in position 5 was able to fit the hydrophobic sphere Z1 thus explaining its major ST activity with respect to the demethoxy derivative.

Taking into account these findings, we focused our efforts on exploring the importance of the two hydrophobic areas (Z1 and Z2) by inserting methoxy groups in different positions of the benzene-fused ring.

Following our modelling studies, the designed molecules were synthesized and tested.

The synthesis of the new benzyl-1H-indole derivatives **24**, **26**–**28**, **30** and **32**–**34** was achieved in a multistep reaction, as previously reported by us.[6,12]



Scheme 1.

Table 2. Inhibition of HIV-1 integrase enzymatic activity, replication of HIV-1 (IIIB) and cytotoxicity in MT-4 cells

Compound	IN enzymatic activity		Activity in MT-4 cells		
	Over-all IC_{50} (μM)[a]	ST IC_{50} (μM)[b]	HIV-1 EC_{50} (μM)[c]	Cytotoxicity CC_{50} (μM)[d]	SI[e]
24	86.08 ± 12.58	92.30 ± 25.16	3.17 ± 1.71	74.0 ± 51.3	24
25	4.60 ± 1.00	7.55 ± 0.05	11.5 ± 8.7	126.0 ± 37.5	11
26	1.96 ± 1.31	2.34 ± 2.14	>100	100	<1
27	4.76 ± 2.74	2.64 ± 1.86	>100	>100	1
28	1.24 ± 0.06	0.66 ± 0.01	4.09 ± 1.29	81.1	20
29	0.86 ± 0.18	2.33 ± 0.83	8.35 ± 0.53	64.06 ± 9.74	8
30	0.08 ± 0.003	0.14 ± 0.11	0.59 ± 0.38	41.1 ± 16.7	70
31	0.30 ± 0.05	0.27 ± 0.05	5.63 ± 1.87	63.6 ± 43.3	12
32	0.19 ± 0.05	0.22 ± 0.21	8.39 ± 2.60	66.0 ± 14.8	8
33	0.27 ± 0.19	0.19 ± 0.14	7.04 ± 2.17	82.9 ± 76.1	12
34	0.06 ± 0.01	0.03 ± 0.01	2.13 ± 0.05	213.3 ± 50.3	100
MK-518	0.009 ± 0.0002	0.007 ± 0.0005	0.013 ± 0.0005	>18	>1387
GS9137	0.004 ± 0.003	0.015 ± 0.002	0.0008 ± 0.00009	2.12 ± 0.36	>2650

All data represent average results ± SD.
[a] Concentration required to inhibit the in vitro overall integrase activity by 50%.
[b] Concentration required to inhibit the in vitro strand transfer step by 50%.
[c] Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells.
[d] Cytotoxic concentration to reduce MT-4 cell viability by 50%.
[e] Selectivity index: ratio CC_{50}/EC_{50}.

The appropriate 1H-indole was first 3-acetylated by the reaction with acetyl chloride and a slight excess of diethylaluminium chloride. With microwave assisted synthesis the reactions were carried out in only 10 min, instead of 2 h using the conventional method.

The obtained derivatives were then N-alkylated by treatment with 4-fluorobenzyl bromide and a small amount of sodium hydride to give intermediates **17**–**22**.

Under the 'classical' method, reactions were performed in 30 min; whereas applying the microwave irradiation to this step of our synthetic strategy took only 5 min, thus confirming that focused microwave irradiation is a highly effective technique for accelerating organic reactions, even at low temperatures. The coupling with diethyl oxalate in the presence of a catalytic amount of sodium methoxide and using microwave irradiation, easily provided derivatives **23**–**28**. The final aryl hydroxyketoacids (**29**–**34**) could be readily produced from the hydrolysis of the corresponding arylhydroxyketoesters (**23**–**28**) in basic medium (Scheme 1). Both analytical and spectral data of all synthesized compounds are in full agreement with the proposed structures. All synthesized compounds were tested for their inhibitory effect on IN enzymatic activity and against HIV-1 replication.

To determine the susceptibility of the HIV-1 integrase enzyme towards synthesized compounds we used enzyme-linked immunosorbent assays. However, their inhibitory effect on the HIV-induced cytopathic effect (CPE) in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT-assay[13] (supplementary data for biological assay methods).

The biological results showed that the DKAs (**30**–**34**) were more active than the corresponding esters (**24**–**28**) in both assays (Table 2). Moreover, all mono methoxy-substituted DKAs exhibited higher potency than the corresponding unsubstituted derivative **29**. In particular, derivative **30** characterized by the presence of one methoxy group at position 4 proved to be the most potent compound in the enzyme assays at nanomolar concentration (Table 2). Interestingly, our idea to increase the IN binding affinity by occupying both the two hydrophobic areas Z1 and Z2 was confirmed by the biological results of compounds **28** and **34** in which we held the 4-methoxy group constant and introduced an additional methoxy moiety at the 7-position of the indole nucleus, obtaining disubstituted derivatives. As expected, the 4,7-dimethoxysubstituted DKA, **34**, showed a best-fit value which was better than monosubstituted analogues (**30**) (supplementary data, Table S2).

This behaviour could be explained by considering that derivative **34** (Fig. 4) was able to occupy both hydrophobic areas Z1 and Z2. In agreement with the

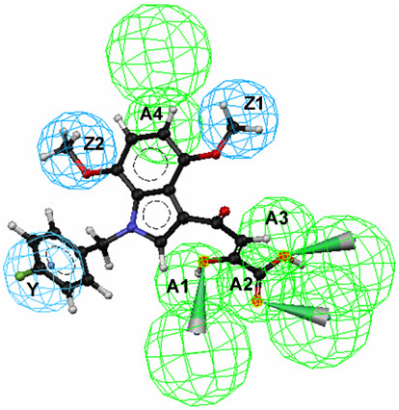


Figure 4. Alignment of compound **34** into pharmacophore Hypo1 by HipHop (A1–A4, hydrogen-bond acceptor, green; Z1–Z2, hydrophobic aliphatic, blue; Y, hydrophobic aromatic, cyan).

pharmacophore mapping values, the introduction of the second methoxy group provided a potent nanomolar INSTI (**34**) and improved the potency of the most active monosubstituted derivative **30**.

Finally, when the synthesized compounds were tested against HIV-1 replication in cell cultures all DKAs showed an EC_{50} lower than 10 μM and proved to be better than corresponding esters.

In summary we have developed a new pharmacophore model and identified a series of novel and potent IN inhibitors based on the benzylindole template.

These results suggest that this new model could be used to improve the potency of known INSTIs by suitable modification. More detailed SAR examination and the exploration of other features suggested by the new pharmacophore model are still ongoing and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.089.

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