Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 529–532

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Combinatorial Library of Indinavir Analogues and Its In Vitro and In Vivo Studies

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Received 9 October 2001; accepted 21 November 2001

Abstract—A combinatorial library of 300HIV protease inhibitors has been synthesized. The library was screened against recombinant wild-type and mutant HIV-1 protease enzymes. The pharmacokinetics of the library was evaluated by dosing in dogs. Compounds that are notably more potent than indinavir and have favorable pharmacokinetic properties were identified.

The clinically effective human immunodeficiency virus (HIV) protease inhibitors are a novel class of drugs that are considered one of the most important therapeutic agents to date for the treatment of HIV infection and AIDS.[1] Although protease inhibitor therapy is highly effective, these drugs all suffer to some degree from short half lives, food restrictions and increasing viral resistance.[2] Indinavir is an orally bioavailable and highly potent HIV protease inhibitor[3] that undergoes significant first pass clearance and is primarily metabolized by CYP3A4 in humans.[4] The P_3, P_1', and P_2' subsites of indinavir had been identified as the major metabolic sites that lend itself well to be explored by solid-phase combinatorial synthesis (Fig. 1).[5] Herein, we describe a combinatorial library of indinavir analogues and present both in vitro and in vivo screening data. We show that screening of a mixture is an effective method for finding compounds with both improved activity and pharmacokinetic parameters.

In preparing the library, we chose a standard solid-phase 'mix and split' protocol[6] to synthesize the initial library mixtures. The solid-phase synthesis of indinavir has been reported previously.[5a,b] The indinavir molecule was divided into three fragments, the hydroxylethylene unit (**X**),[7] the aminoindanol moiety (**Y**) and the pyridylmethyl group (**Z**) (Fig. 2). The synthesis of the library is outlined in Scheme 1. The orthogonally protected **X** subunit was anchored to Rapp TentaGel S COOH resin through the hydroxy group via an ester linkage.[5a] The coupling reaction was performed with EDC and DMAP in DCM.[5b] The five resin bound **X** fragments were archived and mixed. Next the allyl group was removed by $Pd(PPh_3)_4$ and 1,3-dimethylbarbituric acid, then the resulting material was split into four pools. The **Y** subunits were then attached to the resin using the standard coupling conditions. The four pools were archived and mixed and the Boc group was removed under acidic conditions. This resin was then

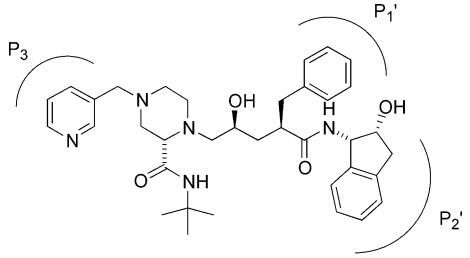

Figure 1. Major metabolic sites of indinavir.

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0960-894X/02/$ - see front matter
PII: S0960-894X(01)00824-1

split into 15 separate pools and the **Z** subunits were installed by reductive amination, amide coupling or sulfonylation.[5b] The Boc group of **Z14** pool was removed by TFA/DCM. The final products were released from the resin by treatment with 10% TEA/MeOH at 50 °C.[5a] The total number of unique X-Y-Z combinations in the library is $5 \times 4 \times 15$, or 300. Each Z pool contained 24 total members.

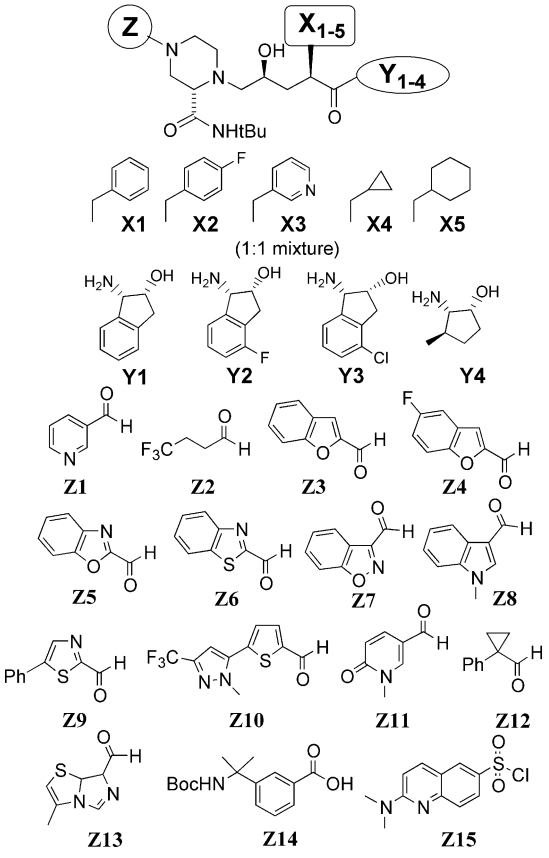
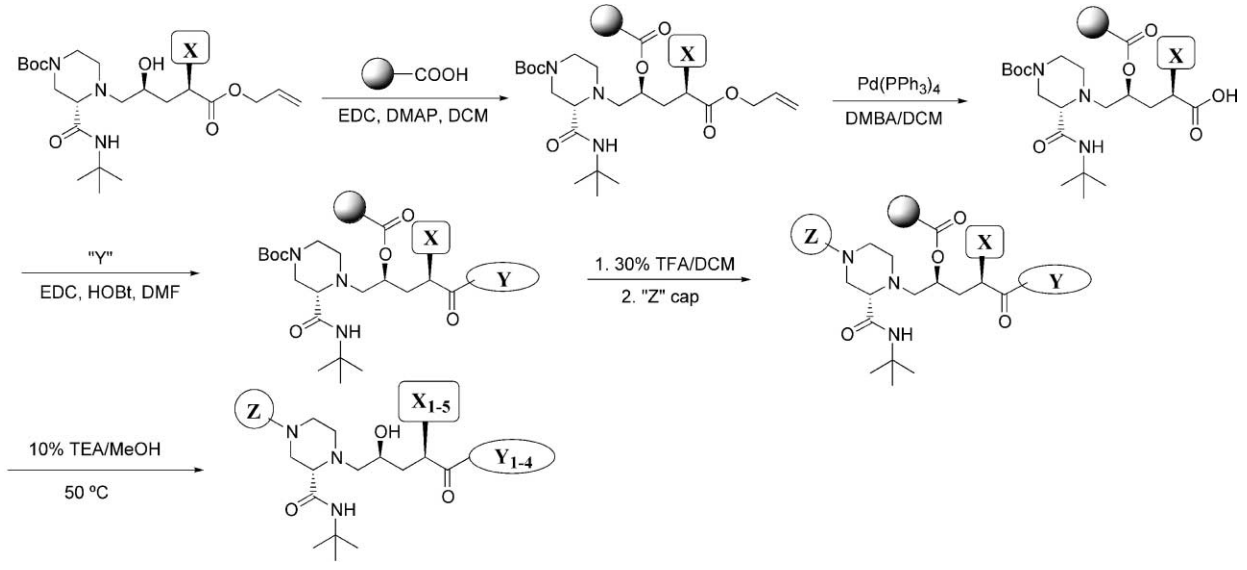


Figure 2. Library components.

The biological activity of the pools is shown in Table 1. The compounds were tested for their ability to inhibit cleavage of a substrate by the wild-type HIV-1 protease enzyme (IC_{50}) and to inhibit the spread of viral infection in MT4 human T-lymphoid cells infected by the IIIb isolate (CIC_{95}).[3] The compounds were also tested with A-44 mutant enzyme variant[8] to investigate their potential potency against PI-resistant HIV virus. The **Z1** pool contains indinavir as one of the components. The results show that eight pools are more potent than the **Z1** pool in both wild-type and mutant enzyme inhibition assays. The **Z3**, **Z9**, **Z10**, and **Z15** pools are also equal to or more potent than the **Z1** pool in the antiviral assay. The Z3 pool contains the P_3 fragment of MK-944a, a potent HIV protease inhibitor with favorable pharmacokinetic profile.[9] Based on the results obtained from the library we synthesized three single compounds **1** (**X1-Y1-Z9**), **2** (**X1-Y1-Z10**) and **3** (**X1-Y1-Z15**) from pool **Z9**, **Z10** and **Z15** (Fig. 3). These three compounds were tested in the assays and they showed significant improvement in both intrinsic potency against wild-type (>16-fold vs indinavir) and A-44 mutant enzyme (>140-fold vs indinavir) and in the ability to inhibit viral spread in infected cells. The potency of these compounds was below the limit of detection in the enzyme inhibition assays (0.1 nM for wild-type and 0.6 nM for A-44 mutant). Both compounds **1** and **2** have a biaryl group at P_3 moiety and compound **3** has a sulfonyl linkage to piperazine. The biological activity of these compounds indicate that variation of the **Z** subunit has an important positive impact on potency against HIV protease enzymes, both in wild-type and mutants.

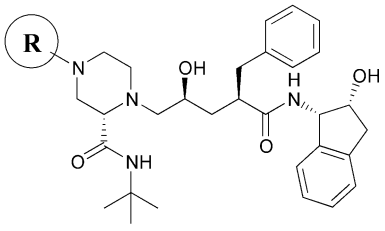
In order to obtain reliable pharmacokinetic data from the library mixtures,[5b,10] we first needed to determine whether all components in the mixture were present in equimolar amounts. To do this, we individually synthesized the 20 single compounds represented in the **Z1** pool and reconstituted an equimolar 20 component pool. By LC–MS analysis[11] of the reconstituted pool we



Scheme 1. Solid-phase synthesis of indinavir analogues library.

Table 1. In vitro potency of compounds and pools

Compd/pool	Wild-type IC_{50} (nM)	Mutant A-44 IC_{50} (nM)	Wild-type CIC_{95} (nM)
Indinavir	0.59	35.3	50.5
Z1	1.6	89.0	50
Z2	15.3	568	>400
Z3	0.49	24.0	50
Z4	0.94	46	200
Z5	1.2	66	400
Z6	1.2	71.7	400
Z7	1.17	71.5	400
Z8	6.7	332.2	>400
Z9	0.26	2.0	50
Z10	0.13	0.6	25
Z11	2.2	131	400
Z12	7.7	257	>400
Z13	6.7	378	>400
Z14	28	392.4	>400
Z15	0.22	2.2	50
1	<0.1	<0.6	25.0
2	<0.1	<0.6	6.0
3	<0.1	<0.6	12.5



1, R = Z9; **2**, R = Z10; **3**, R = Z15

Figure 3. Single compounds.

confirmed that the **Z1** pool from the library was indeed an equimolar mixture of 20 compounds and that the pools in the library were suitable for in vivo dosing. The possibility of drug–drug interactions (i.e., CYP3A4 inhibition) necessitated the use of an internal standard in each pool. Indinavir was a component of the Z1 pool and acted as its internal standard. Subsequently, a compound with a well defined PK profile in dogs (X1Y1Z3) was added to each pool as an internal standard.[9] We dosed eight pools of the library in dogs [0.5 mpk/compound; 20 compounds per dog ($n=2$)].

The concentration versus time profiles for the **Z1** pool are depicted in Chart 1. The pharmacokinetic parameters of the **Z1** pool in dogs are summarized in Table 2. The results show that a cyclopropyl substitution in the P_1' position (**X4Y1–4**) resulted in an increased C_{max} and AUC in dogs. In some cases combinations of the cyclohexyl (**X5** with **Y1**, **Y3**) or 4-fluorophenyl (**X2** with **Y3**) substitution in P_1' with changes in P_2' showed an increased half-life compared to other substituents at P_1'. At the P_2' position, aminohydroxy-cyclopentane (**Y4**) afforded higher C_{max} and AUC than other subunits at P_2'. In order to illustrate whether mixture dosing could provide valuable pharmacokinetic information for single compounds, we synthesized three single compounds from the pool and dosed in dogs (10 mpk per compound). The results are shown in Table 3. The

Table 2. Pharmacokinetics of **Z1** pool in dogs

Components	C_{max} (nM)	T_{max} (nM)	AUC (nM h)	$t_{1/2}$ (min)
X1Y1 (indinavir)	**358**	**40**	**307**	**27.1**
X1Y2	250	40	258	44.5
X1Y3	250	40	256	45.8
X1Y4	**554**	40	**512**	31.5
X2Y1	264	40	258	44.6
X2Y2	195	40	197	48.2
X2Y3	180	40	181	**50.5**
X2Y4	378	40	371	39.2
X3Y1	313	40	291	43.4
X3Y2	268	40	245	28.7
X3Y3	220	40	227	23.9
X3Y4	**524**	40	**464**	23.4
X4Y1	**651**	40	**566**	21.2
X4Y2	**610**	40	**525**	20.2
X4Y3	**626**	40	**566**	23.0
X4Y4	**996**	40	**839**	19.0
X5Y1	232	40	260	**54.4**
X5Y2	148	40	236	48.2
X5Y3	182	40	235	**81.0**
X5Y4	**483**	40	**504**	42.5

Compounds with favorable PK profiles in comparison to that of indinavir.

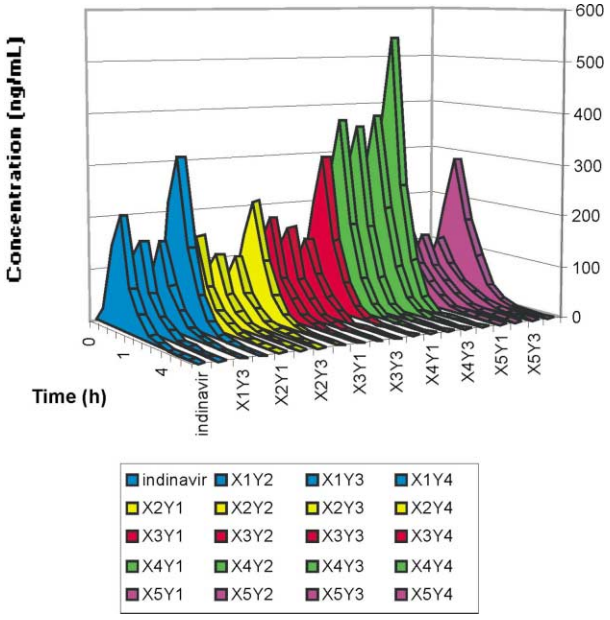
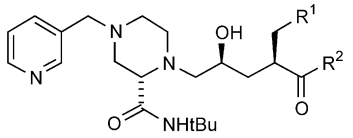


Chart 1. Pharmacokinetic profile of **Z1** pool.

pharmacokinetic profile of the single compounds showed good correlation with the results from the pool.

Compound **6** with **X4** subunit showed higher C_{max} and AUC than **5** with **X5** subunit. Compound **5** which has the **Y4** subunit showed higher C_{max} and AUC than **4** which contains the **Y3** subunit. Compound **4** has the longest half-life in all three compounds. Compound **5** with **X5** subunit showed longer half-life than **6** with **X4** subunit. All three compounds showed improved half-lives compared to indinavir ($t_{1/2}$ 38.5 min). These results demonstrate that individual compounds with optimal pharmacokinetic properties could be identified by screening the mixtures in vivo.

Table 3. Pharmacokinetics of single compounds



4, R^1 = X5, R^2 = Y3; **5**, R^1 = X5, R^2 = Y4;
6, R^1 = X4, R^2 = Y4

Compd (dose)	C_{max} (μM)	T_{max} (min)	AUC (μM h)	$t_{1/2}$ (min)
4 (10 mpk)	0.36	35	0.48	53
5 (10 mpk)	3.76	25	3.83	49
6 (10 mpk)	6.62	45	5.28	41

In summary, we have prepared a HIV protease inhibitor library of indinavir analogues. After in vitro screening, we have identified compounds (**1**, **2**, and **3**) that are substantially more potent than indinavir against both wild-type and mutant enzymes. We have also acquired valuable pharmacokinetic SAR information by dosing pools in dogs. Compounds with higher C_{max} and AUC and longer half-life were identified. Based on the results of this library, preparation and evaluation of several subsequent libraries are underway, and will be published in due course.

Acknowledgements

We thank Nathan A. Yates for performing LC–MS analysis and the Department of Process Research for providing X and Y subunits.

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