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

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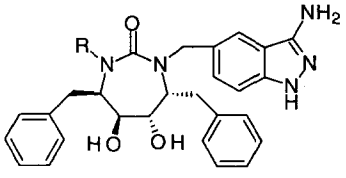
Abstract: A series of alkyl substituted P1/P1' analogs was prepared in an attempt to increase translation of the 3-aminoindazole class of HIV protease inhibitors. Increasing the lipophilicity of the P1/P1' residues dramatically improved translation of enzyme activity to antiviral activity in the whole cell assay.

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.[1] Recently we described a series of unsymmetrical 3-aminoindazole substituted cyclic ureas that are highly potent inhibitors of HIV-Pr.[2] Two promising analogs in this series are **DMP 850** and **DMP 851**, which showed a good pharmacokinetic profile in dogs. Both gave a high Cmax 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**.[3] Although **4a** was a potent inhibitor of the enzyme, it is very polar and showed poor translation to the whole cell assay.[4] Rodgers and coworkers were able to improve the cellular activity of this series by substituting hydrophobic residues on the 3-aminoindazole.[3] 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

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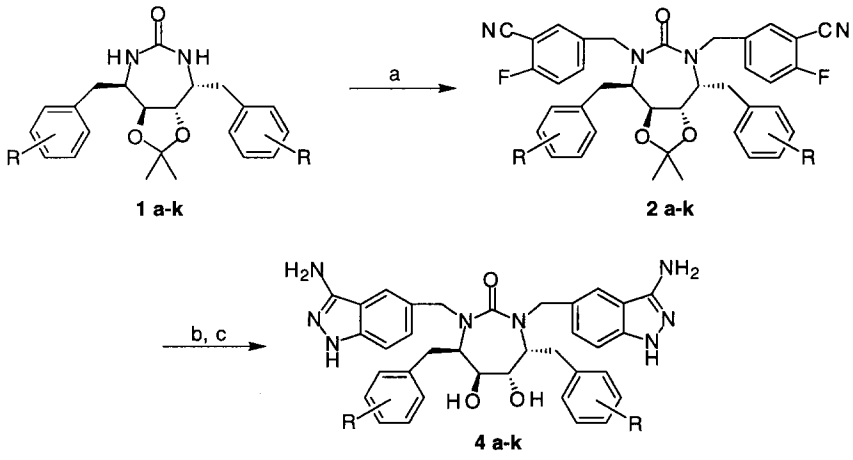
3-aminoindazole and the enzyme we chose to leave the P2/P2' residues unchanged and improve translation by increasing the lipophilicity of the P1/P1' residues.

Chemistry

The synthesis of the symmetrical 3-aminoindazoles is shown in Scheme 1.[3] The P1/P1' substituted cyclic ureas **1b–k** were prepared as previously described using L-tartrate as the chiral precursor.[5] 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*t*-Bu, THF, 0 °C; (b) $H_2NNH_2 \cdot H_2O$, *n*-Butanol, reflux; (c) HCl, MeOH / H_2O.

Results and Discussion

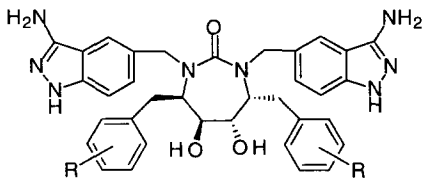
The P1/P1' substituted cyclic ureas were tested in our enzyme binding affinity[6] and whole cell antiviral[7] assays. The results are shown in Table 1. The parent aminoindazole **4a** was the most potent compound in the binding affinity assay with a $K_i < 0.01$ nM. However, this compound was too hydrophilic with a Clog P of 3.5 and did not translate well to the whole cell assay (IC_{90}=2.8 μM). By increasing substitution on the P1/P1' residues the Clog P was increased approximately 0.5 for each additional carbon added.[8] This increase in lipophilicity dramatically increased translation in this series as shown in Graph 1.[9] 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.

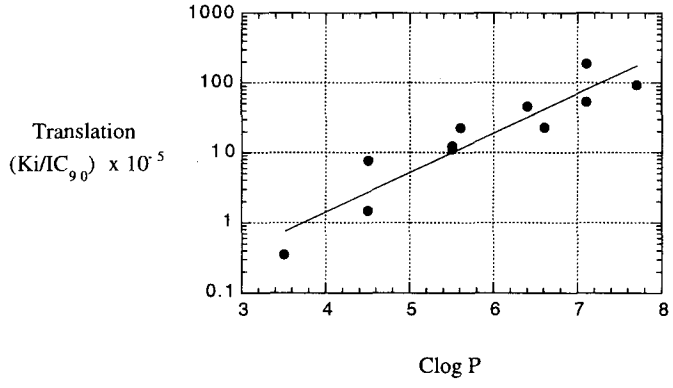
Table 1. Translation and ClogP of P1/P1' substituted cyclic ureas.



Compd	R	K_i (nM)[a]	IC_{90} (nM)[b]	Clog P	Translation[c]
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-*n*-Pr	0.068	290	6.6	23
4h	4-*i*-Pr	0.19	410	6.4	46
4i	4-*t*-Bu	0.19	100	7.1	190
4j	3-*t*-Bu	0.21	390	7.1	54
4k	4-*n*-Bu	0.17	180	7.7	94

[a] Values were measured by cleavage of a fluorescent peptide substrate using HPLC.
[b] Determined by measuring the accumulation of viral RNA transcript after infection of cells with HIV-1.
[c] Values are expressed as $(K_i/IC_{90}) \times 10^{-5}$

Graph 1. Translation vs. Clog P



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4. Poor translation has usually been attributed to the inability of highly polar compounds to penetrate cell membranes however numerous other parameters must be considered including lipid solubility, binding to media, compound stability and intracellular localization.

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8. Computer generated Clog P was calculated using MedChem Software Release 3.32, Ponoma College, Claremont, Ca.

9. Correlation coefficient = 0.67.