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Synthesis, Antiviral Activity and Pharmacokinetics of P1/P1′ Substituted 3-Aminoindazole Cyclic Urea HIV Protease Inhibitors

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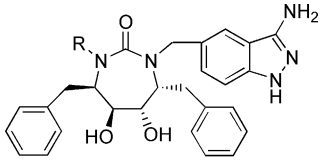
Abstract—A series of P1/P1′ substituted cyclic urea analogues were prepared in an attempt to increase the intra-cellular antiviral potency of the nonsymmetrical 3-aminoindazoles **DMP 850** and **DMP 851**. The effect of alkyl substitution of the P1/P1′ residues on cellular antiviral potency, protein binding, resistance profile and pharmacokinetics are described.

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 the nonsymmetrical 3-aminoindazole cyclic ureas **DMP 850** and **DMP 851.**[2] These clinical candidates are potent inhibitors of HIV-Pr and showed a good pharmacokinetic profile in dogs.



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

DMP 850 and **DMP 851** are highly potent inhibitors of the HIV-Pr enzyme with a K_i of 0.031 and 0.021 nM, and show modest translation in a whole cell antiviral assay with an IC_{90} of 62 and 56 nM, respectively. In view of the excellent enzymatic potency, we felt an increase in cellular potency could be obtained by modifications designed to improve cell penetration. We have previously shown that, in general, the symmetrical bis-3-aminoindazole substituted cyclic ureas are too polar and have limited cellular activity. Increasing the lipophilicity of the 3-aminoindazole P2/P2′ residues[3] or the benzyl P1/P1′ residues[4] by substituting small alkyl substituents resulted in improved antiviral activity. However, with either approach there was a limit on the size of the alkyl groups tolerated. Larger branched substituents significantly decreased binding due to unfavorable steric interactions and limited further improvements in whole cell antiviral activity. We predicted that increasing the lipophilicity of the benzyl P1/P1′ residues in the **DMP 850** and **DMP 851** series would increase the cellular potency. In addition, since **DMP 850** and **DMP 851** had a Clog P[5] of 5.2 an increase of only one or two additional carbons on each P1 residue should be sufficient.

Chemistry

Previous syntheses of the P1/P1′ substituted cyclic ureas **4a**–**e** required the stereoselective reduction of a substituted oxime derived from L-tartaric acid.[6] Although this route was useful for our preliminary

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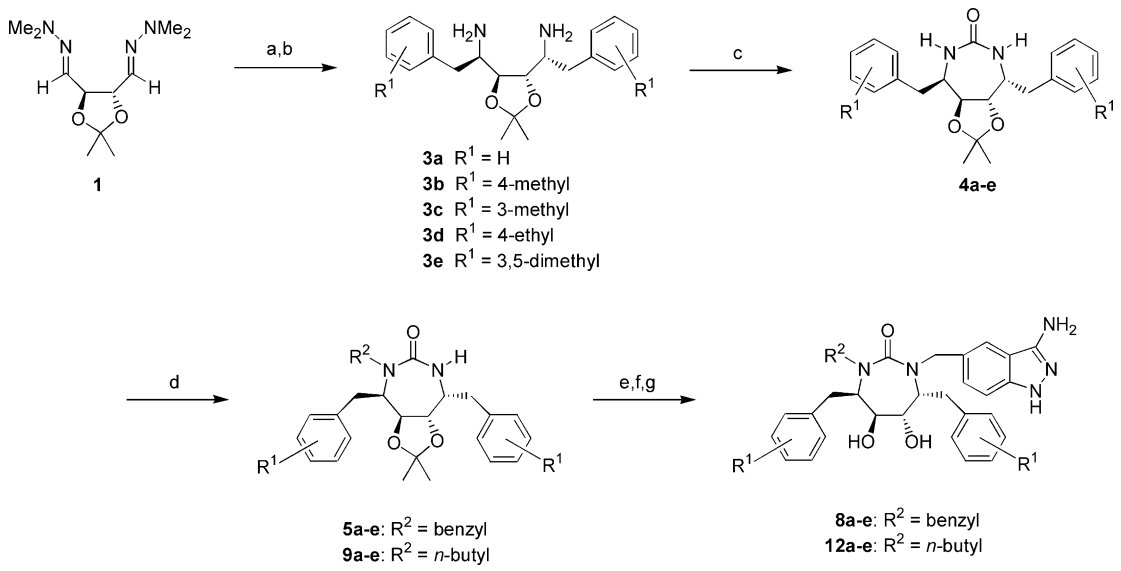


Scheme 1. Reagents and conditions: (a) $CH_3C_6H_4R^1$, *s*-BuLi, THF, −15–0 °C, 60–99%; (b) RaNi, MeOH, H_2 (250 psi), 100 °C, 82–93%; (c) 1,1′-CDI, 1,1,2,2-tetrachloroethane, reflux, 46–60%; (d) BnBr (**5a–e**) or BuI (**9a–e**), KO*t*-Bu, THF, 0 °C, 62–76%; (e) 3-CN-4-F-BnBr, KO*t*-Bu, THF, 0 °C, 72–96%; (f) $H_2NNH_2{\cdot}H_2O$, *n*-butanol, reflux, 80–99%; (g) HCl, MeOH/H_2O, 81–96%.

screening, the cyclic ureas were generally obtained in moderate yields and a more efficient synthesis was desired for scaleup for in vivo studies. One option was an alternate tartrate derived route developed by our chemical process research and development group. In this methodology, the P1/P1′ residues were introduced via a stereoselective addition of benzyllithium to the dihydrazone **1**, followed by reduction to the diamine and cyclization.[7] By modifying this synthetic approach by incorporating appropriately substituted benzyllithiums the P1/P1′ substituted cyclic ureas could be obtained in improved yields as shown in Scheme 1.

For example, by metalating *p*-xylene with *s*-BuLi (1.3 M in cyclohexane) in THF at −15 °C the resulting *p*-methyl-benzyllithium was formed. Addition of the dihydrazone **1** in THF followed by warming to 0 °C gave the bis-hydrazine in 95% yield. Reduction with Raney nickel and hydrogen (250 psi) in methanol gave the diamine **3b** in 83% yield. Similarly, the corresponding *m*-methyl, *p*-ethyl and 3,5-dimethyl substituted diamines **3c–e** could be obtained from *m*-xylene, *p*-ethyltoluene or mesitylene. Cyclization with 1,1′-carbonyldiimidazole in refluxing tetrachloroethane gave the substituted cyclic ureas **4a–e**. Alkylation with 1.1 equivalents of benzyl bromide or butyl iodide gave the mono-alkylated cyclic ureas **5a–e** and **9a–e**. The 3-aminoindazole was introduced by alkylation with 3-cyano-4-fluoro-benzylbromide followed by treatment with hydrazine hydrate in refluxing *n*-butanol. Finally, deprotection of the acetonide provided the non-symmetrical 3-aminoindazoles **8a–e** and **12a–e**.

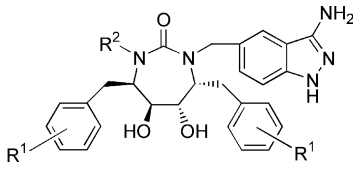
Results and Discussion

The P1/P1′ substituted cyclic ureas were tested in our enzyme binding affinity[8] and whole cell antiviral[9] assays. The results are shown in Table 1. In both the **DMP 850** and **DMP 851** series, mono-substitution of the P1/P1′ residues was tolerated giving only a slight decrease in enzyme binding, and a 2–5-fold improvement in cellular antiviral activity (IC_{90}s). The *p*-methyl analogues **8b** and **12b** improved the IC_{90} over 5-fold to 12 nM and the *m*-methyl analogues **8c** and **12c** improved the IC_{90}s about 2-fold. Similar improvements were seen with the bulkier *p*-ethyl analogues, however, losses in binding were apparent which limited the increase in cellular potency. The 3,5-dimethyl substituted analogues significantly decreased binding and showed no improvement in IC_{90} compared to the unsubstituted parents.

In addition to antiviral potency against wild type viruses, the effect of binding to human plasma proteins and activity against drug resistant viral mutants were important factors in our selection criteria. The relative degree of plasma protein binding was determined by a functional assay where the IC_{90}s were determined in the absence or presence of physiological levels of two human plasma proteins (45 mg/mL human serum albumin and 1 mg/mL alpha-1-acid glycoprotein). The results are reported as the fold increase in IC_{90} (fold shift). The mono-substituted analogues all showed an increase in plasma protein binding over **DMP 850** and **DMP 851**. This, in effect, lowers the amount of free drug available in plasma. By calculating the protein binding adjusted IC_{90}s ($IC_{90}\times$fold shift) it was shown that the substituted analogues were still more potent than **DMP 850** and **DMP 851** but the increased protein binding partially offsets the increases in antiviral potency.

Adequate antiviral potency to inhibit common drug resistant strains of HIV is an additional factor in the selection of our protease inhibitors. Despite the efficacy of the current protease containing antiretroviral regimens, some failures occur due to the development of

Table 1. Binding, protein shift and cellular activity of P1/P1′ substituted cyclic ureas



Compd	R^1	R^2	K_i (nM)[a]	RF IC_{90} (nM)[b]	HSA + AAG (fold shift)[c]	PB adj. IC_{90} (nM)[d]	Virus IC_{90} (nM)				
							HXB2[b]	I84V[e]	V82F/I84V[e]	Abbott[f]	Vertex[g]
DMP850	H	Bn	0.031	62	8×	496	92	370	2100	1520	257
8b	4-Me	Bn	0.047	12	16×	192	17	310	>1700	1880	24
8c	3-Me	Bn	0.062	25	12×	300	26	104	2680	1760	
8d	4-Et	Bn	0.072	19	13×	247	81	410	>1600	12,600	
8e	3,5-Me	Bn	0.20	50	7×	350	100			>7300	
DMP851	H	*n*-Bu	0.021	56	17×	952	69	390	1790	1470	87
12b	4-Me	*n*-Bu	0.037	12	35×	420	16		1750	1700	7
12c	3-Me	*n*-Bu	0.045	31	25×	775	54		1600	1600	
12d	4-Et	*n*-Bu	0.094	10	22×	220	22	260	>1800	4030	
12e	3,5-Me	*n*-Bu	0.19	66			56			7540	

[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]Fold shift of the RNA IC_{90} with added HSA (45 mg/mL) and AAG (1 mg/mL).
[d]Protein binding adjusted IC_{90} (IC_{90}× fold shift).
[e]Values were calculated based on quantitation of viral yield by plaque assay in MT-2 cells.[10]
[f]HIV mutant virus 25-fold resistant to ritonavir (M46I/L63P/A71V/V82F/I84V).
[g]HIV mutant virus 19-fold resistant to amprenavir (M46I/I47V/I50V).

Table 2. Pharmacokinetics of selected compounds in dogs

Compd	R^1	R^2	C_{max} (μM)[a]	$C_{8\ h}$ (μM)	$T_{1/2}$ (h)	F%
DMP850	H	Bn	9.6	2.8	3.5	60
8b	4-Me	Bn	3.6	0.35	3.9	29
8c	3-Me	Bn	1.0	0.24	3.8[b]	20
DMP851	H	*n*-Bu	11.7	7.2	7.7	57
12b	4-Me	*n*-Bu	1.2	0.07	1.6	23

[a]Oral bioavailability in dogs dosed at 10 mg/kg.
[b]Calculated from iv dosing at 5 mg/kg.

viral resistance. Our P1/P1′ analogues were tested against a panel of HIV mutant strains created by site directed mutagenesis including viruses with single, double, triple or quintuple amino acid changes in the protease gene.[11] Although similar improvements in potency were seen with a second wild type viral isolate HXB2, the substituted analogues did not show significantly increased potency against mutations that enlarge the S1/S1′ subsite (I84V, V82F/I84V).[12] For example, the *p*-methyl substituted derivative **8b** is 5-fold more potent against the wild type viruses RF and HXB2 but is only slightly better than **DMP 850** against the I84V single mutant. Other viral mutants that contain the common I84V mutation show a similar trend. The *meta*-methyl and *para*-methyl analogues are approximately equipotent to **DMP 850** and **DMP 851** against the V82F/I84V double and the ritonavir resistant virus. Interestingly, viruses without mutations in the S1/S1′ pockets did not show increased resistance to the P1/P1′ variants. The *p*-methyl analogues **8b** and **12b** gave an 11× improvement in potency compared to **DMP 850** and **DMP 851** against anamprenavir resistant virus which is mutated in both the S2/S2′ pockets and the hinge region of the flap.[13]

In order to examine the effects of P1/P1′ substitution on bioavailability we dosed the *para*-methyl **8b**, **12b** and *meta*-methyl **8c** analogues orally in dogs. The pharmacokinetic parameters are shown in Table 2. Compared to **DMP 850** and **DMP 851** the substituted analogues showed a significantly lower C_{max} and lower bioavailability. In addition, the 8 h trough blood levels were much lower and not sufficient to cover the protein binding adjusted IC_{90}s for the I84V mutant. Based on the overall pharmacokinetics, it was determined that these substituted analogues had insufficient oral bioavailability for further development.

In conclusion, we have shown that increasing the lipophilicity of the nonsymmetrical 3-aminoidazoles **DMP 850** and **DMP 851** by modification of the P1/P1′ residues improved the antiviral potency against wild type HIV. Based on the protein binding adjusted IC_{90}s and the resistance profile against HIV mutants, the substituted *meta*-methyl or *para*-methyl analogues are approximately equipotent to **DMP 850** and **DMP 851**. Unfortunately, the pharmacokinetics outweigh the improvements in the antiviral potency, eliminating these substituted analogues as potential development candidates.

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