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Brief Article

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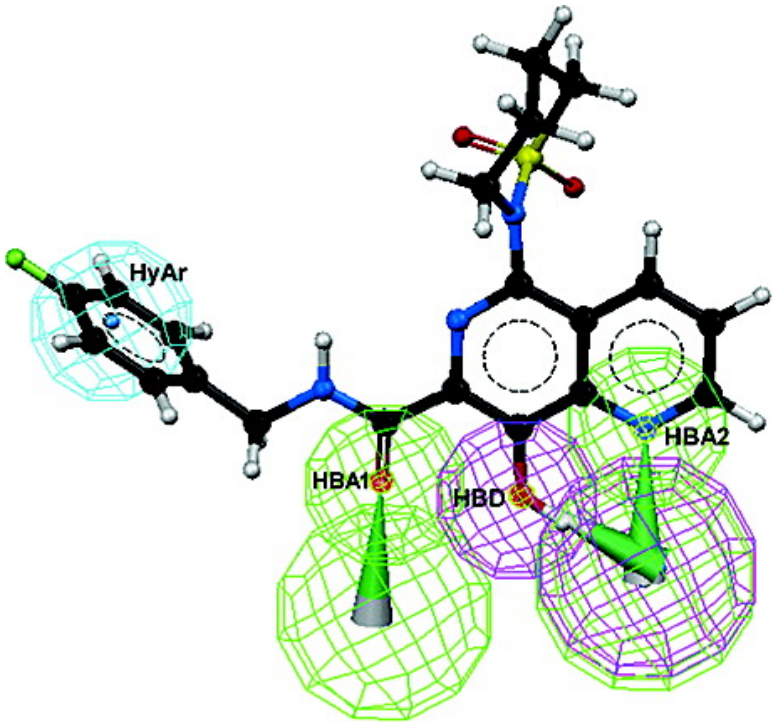

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Pharmacophore-Based Design of HIV-1 Integrase Strand-Transfer Inhibitors

Maria Letizia Barreca,[†,*] Stefania Ferro,[†,*] Angela Rao,[†] Laura De Luca,[†] Maria Zappalà,[†] Anna-Maria Monforte,[†] Zeger Debyser,[§] Myriam Witvrouw,[§] and Alba Chimirri[†]

Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, 98168 Messina, Italy, and Molecular Medicine, Katholieke Universiteit Leuven and IRC KULAK, Kapucijnenvoer 33, B-3000 Leuven, Flanders, Belgium

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Using a training set of diketo-like acid HIV-1 integrase (IN) strand-transfer inhibitors, a 3D pharmacophore model was derived having quantitative predictive ability in terms of activity. The best statistical hypothesis consisted of four features (one hydrophobic aromatic region, two hydrogen-bond acceptors, and one hydrogen-bond donor) with r of 0.96. The resulting pharmacophore model guided the rational design of benzylindoles as new potent IN inhibitors, whose microwave-assisted synthesis and biological evaluation are reported.

Introduction

Human immunodeficiency virus type 1 (HIV-1) encodes three enzymes which are required for viral replication: reverse transcriptase, protease, and integrase (IN). Although drugs targeting reverse transcriptase and protease are widely used and have shown effectiveness particularly when employed in combination, toxicity and development of resistant strains have limited their usefulness.[1] Therefore, there is ever-increasing impetus for the discovery of new agents directed against alternative sites in the viral life cycle.

IN has thus emerged as an attractive target for anti-HIV therapy, both because it is necessary for stable infection and because known functional analogues are lacking in the human host.[2] Integrase inserts a double-stranded DNA copy of the viral RNA genome into the chromosomes of an infected cell through two separate reactions; in the "3′-processing" step, IN removes two nucleotides 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. For the integration reaction, no source of energy (e.g. no ATP) is needed and only divalent cations such as Mn^{2+} or Mg^{2+} are required for the catalytic activity.

Although a wide variety of compounds have been reported as IN inhibitors,[3] drugs active against this enzyme have not as yet been approved by the FDA. One of the major leads in the development of anti-HIV-1 IN drugs is represented by β-diketo acids[4] (DKAs) and their derivatives[3] which have been shown to selectively inhibit the strand transfer step by sequestering the divalent cations bound in the active site of the enzyme,[5] and to block HIV-1 replication in infected cells.

Considering that the only two IN inhibitors so far in clinical trials,[3] Merck's L-870,810 and Shionogi/GlaxoSmithKline's S-1360 (Figure 1, **1** and **19**), belong to the β-diketo-like acid family, we have recently constructed a three-dimensional pharmacophore model for the binding of DKAs to the enzyme.[6] Using this model as a query for virtual screening, we have found several compounds that contain the specified 3D patterns of chemical functions. Biological testing showed that our strategy was successful in searching for new structural leads as HIV-1 IN inhibitors.[6]

We have now extended our investigation to the development of 3D QSAR models for the same family of IN inhibitors. In fact, even if several HIV IN pharmacophores have already been published,[7−12] to the best of our knowledge no attempts to build quantitative models for DKA-like strand-transfer-selective inhibitors have as yet been reported. In fact, the only two previously reported pharmacophores for DKA-like derivatives are "qualitative" hypotheses [6,12] (generated without the use of activity data), whereas we have now developed a more sophisticated "quantitative predictive" model, since the biological activities were taken into account.

On the basis of the best hypothesis, we designed and synthesized a novel potential class of β-diketo acid-containing inhibitors of IN, and the evaluation of the IN inhibitory activity confirmed the strength of our rational approach.

Results and Discussion

Continuing our work in this research field [6,13,14] and in an attempt to identify new and potent IN inhibitors, we tried to generate the simplest hypothesis that could correctly predict the activity of compounds belonging to the DKA class. The 3D QSAR hypotheses obtained should give important guidance for the design of novel HIV-1 integrase inhibitors.

Pharmacophore Hypothesis Generation and Rational Design of New DKA Analogues. The algorithm HypoGen[15] implemented in the Catalyst package[16] was used to derive automated SAR pharmacophore models, called "hypotheses", from a data set consisting of 33 molecules acting as IN strand-transfer-selective inhibitors and including the most representative DKAs and DKA-like derivatives reported in the literature.[3,5,17−20] Among them, 17 compounds were selected as the training set (TS), and the other 16 were used as the prediction set (PS). The structures and IN inhibitory activity values of TS (**1**-**17**) and PS molecules (**18**-**33**) are reported in Figure 1.

* To whom correspondence should be addressed. Phone: (++39) 090-6766464. Fax: (++39) 090-355613. E-mail: barreca@pharma.unime.it; sferro@pharma.unime.it.
† Università di Messina.
§ Katholieke Universiteit Leuven and IRC KULAK.

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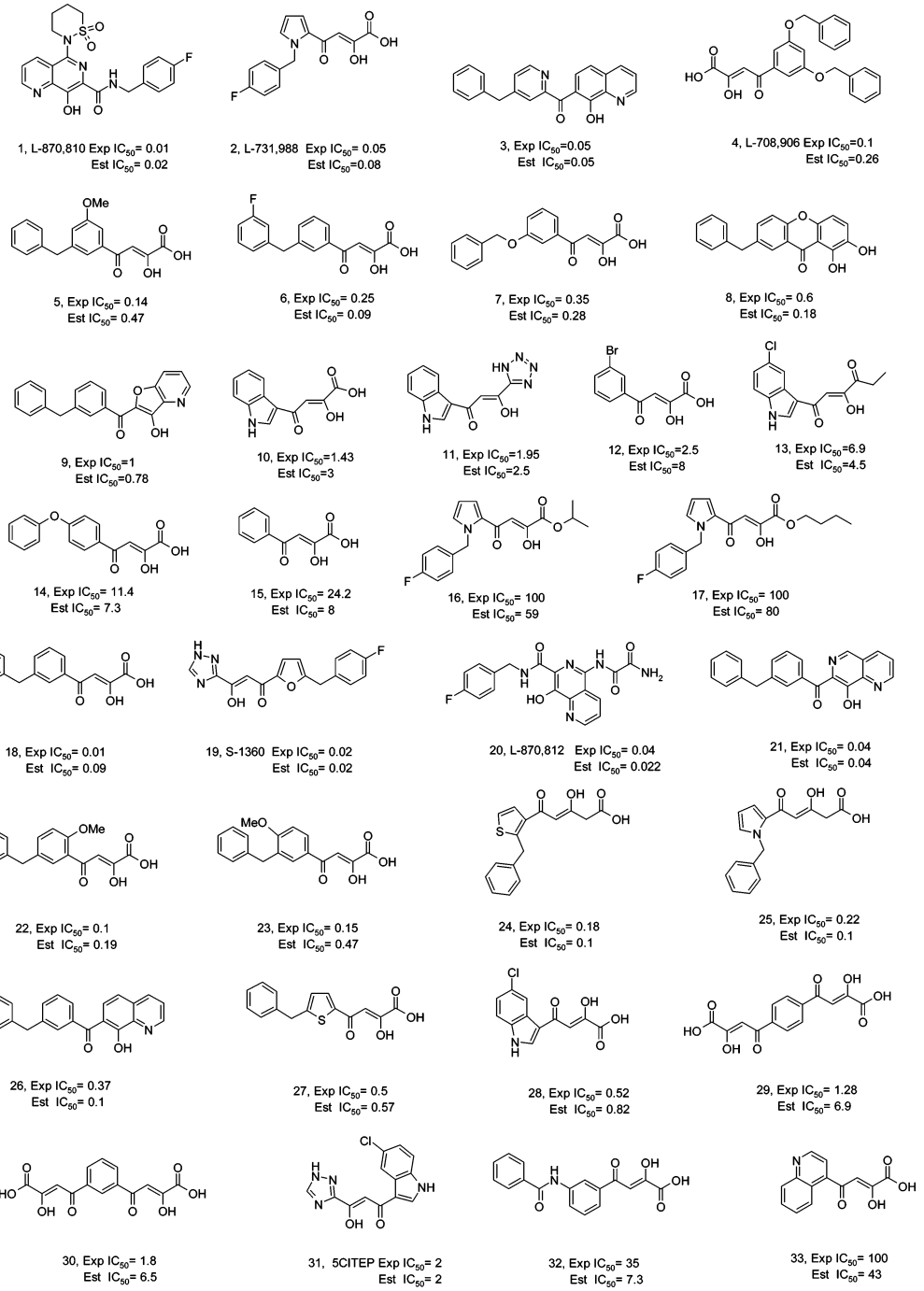


Figure 1. Chemical structures of the 33 data set molecules (TS, **1**−**17** and PS, **18**−**33**) with their experimental (exp IC_{50}) and estimated (est IC_{50}) IN strand transfer inhibitory activities, both expressed in μM.

The range of in vitro IN inhibitory activity, expressed as IC_{50} for strand transfer inhibition, spanned 5 orders of magnitude (0.01−100 μM), making this a good data set for HypoGen module.

It has been suggested that the DKAs act by coordinating the two magnesium ions required for IN activity; in particular, the carboxylic group (or its bioisosteric equivalent) interacts with one Mg^{2+}, while the ketoenol moiety binds the other one.[5]

In the pharmacophore-based investigation of DKA-like derivatives involving Catalyst, on the basis of the chemical features of the TS compounds and their proposed mechanism of action, the following chemical functions were selected in the feature dictionary: hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic (HY), hydrophobic aliphatic (HYAl), and hydrophobic aromatic (HYAr) groups. In fact, even if the metal-binding function is not included in the Catalyst feature dictionary, it can be replaced by a standard hydrogen-bond acceptor/donor function.[15]

Using the selected TS molecules, HypoGen algorithm constructed the 10 simplest hypotheses that showed the best correlation between estimated and measured activities, and the results of statistical significance and predictive ability are presented in Table 1.

The quality of the generated pharmacophore hypotheses was evaluated by considering the cost functions calculated by the HypoGen module during hypothesis generation.[15] The Fixed cost of the 10 top-scored hypotheses was 67.0 bits, well separated from the Null

Table 1. Summary of the HypoGen Runs for the DKA Dataset

Hypo[a]	total cost	cost diff[b]	RMS	correl. (*r*)	features[c]
Hypo1	79.382	89.897	0.819	0.959	HBA,HBA,HBD, HYAr
Hypo2	79.573	89.573	0.956	0.961	HBA,HBA,HBD, HYAr
Hypo3	85.041	84.238	1.008	0.936	HBA,HBA,HBD, HY
Hypo4	86.067	83.238	1.347	0.935	HBA,HBA,HBA,HYAr
Hypo5	87.036	82.243	1.455	0.923	HBA,HBA, HBA,HY
Hypo6	87.439	81.840	1.453	0.923	HBA,HBA, HBD,HY
Hypo7	91.453	77.826	1.541	0.914	HBA,HBA, HBA,HY
Hypo8	105.035	63.825	2.106	0.828	HBA, HBA,HYAr,HYAr
Hypo9	105.454	63.825	2.087	0.832	HBA,HBA,HBD, HYAr
Hypo10	109.082	60.197	2.225	0.805	HBA, HBA,HBA, HYAr

[a] Numbers for the hypothesis are consistent with the numeration as obtained by the hypothesis generation. [b] Difference between the Null hypothesis and the cost of each returned hypothesis. [c] HYar: hydrophobic aromatic; HY: hydrophobic; HBA: hydrogen bond acceptor. HBD: hydrogen bond donor.

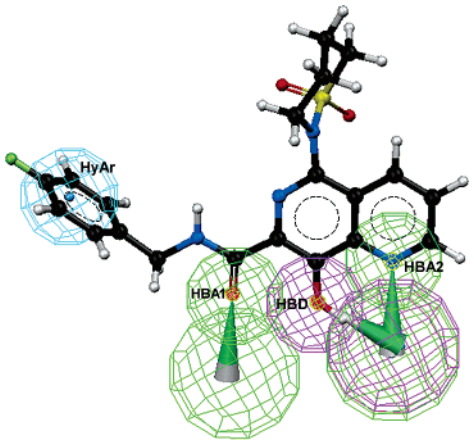


Figure 2. The top scoring HypoGen pharmacophore Hypo1 is mapped to the most active compound in the training set (**1**, L-870,810) (HBD, hydrogen bond donor; HBA, hydrogen bond acceptor; HYAr, hydrophobic aromatic region).

hypothesis cost score of 169.3 bits; the difference between these two theoretical parameters is higher than 70 (102.3) and the Configuration cost was less than 17 bits, thus suggesting a robust correlation.

The top ranked pharmacophore model (Hypo1) had the best predictive power and statistical significance and was characterized by the highest cost difference (89.897), the lowest rms (0.81947), and the best correlation coefficient (0.959). These values indicated a great predictability of the 3D-QSAR pharmacophore and confirmed that it did not come about by chance; Hypo1 was thus retained for further analysis.

The selected 3D hypothesis consisted of one hydrophobic aromatic region (HYAr), two hydrogen-bond acceptor (HBA1 and HBA2), and one hydrogen-bond donor (HBD) sites in a specific three-dimensional orientation. Using the most active molecule (**1**) of the TS, which is also one of the two IN-DKA-like inhibitors in clinical development, a flexible fit of this molecule to Hypo1 is shown in Figure 2.

Compound **1** and its analogues (**3**, **20**, and **21**) can be still considered DKA-like derivatives. In fact, it has been reported that 8-hydroxy-[1,6]-naphthyridine and 8-hydroxyquinoline were a suitable replacement for the 1,3-diketoacid motif, and the molecules obtained act analogously to diketo acids (that is, selective inhibition of strand transfer).[17] Considering the proposed mechanism of action for DKAs, the interaction between **1** and the metal ions in the IN active site was accounted for by our hypothesis of the mapping of the hydrogen bond sites described by the 7-carbonyl-8-hydroxy-[1,6]-naphthyridine moiety. The hydrophobic aromatic feature was instead located at the centroid of the benzyl ring. The lowest-cost hypothesis Hypo1 fitted the input data well (Figure 1 and Table 1 in the Supporting Information).

We also used Hypo1 to perform a regression analysis with the PS of 16 compounds (Figure 1, **18**−**33**) in order to check the predictive power of this model. Linear regression of the predicted activities for PS IN inhibitors versus the experimental ones gave a fairly good correlation coefficient of 0.85, confirming the validity of the most statistically significant HypoGen hypothesis in predicting the IN inhibitory activity of DKAs and DKA-like derivatives.

It is also worth noting that the type and number of features encoded in the automatically generated hypothesis were in full agreement with our previously manually developed ligand-based pharmacophore model.[6] However, the spatial location of HYAr differed a little in the two hypotheses. In particular, the HypoGen runs pointed out that to have compounds with very high IN inhibitory activity, it is necessary to have one hydrophobic feature well separated by the DKA motif; the distance between HYAr and HBA1, and HBD and HBA2, were found to be 6.20 ± 1 Å, 8.82 ± 1 Å and 10.91 ± 1 Å, respectively.

The most active compounds in the data set assumed a conformation that allowed proper mapping of the HYAr feature of the generated hypothesis, whereas most of the less active compounds (**10**−**15**, **29**−**33**) were unable to map this feature, as shown by the presence of an asterisk in the mapping column of the output file of Catalyst run (see Supporting Information).

Among the compounds that failed to map the HYAr feature, compound **10** drew our attention; the alignment of this IN inhibitor on Hypo1 (Figure 3A) clearly showed that its IN inhibitory potency (IC_{50} = 1.4) might be improved by further analogue synthesis.

In fact, since **10** mapped to only three of the four features of the lowest-cost Catalyst-generated DKA hypothesis, we thought that the introduction of functionality in this ligand, which might interact with the fourth feature of the hypothesis (i.e. HYAr), could provide enhanced IN inhibitory activity to the compound. This idea was also supported by comparison of the activity data of compound **15** (IC_{50} = 24.2) and its benzyl derivative **18** (IC_{50} = 0.01)**.** The designed *N*-benzyl derivative of compound **10** (compound **40**, Scheme 1**)** and the corresponding conformational models were thus edited within Catalyst as described in the Supporting Information. For the estimation of **40** we used Hypo1 and the BestEst option of the ViewHypothesis Workbench in Catalyst. The predicted IC_{50} value for **40** was 0.02 μM, and its mapping onto the top-ranked hypothesis is represented in Figure 3B.

The promising molecular modeling results prompted us to plan the synthesis of a series of new 1H-indole derivatives **34**−**45** (Scheme 1) bearing a benzyl or 4-fluorobenzyl substituent at N-1, seeing that an overview of the most active DKA analogues highlighted the presence of a fluorine atom on the benzyl moiety. Furthermore, a chlorine atom or a methoxy group was introduced on the benzene-fused ring of some of the newly designed benzylindole derivatives (i.e. **36**-**39**, **42**-

Table 2. Inhibition of Integration and Anti-HIV-1 Activity, Both Expressed as μM

compd	overall[a]	3′-P[a]	ST[a]	EC_{50}[b]	CC_{50}[c]	SI[d]
34	0.76 ± 0.10	>286.2	5.40 ± 4.16	14.30 ± 2.33	119.7 ± 66.1	8
35	1.20 ± 0.0	>272.20	1.50 ± 0.57	9.25 ± 3.81	27.2 ± 5.4	3
36	0.22 ± 0.04	10.19 ± 5.83	0.03 ± 0.01	4.50 ± 1.00	34.00 ± 6.78	8
37	0.39 ± 0.14	11.47 ± 2.5	0.07 ± 0.05	3.42 ± 2.23	26.53 ± 4.24	8
38	0.15 ± 0.05	162.5 ± 2.4	0.68 ± 0.42	6.07± 0.0	29.04 ± 0.0	5
39	0.22 ± 0.08	130.7 ± 0.0	0.80 ± 0.7	14.34 ± 7.14	127.45 ± 26.5	9
40	0.02 ± 0.0006	8.28 ± 2.21	0.03±0.0006	19.92 ± 15.25	>78	>4
41	0.002 ± 0.001	5.3 ± 1.25	0.015 ± 0.003	5.31 ± 0.88	>74	>14
42	0.010 ± 0.003	0.97 ± 0.18	0.10 ± 0.01	4.78 ± 0.98	37.24 ± 1.54	8
43	0.20 ± 0.08	1.59 ± 1.08	0.01 ± 0.001	2.78 ± 0.39	53.42 ± 6.69	19
44	0.017 ± 0.006	2.16 ± 0.68	0.021 ± 0.007	5.32 ± 0.24	86.38 ± 52.79	16
45	0.019 ± 0.008	2.71± 0.0	0.004 ± 0.001	5.81 ± 1.93	63.54 ± 39.53	11
1	0.0005 ± 0.0003	0.12 ± 0.03	0.0025 ± 0.0007	0.0047 ± 0.0007	2.15 ± 0.15	457

[a] Concentration required to inhibit by 50% the in vitro integrase activity assays as IC_{50} (μM). [b] Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells. [c] Cytotoxic concentration to reduce MT-4 cell viability by 50%. [d] Selectivity index: ratio CC_{50}/EC_{50}.

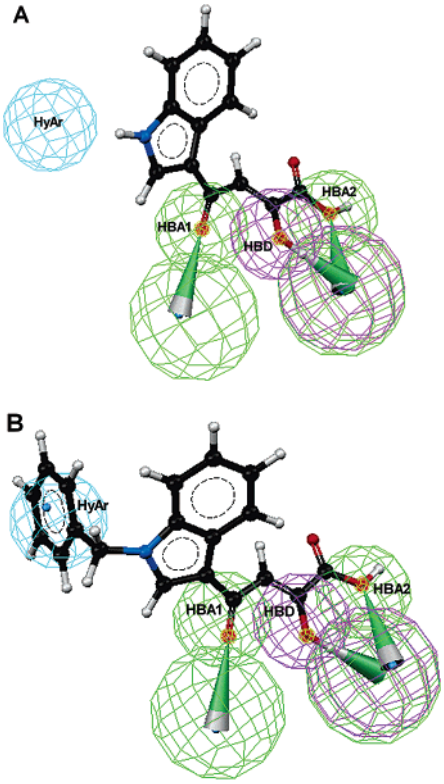


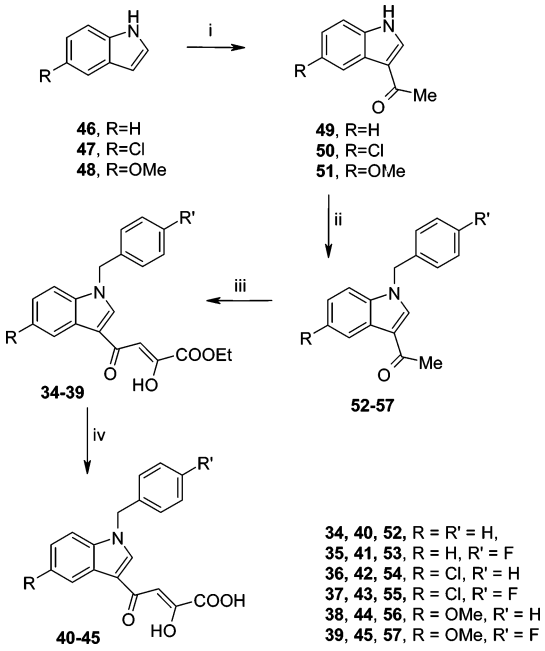
Figure 3. Compound **10** (A) and **40** (B) aligned on the lowest cost pharmacophore model generated for DKA-like strand transfer inhibitors (HBD, hydrogen bond donor; HBA, hydrogen bond acceptor; HYAr, hydrophobic aromatic region).

45) based on the observation that chloro-substituted compound **28** (IC_{50} = 0.52) was 2-fold more potent than **10** (IC_{50} = 1.4), and that a methoxy group was present in potent IN inhibitors such as **5**, **22**, and **23**.

Inhibition of HIV-1 IN Activity. All the synthesized compounds were tested in IN inhibition assays which have been recently reviewed.[22,23] The results showed that the diketo acid derivates were generally more potent than the corresponding esters and that most of the tested compounds showed 50−100 fold selectivity toward inhibition of strand transfer in comparison to 3′-processing (Table 2).

Our lead compound (**40**) had a strand stransfer inhibitory activity of 0.03 μM; the introduction of a chlorine atom on the indolic ring (**42**) led to a significant decrease of anti-IN activity, while the presence of a methoxy group in the same position (**44**) did not influence the potency.

Scheme 1[a]



[a] Reagents and conditions: (i) AcCl, Et_2AlCl, CH_2Cl_2, 0 °C, 2 h; (ii) benzyl or 4-fluorobenzyl bromide, NaH, DMF, 0 °C, 30 min; (iii) diethyl oxalate, dry C_2H_5ONa, THF, two separated steps under the same conditions: 50 °C, 2 min, 250 W, 300 psi; (iv) 2 N NaOH, MeOH, rt, 1.5 h.

The best activity was displayed when a *p*-fluorine atom was present on the benzyl moiety; in fact compound **41** was 2-fold more potent compared to the unsubstituted parent **40,** and fluoro derivatives **43** and **45** were the most active compounds of the series with IC_{50} = 0.01 μM and IC_{50} = 0.004 μM, respectively.

In particular the contemporary presence of a fluorine atom on the benzyl moiety and a methoxy group on the indole system increseased the IN inhibitor activity, and compound **45** showed potency comparable to that of L-870,810, one of the two IN inhibitors in clinical trials. The relevance of our modeling assumptions is worth noting as documented by the measured strand stransfer inhibitory activity of **40** (IC_{50} = 0.03 μM), which compared well with the predicted value of 0.02 μM.

The better inhibition of strand transfer activity by compound **40** with respect to that reported by Sechi et al.[21] (IC_{50} = 1 μM) is probably due to the fact that we determined strand transfer activity independent of DNA binding and 3′-processing.

In Vitro Anti-HIV Assay and Drug Susceptibility Assay. The antiviral activity of our compounds on the HIV-induced CPE in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT-assay.[24] All compounds proved to be effective inhibitors of HIV-1 replication at micromolar concentration (Table 2), without correlation between anti-HIV and anti-IN activities. This behavior might be due to the physical−chemical properties of our molecules. Extensive further modifications are in progress aiming at modifying the solubility and decreasing the toxicity in this series of IN strand-transfer-selective inhibitors.

Experimental Section

Chemistry. The synthesis of 1*H*-indole derivatives **34**−**45** was accomplished according to the reaction sequence reported in Scheme 1. The appropriate 1*H*-indole (**46**−**48**) was 3-acetylated by reaction with acetyl chloride using diethylaluminum chloride as catalyst and then N-alkylated by treatment with the suitable benzyl bromide in the presence of sodium hydride to give intermediates **52**−**57**. These derivatives were successively condensed with diethyl oxalate and a catalytic amount of sodium methoxide to give ethyl esters **34**−**39**. This reaction was performed under microwave irradiation: reaction times were strikingly reduced (i.e. 4 min), yields were almost quantitative, and transesterification that may occur in this synthetic route did not take place. Finally, esters **34**−**39** were converted by basic hydrolysis into the corresponding acids **40**−**45**.

After designing and synthesizing our benzyl derivatives (**34**−**45**) and while the present paper had still to be completed, Sechi et al. published the IN inhibitory activity of derivative **40**.[21] However, we used different synthetic conditions for obtaining this compound. In particular, in our work the synthesis of **40** was achieved with the support of microwave irradiation techniques, thus reaching a drastic reduction in reaction time, higher yields, and cleaner reactions.

Conclusion

This paper shows the generation of a quantitative model for DKA-like derivatives acting as inhibitors of HIV-1 IN. The statistically most significant HypoGen hypothesis consisted of four features (one hydrophobic aromatic region, two hydrogen-bond acceptors, and one hydrogen-bond donor) that enabled us to rationally design new DKAs containing a benzylindole skeleton. Synthesized molecules proved to be potent IN-inhibitors by blocking the strand transfer process.

These results suggested that our 3D QSAR model can be useful and predictive to identify new promising compounds. We are presently using the best HypoGen pharmacophore as a 3D query for the identification of novel potential IN inhibitors in large 3D databases of molecules.

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Note Added after ASAP Publication. Reference 21 in the version of the paper posted October 6, 2005, has been corrected in the new version posted October 18, 2005.

Supporting Information Available: Additional experimental data are available free of charge via Internet at http://pubs.acs.org

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