

## INCREASED ANTIVIRAL ACTIVITY OF CYCLIC UREA HIV PROTEASE INHIBITORS BY MODIFYING THE P1/P1' SUBSTITUENTS

Robert F. Kaltenbach III,\* Ronald M. Klabe, Beverly C. Cordova, and Steven P. Seitz

*DuPont Pharmaceuticals Company, Experimental Station, Box 80500,  
Wilmington, DE 19880-0500, U.S.A.*

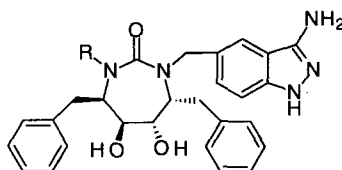
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**Abstract:** A series of alkyl substituted P1/P1' analogs was prepared in an attempt to increase translation of the 3-aminoindazole class of HIV protease inhibitors. Increasing the lipophilicity of the P1/P1' residues dramatically improved translation of enzyme activity to antiviral activity in the whole cell assay. © 1999 Published by Elsevier Science Ltd. All rights reserved.

**Keywords:** antiproliferative agents; antivirals; enzyme inhibitors; substituent effects.

### Introduction

There has been an intense effort to find new therapeutics that inhibit the Human Immunodeficiency Virus Protease (HIV-Pr). In clinical trials, inhibition of this enzyme was shown to reduce the levels of infectious virus and increase CD4 cell counts in patients infected with HIV.<sup>1</sup> Recently we described a series of unsymmetrical 3-aminoindazole substituted cyclic ureas that are highly potent inhibitors of HIV-Pr.<sup>2</sup> Two promising analogs in this series are **DMP 850** and **DMP 851**, which showed a good pharmacokinetic profile in dogs. Both gave a high C<sub>max</sub> with relatively low clearance and had adequate blood levels at 8 h to cover the worst single mutant for cyclic ureas, the I84V protease mutation. **DMP 850** and **DMP 851** were selected for evaluation in clinical trials.



**DMP 850** R = Benzyl  
**DMP 851** R = *n*-Butyl

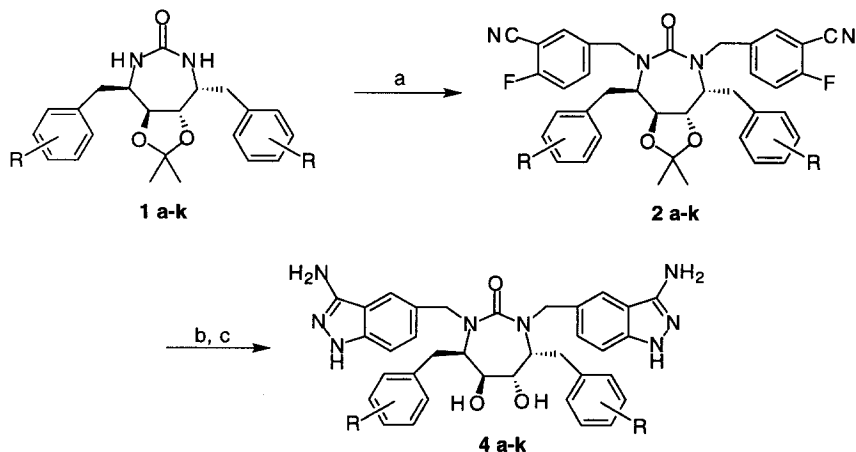
The unsymmetrical cyclic ureas evolved from the symmetrical 3-aminoindazole parent **4a**.<sup>3</sup> Although **4a** was a potent inhibitor of the enzyme, it is very polar and showed poor translation to the whole cell assay.<sup>4</sup> Rodgers and coworkers were able to improve the cellular activity of this series by substituting hydrophobic residues on the 3-aminoindazole.<sup>3</sup> Although this approach succeeded in increasing potency, substitution of the P2/P2' groups also decreased enzyme inhibition due to unfavorable steric interactions. In addition, an X-ray crystal structure of **4a** bound to HIV-Pr showed four hydrogen bonds from each 3-aminoindazole to the enzyme. The excellent resistance profile observed with **4a** (no loss to the I84V mutant) is attributed to the extremely tight binding resulting from these hydrogen bonds. In order to preserve the important interactions between the

3-aminoindazole and the enzyme we chose to leave the P2/P2' residues unchanged and improve translation by increasing the lipophilicity of the P1/P1' residues.

## Chemistry

The synthesis of the symmetrical 3-aminoindazoles is shown in Scheme 1.<sup>3</sup> The P1/P1' substituted cyclic ureas **1b–k** were prepared as previously described using L-tartrate as the chiral precursor.<sup>5</sup> Alkylation with 3-cyano-4-fluorobenzyl bromide gave the bis-alkylated cyclic ureas **2a–k** in excellent yields. Treatment with hydrazine hydrate in refluxing *n*-butanol formed the 3-aminoindazole ring in quantitative yields. Deprotection provided the bis-alkylated 3-aminoindazoles **4a–k**.

**Scheme 1**



Reagents: (a) 3-CN-4-F-BnBr, KO<sup>t</sup>-Bu, THF, 0 °C; (b) H<sub>2</sub>NNH<sub>2</sub>•H<sub>2</sub>O, *n*-Butanol, reflux; (c) HCl, MeOH / H<sub>2</sub>O.

## Results and Discussion

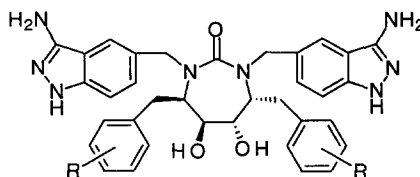
The P1/P1' substituted cyclic ureas were tested in our enzyme binding affinity<sup>6</sup> and whole cell antiviral<sup>7</sup> assays. The results are shown in Table 1. The parent aminoindazole **4a** was the most potent compound in the binding affinity assay with a  $K_i < 0.01$  nM. However, this compound was too hydrophilic with a Clog P of 3.5 and did not translate well to the whole cell assay ( $IC_{90} = 2.8$   $\mu$ M). By increasing substitution on the P1/P1' residues the Clog P was increased approximately 0.5 for each additional carbon added.<sup>8</sup> This increase in lipophilicity dramatically increased translation in this series as shown in Graph 1.<sup>9</sup> As seen from **4b–g**, the addition of small straight chain alkyl residues gave a slight decrease in enzyme binding but whole cell antiviral activity ( $IC_{90}$ 's) improved. For example, addition of four carbons increased the Clog P to 5.5 and gave over an order of magnitude improvement in translation resulting in an  $IC_{90}$  of 300–540 nM. Unfortunately, as seen with

the P2/P2' substituted 3-alkylaminoindazoles, increasing the size of the alkyl substituent caused a progressive decrease in enzyme inhibitory activity. Large and branching alkyl substituents **4h–k** significantly decreased enzyme inhibition even though translation continued to improve. For example, while the *t*-butyl analog **4i** showed almost three orders of magnitude improvement in translation, the diminished  $K_i$  of 0.19 nM resulted in an  $IC_{90}$  of only 100 nM.

## Conclusions

We have shown that increasing the lipophilicity of symmetrical 3-aminoindazoles by modification of the P1/P1' residues increased translation and ultimately improved antiviral activity in the whole cell assay. The substitution of small alkyl groups was tolerated giving a substantial increase in antiviral activity. However, there is a limit on the size of the alkyl group. Larger branched substituents significantly decreased binding and limited further improvements in whole cell antiviral activity.

**Table 1.** Translation and ClogP of P1/P1' substituted cyclic ureas.

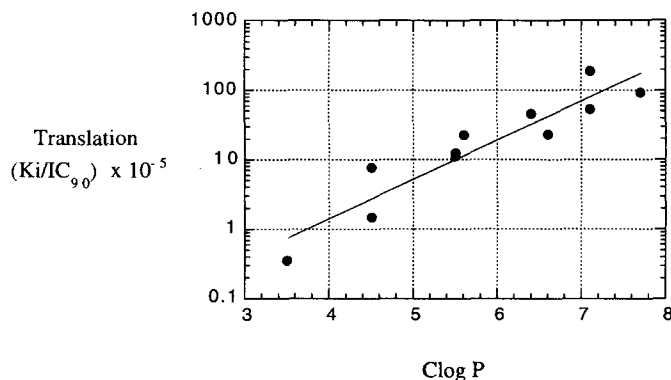


Compd	R	$K_i$ (nM) <sup>a</sup>	$IC_{90}$ (nM) <sup>b</sup>	Clog P	Translation <sup>c</sup>
4a	H	<0.01	2760	3.5	<0.36
4b	4-Me	0.061	780	4.5	8
4c	3-Me	0.011	740	4.5	1.5
4d	3,5-diMe	0.067	540	5.5	12
4e	2,5-diMe	0.034	300	5.5	11
4f	4-Et	0.068	300	5.6	23
4g	4- <i>n</i> -Pr	0.068	290	6.6	23
4h	4- <i>i</i> -Pr	0.19	410	6.4	46
4i	4- <i>t</i> -Bu	0.19	100	7.1	190
4j	3- <i>t</i> -Bu	0.21	390	7.1	54
4k	4- <i>n</i> -Bu	0.17	180	7.7	94

<sup>a</sup> Values were measured by cleavage of a fluorescent peptide substrate using HPLC.

<sup>b</sup> Determined by measuring the accumulation of viral RNA transcript after infection of cells with HIV-1.

<sup>c</sup> Values are expressed as  $(K_i/IC_{90}) \times 10^{-5}$

**Graph 1.** Translation vs. Clog P

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9. Correlation coefficient = 0.67.