Efficient 3D Database Screening for Novel HIV-1 IN Inhibitors

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Received December 19, 2003

We describe the use of pharmacophore modeling as an efficient tool in the discovery of novel HIV-1 integrase (IN) inhibitors. A three-dimensional hypothetical model for the binding of diketo acid analogues to the enzyme was built by means of the Catalyst program. Using these models as a query for virtual screening, we found several compounds that contain the specified 3D patterns of chemical functions. Biological testing shows that our strategy was successful in searching for new structural leads as HIV-1 IN inhibitors.

INTRODUCTION

HIV-1 integrase (IN) is one of the three viral-encoded enzymes required for the replication of the virus. In recent years, IN has gained much attention as a potential drug target[1,2] mainly due to the emergence of drug-resistant strains that are becoming difficult to treat with currently available therapeutic agents that inhibit the other two enzymes: the reverse transcriptase and the protease. Considering that there is no known cellular counterpart of IN in human cells and that IN inhibitors used in a cocktail regimen with current drugs will help to reduce the chance for HIV to develop drug resistance, there is considerable interest in discovering effective and selective inhibitors of the HIV integration process.

The HIV-1 IN enzyme catalyzes the insertion of proviral DNA into the host genome through two separate reactions, called "3′-processing" and "strand transfer".[3] In vitro, integrase can also carry out an apparent reversal of the strand transfer reaction, termed "disintegration".[4] Moreover, it has long been established that divalent metal cations are essential for both steps.[5−8]

However, the HIV-1 IN crystal structures available to date show a single binding site for Mg^{2+} or Mn^{2+};[9−12] on the other hand, the structures of the avian sarcoma virus (ASV) IN catalytic domain contain one or two cofactors bound to the active site.[13]

Although HIV integrase is an attractive target for antiviral therapy and a wide variety of compounds have been reported as IN inhibitors,[2,14−18] no drug active against this enzyme has as yet been approved by the U.S. FDA. Promising anti-integrase drugs are being developed with two compounds in early clinical trials: Shionogi/GlaxoSmithKline S-1360[19] in Phase II and Merck's L-870,810[20] in Phase I (Figure 1).

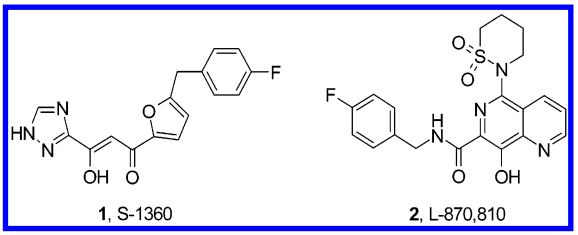

Figure 1. Two integrase inhibitors currently in human studies.

Unfortunately, receptor-based design of novel HIV-1 IN inhibitors is currently hampered by the fact that the complete three-dimensional bioactive structure of HIV-1 integrase is still unknown. Furthermore, although to date two X-ray crystal structures of an IN−ligand complex are available,[11,21] these studies were accomplished with the catalytic domain alone and not with the full-length enzyme and the substrate DNA. Due to this lack of complete structural information, it is necessary to focus attention on alternative strategies for discovering new HIV-1 IN inhibitors.

Considering that S-1360 and L-870,810 belong to the only class of compounds, named β-diketo acid (DKA) analogues[15,22−29] (Figure 2), which have been introduced into clinical studies, we first built a 3D model for the binding of this series of HIV-1 IN inhibitors to the enzyme, and later we used the putative model as a query to screen 3D databases, providing candidates for use in IN assays and thus saving efforts for chemical synthesis. Biological results of some identified compounds are also discussed in this report.

MATERIALS AND METHODS

Generation of the Hypothetical Model. A 3D pharmacophore model was constructed using as a starting point the crystallographic structure of 5CITEP (**3**, Figure 2) and the proposed mechanism of action for DKA analogues.[30] The compound 5CITEP was extracted from the Brookhaven Protein Data Bank file 1QS4[11] and imported into the Catalyst 4.7 software package.[31] On the basis of the information provided by a recently reported 2D-model for the interaction

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10.1021/ci034296e CCC: $27.50
Published on Web 05/15/2004

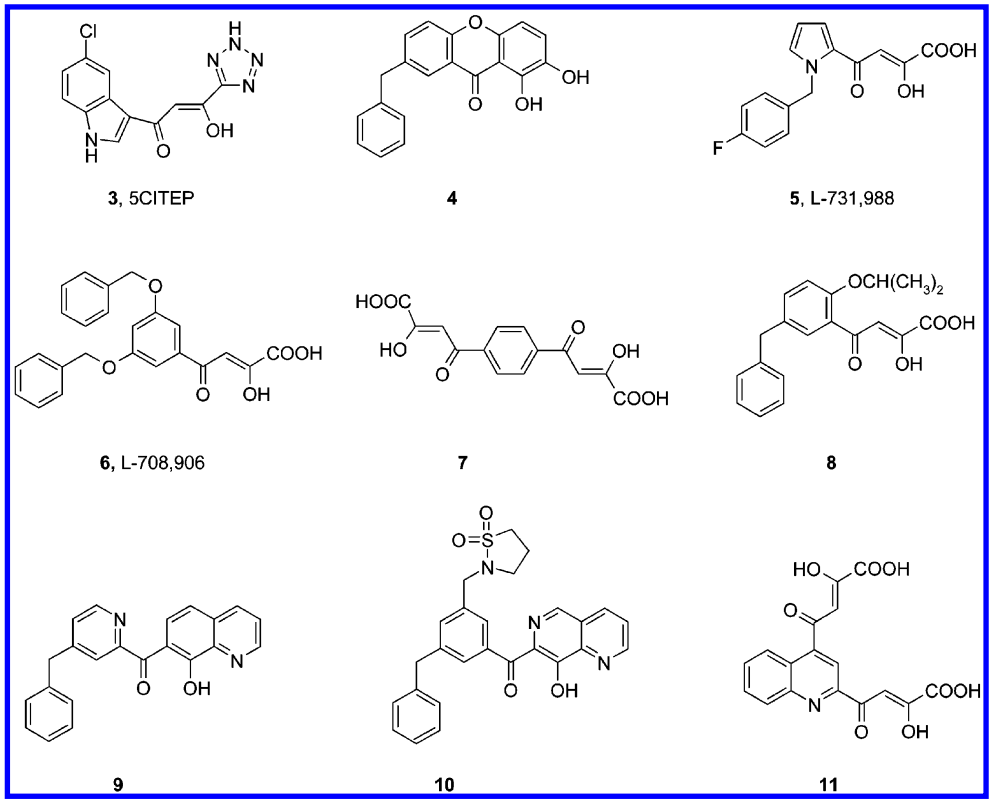

Figure 2. Chemical structures of the most representative DKA analogue integrase inhibitors.

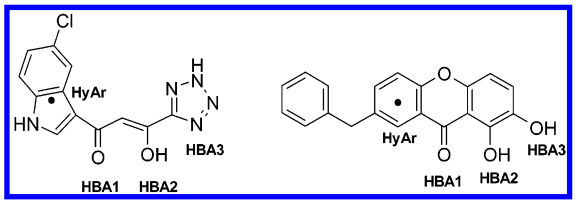

Figure 3. Mapping of chemical functions over 5CITEP (left) and compound **4** (right).

Table 1. Calculated and Specified Distance in 5CITEP, **4**, and Our Pharmacophore Model

	distance (Å)			
molecules	HBA1-HBA2	HBA2-HBA3	HBA1-HBA3	HBA1-HyAr
3, 5CITEP	2.70	2.99	5.38	3.71
4	2.79	2.70	5.25	3.69
3D model	2.5−3.0	2.5−3.2	5.0−5.6	3.5−4.2

of this class of inhibitors with IN,[30] the 5CITEP bioactive conformer was used as a template to place functions with the same spatial relationships as the chemical groups in our reference compound.

A four-point 3D model was thus created by assembling three hydrogen-bond acceptors, mapped over the ketoenol moiety (HBA1 and HBA2) and the position N1 of the tetrazole ring (HBA3), and one hydrophobic−aromatic group (HyAr), mapped over the indole centroid. To restrict the 3D spatial arrangement for the next database search, we added several distance constraints among HBAs−HBAs and HBAs−HyAr functions, according to the corresponding distances calculated in compound **3** and in another DKA analogue, **4** (Figure 3 and Table 1).

While 5CITEP is a perfect point of reference for distances since we obtained its structure from crystallographic data, molecule **4** was chosen among the most potent DKA analogue IN inhibitors because the 1,3-diketo acid moiety is incorporated in a highly rigid system,[29] thus providing useful and unambiguous geometric information. The latter compound was constructed using standard bond lengths and angles from Sybyl[32] fragment library and fully optimized by the semiempirical quantum mechanical method AM1.

In the distance measurement operation, the heavy atoms of the hydrogen-bond acceptors (i.e., O and N) were selected, whereas for the distance HBA1-HyAr, we defined ring centroids, i.e., the centroid of indole (5CITEP) and fused benzene (compound **4**) rings. Finally, we imposed location constraints (i.e., the volume in which the functions can reside) specifying the radius of the spheres according to the default values suggested by Catalyst. A prediction set was later used in order to validate the reliability of the postulated 3D pharmacophore hypothesis; this included the only two integrase inhibitors currently in human studies, S-1360 and L-870,810 (Figure 1), as well as eight molecules among the most representative DKA-based agents so far reported (Figure 2, **4**−**11**).[22,24,28,29]

The 10 compounds of the prediction set were generated using the 2D/3D editor sketcher in the Catalyst program and energy minimized to the closest local minimum using a molecular mechanics approach. To build conformational models of up to 250 conformers for each molecule, the best conformer generation option and a 10 kcal/mol energy cutoff were chosen.

Finally, a 3D query of the CAP2002 database using the putative four-point pharmacophore model was accomplished by means of Catalyst. The CAP2002 3D coordinate database was supplied from Accelerys, Inc. with the Catalyst software.

Substrate and Target DNA Used in the Enzymatic Integrase Assay. The following high-performance liquid chromatography-purified deoxyoligonucleotides, correspond-

ing to the U5 end of the HIV-1 LTR, were purchased from Amersham Biosciences (Piscataway, NJ): INT1 (5′-TGT GGA AAA TCT CTA GCA GT), INT2 (5′-ACT GCT AGA GAT TTT CCA CA). The oligonucleotide INT1 was purified through a 20% denaturing polyacrylamide/urea gel and was 5′-end labeled using polynucleotide T4 kinase and [γ-^{32}P]-ATP (Amersham Biosciences). The DNA substrate for the IN reactions was made by annealing INT1 and INT2. An equimolar mixture of the two oligonucleotides in the presence of 100 mM NaCl was heated shortly at 95 °C and allowed to cool slowly to room temperature. Likewise, annealing of SK70 and T35 resulted in a 35-bp dsDNA molecule that was used as a target DNA molecule (T35/SK70).

Overall Integration Assays. The enzymatic integration reactions were carried out as described previously with minor modifications.[33,34] The final reaction mixture for the overall integration assay contained 20 mM HEPES, pH 7.5, 5 mM dithiothreitol (DTT), 10 mM $MgCl_2$, 75 mM NaCl, 15% (v/v) poly(ethylene glycol) 8000, 15% DMSO, 20 nM of the oligonucleotide substrate, and 0.7 μM of the His-tag IN (10 μL as final volume). Reactions were started by the addition of the enzyme. To determine the susceptibility to different inhibitors, compounds were incubated shortly with the reaction components before the addition of IN. Reactions were allowed to proceed at 37 °C for 60 min and stopped by the addition of formamide loading buffer (95% formamide, 30 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue, 0.1% SDS). Subsequently, products were separated in a 15% denaturing polyacrylamide/urea gel. Quantification of the results was performed using the PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

RESULTS AND DISCUSSION

HIV-1 IN plays a key role in stable and productive viral infection of cells. To date, DKA analogues represent the major leads in the development of anti-HIV-1 integrase drugs, seeing that the only two IN inhibitors undergoing clinical trials belong to this family.

For the above reasons and as a continuation of our work in this research field,[35,36] we decided to build a 3D pharmacophore model representing the distinguishing chemical features of this class of compounds to obtain a useful tool for further discovery of novel IN inhibitors.

The following considerations help to explain how we built our hypothetical model.

First, even if this family of IN inhibitors is commonly named "diketo" derivatives, ^{1}H NMR data are in agreement with a more stable "ketoenol" structure, and so we decided to consider the molecule in this tautomeric form.

Second, 5CITEP (**3,** Figure 2), the only DKA analogue inhibitor of HIV-1 IN for which the biologically relevant conformation is available from experimental data (both X-ray crystallography[11] and molecular dynamics simulations[36]), belongs to the DKA family since the tetrazole ring can be considered as an isostere of the carboxylic acid group.[37]

Third, both biochemical and structural studies put forward a plausible two-metal model for the catalytic center of IN;[13,25,38−40] in particular, Beese and Steitz proposed that, by analogy to the 3′-5′ exonuclease of *Escherichia coli* DNA polymerase I, three conserved acidic residues in the enzyme active site coordinate two divalent cations, forming a template for DNA binding and catalysis.[40]

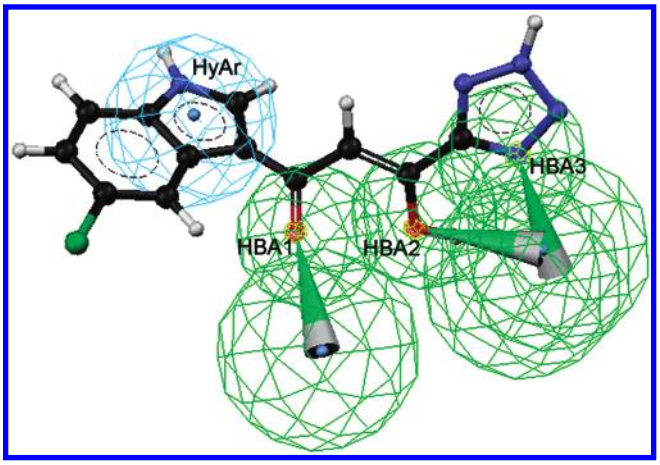

Figure 4. Four-point hypothetical model for DKA analogue IN inhibitors built using the Catalyst program (HBA, green, hydrogen-bond acceptor feature; HyAr, cyan, hydrophobic aromatic function). The vectors represent the putative interactions between the DKA analogues and the metal ions. The structure of 5CITEP is superimposed on the model.

Fourth, it has been suggested that the DKA analogues act by sequestering the metal ions that are required for IN activity; in particular, the carboxylic acid function or its isosteric replacement interacts with one Mg^{2+} at site I, while the ketoenol moiety binds the other Mg^{2+} at site II. [25,30]

In view of the above information and using the 5CITEP atomic coordinates available from X-ray crystallography data, we developed a 3D model (Figure 4) which was consistent with the proposed mechanism of action for this family of IN inhibitors (see the Materials and Methods section for a detailed description). In fact, the interaction between the ligand 5CITEP and the metal ions in the IN active site is accounted for by our hypothesis of the mapping of HBA sites described by the ketoenol functionality and the N-1 atom of the tetrazole moiety.

Moreover, a hydrophobic aromatic region (HyAr) is positioned over the indole ring. It has been repeatedly reported that an aromatic moiety seems to be an essential structural element for anti-IN activity, because it might interact at the catalytic center with the side chain of Tyr143 through "$\pi-\pi$" interactions.[25] In addition, this functionality might also be involved in Coulombic-like interactions (i.e., "cation$-\pi$"[41]) with the positively charged ions in the active site.

Despite the importance of this functionality, this is the first time that the aromatic feature is explicitly included in a three-dimensional pharmacophore model of IN inhibitors to be used in database searching; the earlier models for HIV-1 IN inhibition published to date consist only of hydrogen-bond acceptor/donor sites as also other atoms that could form complexes with the metal cofactors in the active site of the enzyme.[41−46] Furthermore, to the best of our knowledge, no attempts to build a 3D ligand-based pharmacophore model of IN inhibitors using Catalyst has been yet reported.

As reported in the Materials and Methods section, in building our hypothesis we also specified the geometric interrelationships among the chemical functions (Table 1), which are located within reasonable distances of each other as appropriate for DKA-containing IN inhibitors. The reli-

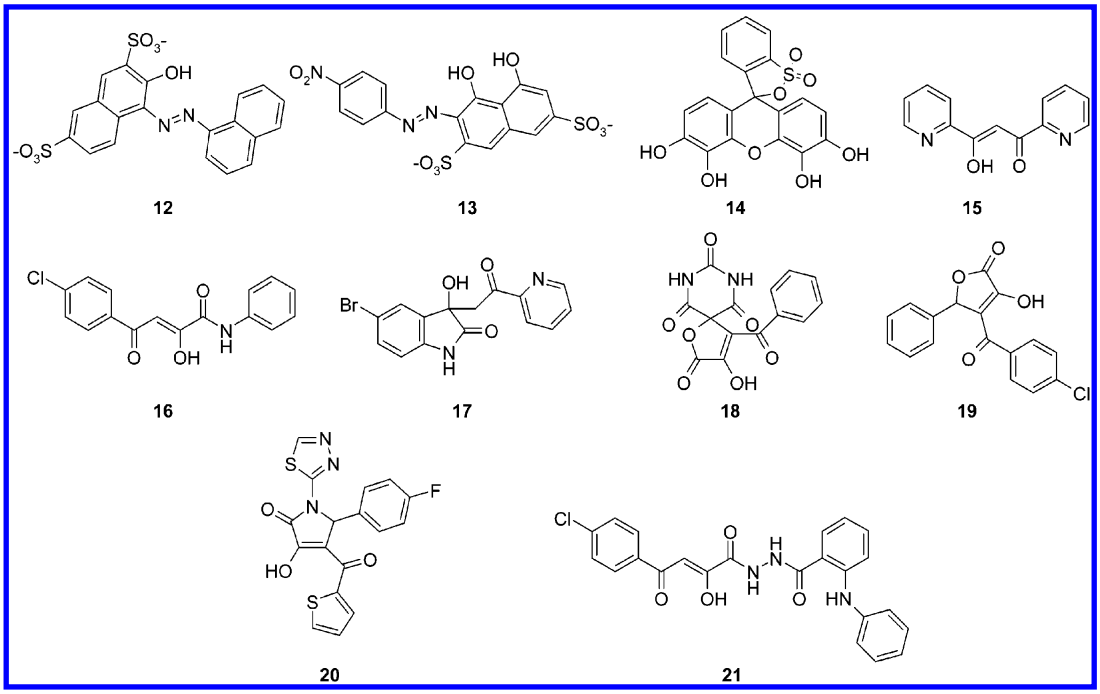

Figure 5. Ten compounds retrieved from the CAP2002 database and assayed for IN inhibition.

ability of the postulated pharmacophore model was later validated by mapping highly active DKA derivatives (Figures 1 and 2; **1, 2, 4**−**11**).

While it is evident that **5**−**8** and **11** are diketo acids, the other molecules are considered DKA analogues on the basis of the following observations. Compound **1,** S-1360 is a DKA-based derivative since the triazole moiety can be considered an isosteric replacement of the tetrazole ring. Furthermore, the rational design of molecules **2**, **4**, **9**, and **10** as novel diketo acid equivalents has recently been reported.[29]

All compounds of the prediction set were found to fit the pattern of the four structural features in at least one conformation. The different alignments for the 10 molecules are provided as Supporting Information. This observation suggests that this hypothetical model, representing the 3D collection of functional groups in a molecule in order to obtain IN inhibition, might be a useful tool for the discovery of potential DKA-based lead compounds.

Virtual Screening. The putative pharmacophore model was used as a search query to identify structural templates from 3D small molecule databases. It is worth noting that some geometric relationships between chemical features were fixed in order to make the model more restrictive and consequently the search query more specific. In fact, by preserving the metal-coordinating features with distance restraints, we made the reasonable assumption that only molecules that shared a metal coordination pattern similar to that of 5CITEP (i.e., of our pharmacophore) could be retrieved from the database. The Fast Flexible search routine in Catalyst was thus selected to screen the commercial Catalyst/CAP2002, which is a collection of compounds from chemical suppliers, and returned about 4000 compounds that contained, in some conformation, the specified 3D location of chemical functions.

A subset of these structures was then chosen by removing compounds that did not satisfy the well-known Lipinski rules describing properties of drug-like compounds.[47] The remaining molecules were overlaid with the pharmacophore by using the Best Fit option, and the top 100 hits were visually reviewed.

Table 2. Inhibition of HIV-1 Integrase Activity and Fit Values

compound	overall integration IC_{50} (μM)[a]	fit value
12	83.6	3.05
13	97	3.12
14	2.8	3.68
15	61.4	2.34
16	1.9	3.85
17	>100	2.08
18	>100	3.14
19	0.9	3.80
20	>100	2.97
21	21.2	3.60

[a] Concentration of inhibitor that inhibits the overall integration in the oligonucleotide-based assay by 50%. Median values of three separate experiments are shown.

Not surprisingly, most compounds within this set were structurally similar to the known DKA analogue HIV-1 IN inhibitors. However, the database search also revealed several novel and structurally unrelated potential IN inhibitors. Finally, a total of 10 compounds (**12**−**21**, Figure 5) were selected for assaying in a stepwise fashion on the basis of (1) their fit value (which measures how closely the functional groups in the hits match the hypothesis features), (2) log *P*, (3) chemical diversity, and (4) availability and cost. The biological results, namely, the inhibition of HIV-1 integrase activity in an enzymatic assay, suggest that our approach might be effective in identifying possible IN inhibitor lead candidates for further development (Table 2).

In fact, out of the 10 compounds tested, seven inhibited the HIV-1 integrase enzymatic activity. Of these, three (**14**, **16**, and **19)** showed potency below 10 μM; in particular, compounds **16** and **19** were the most active IN inhibitors with a 50% inhibitory concentration (IC_{50}) in the overall integration assay of 1.9 and 0.9 μM, respectively. The

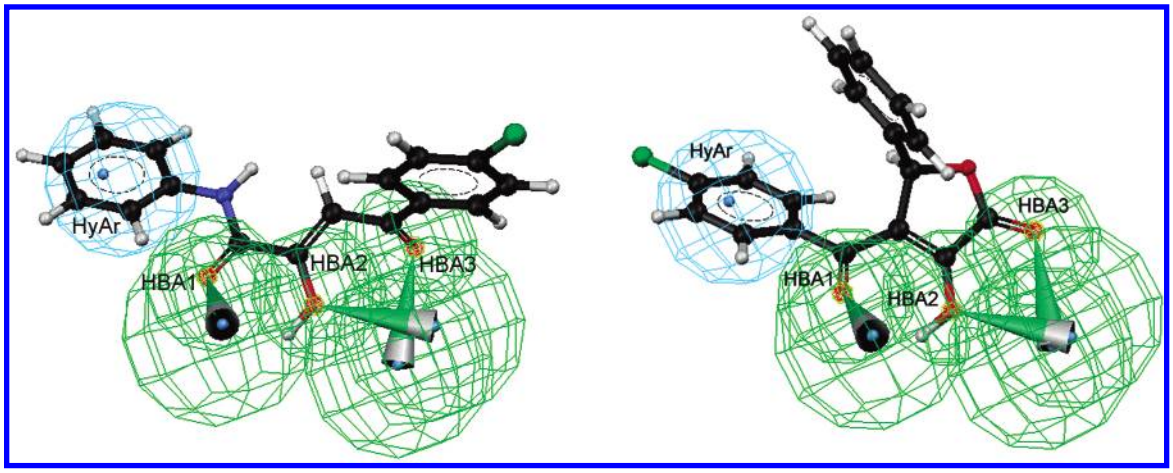

Figure 6. Compounds **16** (left) and **19** (right) mapped to the proposed pharmacophore model for HIV-1 IN inhibition (HBA, green, hydrogen-bond acceptor; HyAr, cyan, hydrophobic aromatic).

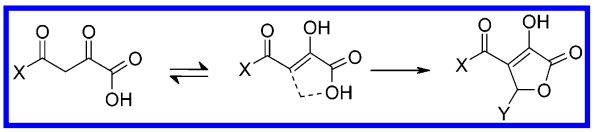

Figure 7. Structural analogy between the diketo acid motif and the 4-keto-3-hydroxy-furan-2-one system.

superimposition of the 10 molecules against the hypothetical hypothesis revealed that the four features of the pharmacophore were well matched by the chemical groups of the molecules. As an example, Figure 6 illustrates the alignment of compounds **16** and **19** onto the plausible 3D pharmacophore model for DKA analogues.

In particular, the most potent IN inhibitor **19,** 4-(4-chlorobenzoyl)-3-hydroxy-5-phenyl-5*H*-furan-2-one, belongs to a class of compounds which is different from previously described DKA analogue IN inhibitors. However, **19** interestingly incorporates the 1,3-diketo acid motif into a 4-keto-3-hydroxy-furan-2-one ring (Figure 7); in fact, as already pointed out, the 1,3-diketo acid moiety enolizes at the α-position, so this molecule can be considered a "closed-form" of the above-mentioned chemical functionality and a new bioactive scaffold for further optimization. These encouraging biological results give hopes that suitable chemical modifications and future structure−activity studies of the retrieved molecules could lead to new compounds with improved inhibitory activity toward HIV-1 IN enzyme.

CONCLUSION

Supporting the hypothesis that promising molecules discovered as HIV-1 IN inhibitors (i.e., DKA analogues, act by chelating the divalent metal in the IN active site), we have developed a three-dimensional pharmacophore model for this series of compounds. The model, consisting of three hydrogen-bond acceptor groups and one hydrophobic aromatic function, was created by means of Catalyst and later used as a 3D query to search the CAP2002 database.

Among the identified molecules which met the specified spatial arrangements of chemical features, 10 were selected for determination of their inhibitory effects against HIV-1 IN activity. The biological results suggest that this modeling approach is reliable and might be a useful procedure to identify inhibitor leads for further development.

ACKNOWLEDGMENT

These investigations were supported by the Ministero dell'Istruzione, dell'Università e della Ricerca (COFIN 2002, Rome, Italy), the Stimulating Flemish Participation in EU Research Programs (Verkennende Internationale Samenwerking-VISA6895, Vlaamse Gemeenschap), and the United States National Institutes of Health (J.M.B.). B.V.M. is funded by a grant from the Flemish Institute supporting Sience-Technological Research in Industry (IWT). We are grateful to Martine Michiels for excellent technical assistance.

Supporting Information Available: The alignment of the 10 molecules of the prediction set onto the pharmacophore model. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

(1) Nair, V. HIV integrase as a target for antiviral chemotherapy. *Rev. Med. Virol.* **2002**, *12*, 179−193.
(2) Gupta, S. P.; Nagappa, A. Design and development of integrase inhibitors as anti-HIV agents. *Curr. Med. Chem.* **2003**, *10*, 1779−1794.
(3) Engelman, A.; Mizuuchi, K.; Craigie, R. HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. *Cell* **1991**, *67*, 1211−1221.
(4) Chow, S. A.; Vincent, K. A.; Ellison, V.; Brown, P. O. Reversal of integration and DNA splicing mediated by integrase of human immunodeficiency virus. *Science* **1992**, *255*, 723−726.
(5) Hazuda, D. J.; Felock, P. J.; Hastings, J. C.; Pramanik, B.; Wolfe, A. L. Differential divalent cation requirements uncouple the assembly and catalytic reactions of human immunodeficiency virus type 1 integrase. *J. Virol.* **1997**, *71*, 7005−7011.
(6) Wolfe, A. L.; Felock, P. J.; Hastings, J. C.; Blau, C. U.; Hazuda, D. J. The role of manganese in promoting multimerization and assembly of human immunodeficiency virus type 1 integrase as a catalytically active complex on immobilized long terminal repeat substrates. *J. Virol.* **1996**, *70*, 1424−1432.
(7) Ellison, V.; Brown, P. O. A Stable Complex between Integrase and Viral-DNA Ends Mediates Human-Immunodeficiency-Virus Integration in-Vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7316−7320.
(8) Mazumder, A.; Neamati, N.; Pilon, A. A.; Sunder, S.; Pommier, Y. Chemical trapping of ternary complexes of human immunodeficiency virus type I integrase, divalent metal, and DNA substrates containing an abasic site − Implications for the role of lysine 136 in DNA binding. *J. Biol. Chem.* **1996**, *271*, 27330−27338.
(9) Bujacz, G.; Jaskoski, M.; Alexandratos, J.; Wlodawer, A.; Merkel, G.; et al. High-Resolution Structure of the Catalytic Domain of Avian-Sarcoma Virus Integrase. *J. Mol. Biol.* **1995**, *253*, 333−346.
(10) Goldgur, Y.; Dyda, F.; Hickman, A. B.; Jenkins, T. M.; Craigie, R.; et al. Three new structures of the core domain of HIV-1 integrase: an active site that binds magnesium. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9150−9154.
(11) Goldgur, Y.; Craigie, R.; Cohen, G. H.; Fujiwara, T.; Yoshinaga, T.; et al. Structure of the HIV-1 integrase catalytic domain complexed

with an inhibitor: a platform for antiviral drug design. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13040−13043.
(12) Wlodawer, A. Crystal structures of catalytic core domains of retroviral integrases and role of divalent cations in enzymatic activity. *Adv. Virus Res.* **1999**, *52*, 335−350.
(13) Bujacz, G.; Alexandratos, J.; Wlodawer, A.; Merkel, G.; Andrake, M.; et al. Binding of different divalent cations to the active site of avian sarcoma virus integrase and their effects on enzymatic activity. *J. Biol. Chem.* **1997**, *272*, 18161−18168.
(14) Neamati, N. Patented small molecule inhibitors of HIV-1 integrase: a 10-year saga. *Expert Opin. Ther. Pat.* **2002**, *12*, 709−724.
(15) Young, S. D. Inhibition of HIV-1 integrase by small molecules: the potential for a new class of AIDS chemotherapeutics. *Curr. Opin. Drug Discov. Devel.* **2001**, *4*, 402−410.
(16) Pommier, Y.; Marchand, C.; Neamati, N. Retroviral integrase inhibitors year 2000: update and perspectives. *Antiviral Res.* **2000**, *47*, 139−148.
(17) Chen, I. J.; Neamati, N.; MacKerell, A. D., Jr. Structure-based inhibitor design targeting HIV-1 integrase. *Curr. Drug Targets Infect. Disord.* **2002**, *2*, 217−234.
(18) Pannecouque, C.; Pluymers, W.; Van Maele, B.; Tetz, V.; Cherepanov, P.; et al. New class of HIV integrase inhibitors that block viral replication in cell culture. *Curr. Biol.* **2002**, *12*, 1169−1177.
(19) Billich, A. S-1360 Shionogi-GlaxoSmithKline. *Curr. Opin. Invest. Drugs* **2003**, *4*, 206−209.
(20) Pais, G. C.; Burke, T. R., Jr. Novel aryl diketo-containing inhibitors of HIV-1 integrase. *Drugs Future* **2002**, *27*, 1101−1111.
(21) Molteni, V.; Greenwald, J.; Rhodes, D.; Hwang, Y.; Kwiatkowski, W.; et al. Identification of a small-molecule binding site at the dimer interface of the HIV integrase catalytic domain. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2001**, *57*, 536−544.
(22) Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; et al. Inhibitors of strand transfer that prevent integration and inhibit HIV− 1 replication in cells. *Science* **2000**, *287*, 646−650.
(23) Neamati, N. Structure-based HIV-1 integrase inhibitor design: a future perspective. *Expert Opin. Invest. Drugs* **2001**, *10*, 281−296.
(24) Wai, J. S.; Egbertson, M. S.; Payne, L. S.; Fisher, T. E.; Embrey, M. W.; et al. 4-Aryl-2,4-dioxobutanoic acid inhibitors of HIV-1 integrase and viral replication in cells. *J. Med. Chem.* **2000**, *43*, 4923−4926.
(25) Pais, G. C.; Zhang, X.; Marchand, C.; Neamati, N.; Cowansage, K.; et al. Structure activity of 3-aryl-1,3-diketo-containing compounds as HIV-1 integrase inhibitors. *J. Med. Chem.* **2002**, *45*, 3184−3194.
(26) Vandegraaff, N.; Kumar, R.; Hocking, H.; Burke, T. R., Jr.; Mills, J.; et al. Specific inhibition of human immunodeficiency virus type 1 (HIV-1) integration in cell culture: putative inhibitors of HIV-1 integrase. *Antimicrob. Agents Chemother.* **2001**, *45*, 2510−2516.
(27) Pluymers, W.; Pais, G.; Van Maele, B.; Pannecouque, C.; Fikkert, V.; et al. Inhibition of human immunodeficiency virus type 1 integration by diketo derivatives. *Antimicrob. Agents Chemother.* **2002**, *46*, 3292−3297.
(28) Dayam, R.; Neamati, N. Small-molecule HIV-1 integrase inhibitors: the 2001−2002 update. *Curr. Pharm. Des.* **2003**, *9*, 1789−1802.
(29) Zhuang, L.; Wai, J. S.; Embrey, M. W.; Fisher, T. E.; Egbertson, M. S.; et al. Design and synthesis of 8-hydroxy-[1,6]naphthyridines as novel inhibitors of HIV-1 integrase in vitro and in infected cells. *J. Med. Chem.* **2003**, *46*, 453−456.
(30) Grobler, J. A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; et al. Diketo acid inhibitor mechanism and HIV-1 integrase: implications for metal binding in the active site of phosphotransferase enzymes. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6661−6666.
(31) *Catalyst 4.7*; Accelrys Inc.: San Diego, CA, 2002.
(32) *Sybyl 6.9*; Tripos Associate Inc.: St. Louis, MO, 2003.
(33) Cherepanov, P.; Este, J. A.; Rando, R. F.; Ojwang, J. O.; Reekmans, G.; et al. Mode of interaction of G-quartets with the integrase of human immunodeficiency virus type 1. *Mol. Pharmacol.* **1997**, *52*, 771−780.
(34) Debyser, Z.; Cherepanov, P.; Van Maele, B.; De Clercq, E.; Witvrouw, M. In search of authentic inhibitors of HIV-1 integration. *Antivir. Chem. Chemother.* **2002**, *13*, 1−15.
(35) De Luca, L.; Pedretti, A.; Vistoli, G.; Barreca, M. L.; Villa, L.; et al. Analysis of the full-length integrase-DNA complex by a modified approach for DNA docking. *Biochem. Biophys. Res. Commun.* **2003**, *310*, 1083−1088.
(36) Barreca, M. L.; Lee, K. W.; Chimirri, A.; Briggs, J. M. Molecular dynamics studies of the wild-type and double mutant HIV-1 integrase complexed with the 5CITEP inhibitor: mechanism for inhibition and drug resistance. *Biophys. J.* **2003**, *84*, 1450−1463.
(37) Marchand, C.; Zhang, X.; Pais, G. C.; Cowansage, K.; Neamati, N.: et al. Structural determinants for HIV-1 integrase inhibition by beta-diketo acids. *J. Biol. Chem.* **2002**, *277*, 12596−12603.
(38) Lins, R. D.; Straatsma, T. P.; Briggs, J. M. Similarities in the HIV-1 and ASV integrase active sites upon metal cofactor binding. *Biopolymers* **2000**, *53*, 308−315.
(39) Brautigam, C. A.; Steitz, T. A. Structural and functional insights provided by crystal structures of DNA polymerases and their substrate complexes. *Curr. Opin. Struct. Biol.* **1998**, *8*, 54−63.
(40) Beese, L. S.; Steitz, T. A. Structural basis for the 3′-5′ exonuclease activity of *Escherichia coli* DNA polymerase I: a two metal ion mechanism. *EMBO J.* **1991**, *10*, 25−33.
(41) Nicklaus, M. C.; Neamati, N.; Hong, H.; Mazumder, A.; Sunder, S.: et al. HIV-1 integrase pharmacophore: discovery of inhibitors through three-dimensional database searching. *J. Med. Chem.* **1997**, *40*, 920−929.
(42) Neamati, N.; Hong, H.; Sunder, S.; Milne, G. W.; Pommier, Y. Potent inhibitors of human immunodeficiency virus type 1 integrase: identification of a novel four-point pharmacophore and tetracyclines as novel inhibitors. *Mol. Pharmacol.* **1997**, *52*, 1041−1055.
(43) Neamati, N.; Hong, H.; Mazumder, A.; Wang, S.; Sunder, S.; et al. Depsides and depsidones as inhibitors of HIV-1 integrase: discovery of novel inhibitors through 3D database searching. *J. Med. Chem.* **1997**, *40*, 942−951.
(44) Hong, H.; Neamati, N.; Wang, S.; Nicklaus, M. C.; Mazumder, A.; et al. Discovery of HIV-1 integrase inhibitors by pharmacophore searching. *J. Med. Chem.* **1997**, *40*, 930−936.
(45) Hong, H.; Neamati, N.; Winslow, H. E.; Christensen, J. L.; Orr, A.; et al. Identification of HIV-1 integrase inhibitors based on a four-point pharmacophore. *Antiviral Chem. Chemother.* **1998**, *9*, 461−472.
(46) Carlson, H. A.; Masukawa, K. M.; Rubins, K.; Bushman, F. D.; Jorgensen, W. L.; et al. Developing a dynamic pharmacophore model for HIV-1 integrase. *J. Med. Chem.* **2000**, *43*, 2100−2114.
(47) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3−25.

CI034296E