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Combinatorial Diversification of Indinavir: In Vivo Mixture Dosing of an HIV Protease Inhibitor Library

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Abstract—An efficient combination solution-phase/solid-phase route enabling the diversification of the P_1', P_2', and P_3 subsites of indinavir has been established. The synthetic sequence can facilitate the rapid generation of HIV protease inhibitors possessing more favorable pharmacokinetic properties as well as enhanced potencies. Multiple compound dosing in vivo may also accelerate the identification of potential drug candidates. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

The human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of acquired immunodeficiency syndrome (AIDS). The discovery of clinically effective HIV protease inhibitors[1] in the recent past has significantly improved the lifestyle of many individuals afflicted with the virus. Never-the-less, current protease inhibitors suffer to some extent from issues not limited to first-pass metabolism, toxicities and food restrictions which often times contribute to patient non-compliance. More recently, the emergence of multi-drug resistant viral variants has been confirmed,[2] further compromising the effectiveness of current PI therapy. Indinavir, a potent and specific orally bioavailable HIV protease inhibitor, is metabolized by P450 isoforms in the CYP3A subfamily.[3] In order to further address the pharmacokinetic properties as well as in vivo potencies of indinavir, we have initiated an investigation directed toward the examination of its metabolically labile sites via generation of an 'indinavir-based' combinatorial library.[4] Depicted in Figure 1 are the major sites of metabolism of indinavir.[3] We postulated that diversification of these sites would lead to a second generation HIV protease inhibitor possessing improved pharmacokinetic and potency profiles.

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Chemistry

Our synthetic endeavor began with lactone **1** (Scheme 1) as previously prepared by Dorsey et al.[5] Hydrolysis of **1** employing aqueous LiOH in DME followed by removal of the water and subsequent exposure to allyl bromide provided hydroxyethylene isostere **2** (containing the 'X' dimension of the library). Tethering the secondary alcohol of **2** to the resin was determined to provide the greatest synthetic flexibility. Accordingly, esterification of Rapp TentaGel S CO_2H resin[6] employing **2** as the nucleophile provided the polymer bound orthogonally protected hydroxyethylene isostere **3**. Noteworthy is the fact that the orthogonal nature of the protecting groups permits either the P_2' or the P_3 subsite to be spatially addressed.

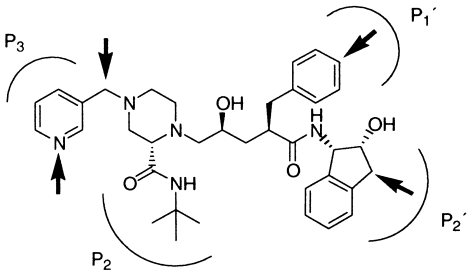

Figure 1. ←Denotes metabolism by CYP3A4.

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Examination of historical data suggested that the P_3 substituent plays an important role in maintaining activity in the HIV viral spread assay. Therefore, we initially chose to spatially address P_3.

Toward that end, deprotection of the allyl ester employing $Pd(PPh_3)_4$ and 1,3-dimethylbarbituric acid provided the carboxylic acid.[7] Amide formation using standard carbodiimide coupling incorporated the requisite amino indanol moiety.[8] Completion of the resin bound indinavir molecule involved acid catalyzed removal of the Boc group followed by reductive amination. Finally, release of the protease inhibitor from the resin was achieved by mild base catalyzed transesterification,[6] providing indinavir in 63% yield and 87% purity as determined by HPLC.[9]

With a viable solid-phase synthesis of indinavir now secured, we turned our attention toward construction of a small library to demonstrate proof of concept. The P_2' and P_3 ligands could easily be diversified by simply varying the amines and aldehydes employed in those respective positions. However, diversifying P_1' required retreating several steps to the lithium enolate progenitor of lactone **1**. By varying the alkylating agent, diversification of P_1' could be achieved. The requisite hydroxyethylene isosteres were then attached to the solid phase to begin construction of the library. The selection of these X fragments was directed toward either filling the hydrophobic S_1' pocket or blocking metabolism. With the differentiation of P_1' now complete, efforts were directed toward the selection of the 'Y' (P_2') and 'Z' (P_3) dimensions. Based on historical data, the Z dimension was selected to control the potency of the target protease inhibitors (see Table 1 for the IC_{50}/CIC_{95} values of single pure compounds $X_1Y_1Z_1$, $X_1Y_1Z_2$, and $X_1Y_1Z_3$) whilst the Y dimension would be used to probe modest amino indanol modifications.[10] Figure 2 depicts the generic structure of the library and the X, Y, and Z subunits employed.

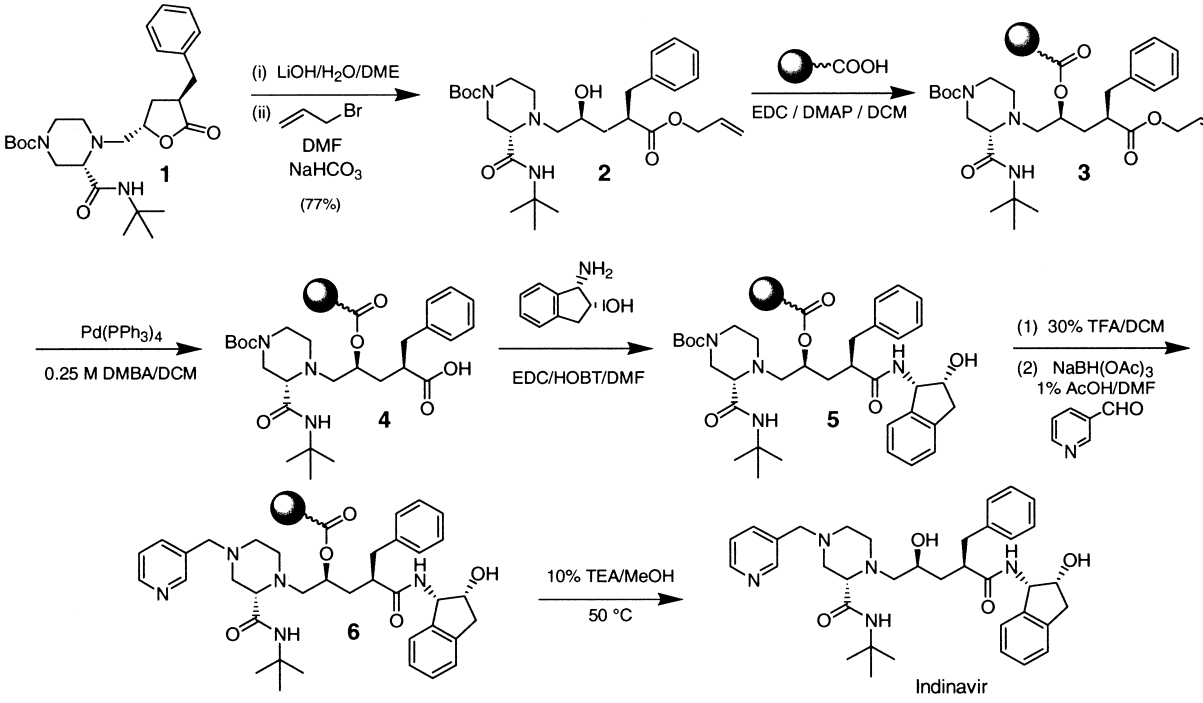
Table 1. In vitro enzyme inhibition and potency of pools/compounds

Pool/compound	Enzyme inhibition IC_{50}, nM[a]	Inhibition of viral spread (IIIb) CIC_{95}, nM[a]
Z_1	2.5	< 160
Z_2	4.3	1250
Z_3	21.8	10,000
Indinavir ($X_1Y_1Z_1$)	0.59[b]	50.5[c]
$X_1Y_1Z_2$	1.1	200
$X_1Y_1Z_3$	1.6	400

[a]Except where noted, values are for $n = 1$.
[b]Average for $n = 178$.
[c]Average for $n = 106$.

Preparation of the library was as follows: the 5 resin bound hydroxyethylene isostere fragments were archived and mixed. The allyl group was removed on a bulk two-thirds of the resin and this material further split into 4 separate pools in order to attach the Y subunits. After doing so, the 4 pools were archived, mixed and the Boc group was removed. This resin was then split into 2 separate pools followed by reductive amination employing the carboxaldehyde Z subunits. Finally, the protease inhibitors were released from the resin by gentle warming in 10% TEA/MeOH.

A separate investigation into the sulfonylation of the piperazine was also ongoing. We demonstrated that spatially addressing the Y dimension was also possible by first removing the Boc group of **3** with TFA followed by sulfonylation[11] of the resultant secondary amine (Scheme 2).



Scheme 1. Solid-phase synthesis of indinavir.

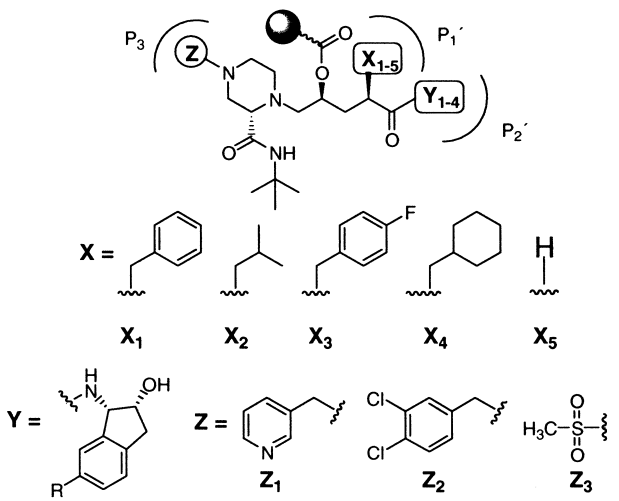
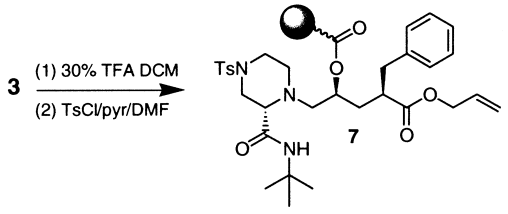


Figure 2. Library and subunits. Y_1 R = H; Y_2 R = Cl; Y_3 R = Br; Y_4 R = F.



Scheme 2. Sulfonylation of N4 piperazine.

Removal of the allyl group of **7**, amide formation and cleavage cleanly provided the 'tosylated' protease inhibitor (not shown). This strategy was utilized for processing the remaining one-third of the resin. Removal of the Boc group was followed by exposing the resin to 10 equiv of MsCl in 1:1 pyridine:DMF (2×1 h). The resin was split into 4 pools, the allyl group was removed and the Y subunits coupled. Cleavage from the resin provided the mixture of mesylated protease inhibitors now spatially addressed in the Y dimension.[12] In order to comply with our original design of spatially addressing the Z dimension, equimolar portions of each of these mixtures were combined and grouped with the previously prepared wells for submission to our HIV viral spread assay.[13]

Results

The biological activities of the 3 pools are displayed in Table 1. The compounds were tested for their ability to prevent cleavage of a substrate by the protease enzyme (IC_{50}) and to inhibit the spread of viral infection in MT4 human T-lymphoid cells infected with the IIIb isolate (CIC_{95}).[14] The results in Table 1 indicate that mixtures had no deleterious effects on our assays. As anticipated, the pool containing the Z_1 ligand (present in indinavir) displayed the highest affinity for the HIV protease enzyme in the binding assay and elicited the best potency in the viral spread assay. This is largely due to the 3-pyridylmethyl group providing both lipophilicity for binding to the protease as well as a weakly basic nitrogen which increases aqueous solubility. The latter is critical in cell based assay potency allowing inhibitors to cross the cell membrane.[5] Although large and highly lipophilic ligands are well tolerated at P_3, they display lower potencies for the reasons stated above, as exemplified by the Z_2 pool. Replacement of the 'arylmethyl' substituents with a sulfonyl moiety resulted in a pool of moderate potency in the binding assay, however, the potency in the cell based spread assay was severely compromised. This is almost certainly due to the dramatic decrease in the pK_a of the N4 piperazine nitrogen resulting in inhibitors of lower aqueous solubility, ultimately restricting the potential of these inhibitors to penetrate the cell membrane.[5] Notably, the intrinsic potencies as well as the inhibition of the spread of viral infection achieved by each pool exactly conforms to our predicted results, as shown in Table 1. Prioritization of the pools for in vivo analysis would thus be centered on the viral spread assay since that assay is clearly more predictive of the in vivo results.

Pharmacokinetics

The next challenge we faced was the multiple component in vivo dosing of our library (0.5 mpk/compound; 20 compounds per dog [$n = 2$], 0.05 M citric acid solution). We postulated that individual compounds possessing optimal pharmacokinetic properties would be readily discernible within the mixture.[15] This strategy would accelerate the identification of those compounds using only 2 dogs rather than the 40 dogs required to dose 20 individual compounds. However, if accurate pharmacokinetic data were to be interpreted from the results, it was imperative that the components of each pool be as close to equimolar as possible. Clearly, it would be unreasonable to establish whether the lack of PK data for one or more compound in the mixture was due to either poor bioavailability or absence of that component in the mixture. In order to determine the composition of pool Z_1, all 20 single pure protease inhibitors were individually prepared via the route described in Scheme 1.[9] From that array of compounds, an equimolar 20 component pool was reconstituted and delivered for LC/MS/MS analysis along with the 20 individual compounds. By comparison, it was concluded that the Z_1 pool was indeed an equal mixture of 20 compounds suitable for in vivo dosing. Analysis of the anticipated data required a thorough understanding of the P450 substrate-structure–activity relationship. Incorporation of the P_3 ligand of indinavir (which is responsible for P450 inhibition) as the Z ligand renders all of the components in the Z_1 pool as P450 inhibitors/substrates, therefore averaging the 3A4 inhibitory contribution of any one compound.[16] Chart 1 depicts the results of the 20 compound combinatorial mixture (Z_1 pool) dosed in dogs. Close examination of Chart 1 indicates that a significant amount of SAR can be gleaned from the resulting data. It is clearly evident that the set of compounds $X_2Y_{1\text{-}4}Z_1$ (isobutyl in P_1') achieved the highest C_{max} levels and AUC's in both dogs, followed by the set of compounds $X_1Y_{1\text{–}4}Z_1$ (benzyl in P_1'). However, only modest differences in the t1/2's were realized. Furthermore, the

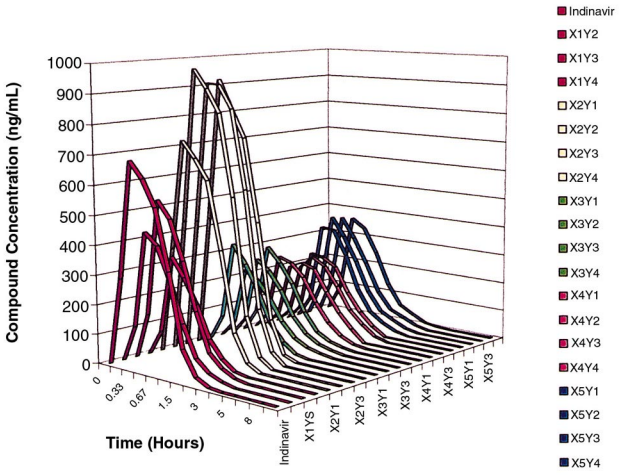


Chart 1. Pharmacokinetic profile of Z_1 pool. Pool was delivered orally as a solution in 0.05 M citric acid.

more highly lipophilic compounds (sets $X_3Y_{1-4}Z_1$ and $X_4Y_{1-4}Z_1$) elicited the lowest AUC's. This is most likely due to the effects of decreased water solubility. The fact that compound $X_1Y_1Z_1$ (indinavir) displayed the fifth highest C_{max} and AUC confirms our hypothesis that pharmacokinetically attractive protease inhibitors would not escape detection in a mixture of 20 compounds. Overall, the results in Chart 1 do provide valuable information on the in vivo dosing of multiple component mixtures.

Conclusion

In summary, we have established a flexible solid-phase synthesis enabling the diversification of the P_1', P_2', and P_3 subsites of indinavir. The synthetic route can be utilized in the generation of an 'indinavir-based' library or libraries directed toward identification of a second generation HIV protease inhibitor possessing improved metabolic and potency profiles. We have also demonstrated that in vitro potency can be measured accurately on mixtures obtained from libraries. Furthermore, reliable pharmacokinetic data can be acquired via oral dosing of combinatorial mixtures in dogs. The design, preparation and analysis of much larger libraries of HIV protease inhibitors is currently underway and will be reported in the near future.

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7. Washing the resin with glacial acetic acid efficiently removed palladium based residues. Standard washing protocols throughout the synthesis typically involved various combinations of DMF, DCM, THF, and IPA. Final resin washing included either DCM or DMF, depending on the next reaction sequence.
8. The initial amide coupling conditions employed were amine (10 equiv), EDC (15 equiv), and HOBT (15 equiv) for 24 h. The conditions were later improved by employing HBTU (2.5 equiv), HOBT (3.75 equiv), DIEA (5 equiv), and amine (2.5 equiv) for 1 h.
9. Analysis by HPLC (4 × 250 mm LiChrospher 100 RP-18 5 μm particle size. Gradient elution 25/75 to 90/10 MeCN/H_2O containing 0.1% TFA, 1.0 mL/min for 15 min) by area integration at 210 nm and 254 nm. The structure assigned to each new compound is in accord with its 400 MHz NMR spectrum as well as appropriate ion identification by mass spectrometry.
10. The synthesis of these amino indanol analogues will be reported elsewhere.
11. The resin was neutralized by washing with 30% TEA/THF, then THF or DCM prior to initiating the sulfonylation reaction.
12. Spatially addressing the Y dimension is the subject of a subsequent library and will be reported in due course.
13. Overall, this strategy afforded 3 distinct pools diversified at X, Y, and spatially addressed at Z.
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16. Future libraries would incorporate a well absorbed CYP3A4 substrate in each pool as a standard to monitor P450 activity. An increase in the C_{max} of this substrate would indicate the presence of a CYP3A4 inhibitor in that particular pool.