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POTENT CYCLIC UREA HIV PROTEASE INHIBITORS WITH BENZOFUSED HETEROCYCLES AS P2/P2' GROUPS

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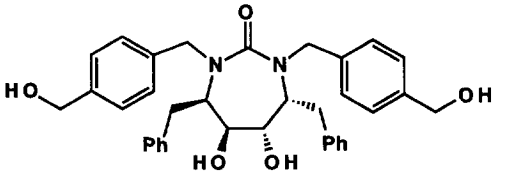
Abstract: A series of benzofused heterocycles was examined to replace the metabolically unstable benzyl alcohol P2/P2' groups of DMP 323 (**1**). Extremely potent inhibitors of HIV protease (Ki < 0.01 nM) and excellent antiviral activity (IC_{90} = 8 nM) were found. An X-ray crystal structure of benzimidazolone **5a** bound to HIV protease showed H-bonds to Asp30 and a bridging water molecule to Gly48.

Over the past decade, intensive research has provided insight into the life cycle of the human immunodeficiency virus (HIV), the causative agent of AIDS. Once the mechansims for HIV replication were understood, key enzymes were identified which then became targets for rational drug design. Our research efforts have focused on combating AIDS through inhibition of HIV protease, an essential enzyme for viral propagation.[1] Recent clinical trials support using HIV protease inhibitors to fight AIDS by reducing viral load and increasing CD4 cell count.[2]



DMP 323
Ki = 0.31 nM
IC_{90} = 100 nM

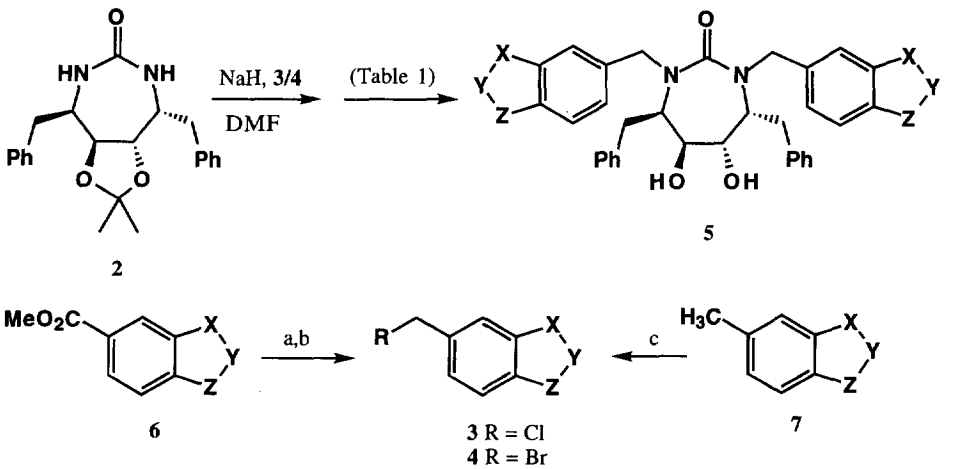
1

Previously, we reported that cyclic urea **1** is a potent inhibitor of HIV protease.[3] Unfortunately, clinical trials with **1** were abandoned due to low oral bioavailability.[4-6] A combination of poor solubility and metabolic instability of the benzylic hydroxymethyl groups accounts for the inadequate pharmacokinetic profile. In this manuscript, we report attempts to overcome the metabolic instability and increase the potency of **1** by incorporating the P2/P2' hydrogen bond donor/acceptor functionality into various benzofused heterocycles.

Attachment of heterocyclic P2 groups was accomplished by alkylation of urea **2**[6] with protected benzylic halides **3** or **4** (Scheme 1). The benzylic chlorides **3** were prepared starting from appropriately substituted esters **6**. Acidic N-H groups were protected with SEM groups (NaH, SEM-Cl, DMF), and the 2-amino group of

benzothiazole **3p** and benzimidazole **3r** (Table 1) was protected as the dimethylpyrrole (2,5-hexanedione, TsOH, $PhCH_3$). Reduction of the ester **6** with DIBAL-H gave the benzylic alcohol, which was converted to the chloride **3** with MsCl and i-Pr_2EtN in DMF. Benzylic bromides **4** were prepared by free radical bromination of the appropriately substituted, SEM protected toluene **7** [NBS, cat. $(PhCO_2)_2$, CCl_4]. Condensation of the benzylic halides **3** or **4** with urea **2** (NaH, DMF) gave symmetrical ureas, which were modified to the target heterocycles **5** as described in Table 1.

Scheme 1

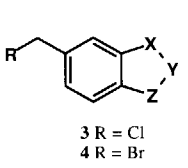


(a) DIBAL-H, CH_2Cl_2, -78 °C (b) MsCl, i-Pr_2EtN, DMF (c) NBS, cat. $(PhCO_2)_2$, CCl_4, reflux

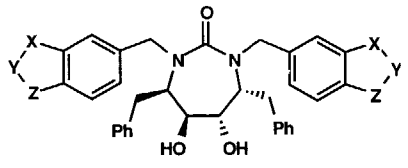
In varying the position of hydrogen bond donor/acceptor functionality, it became apparent that having a hydrogen bond acceptor at position Y (either sp^2 nitrogen or carbonyl) dramatically increased potency against HIV protease. In comparing indole **5f** (Ki = 1.3 nM) with oxindole **5d** (Ki = 0.14 nM) and oxazolidinone **5i** (Ki = 0.18), an order of magnitude improvement in Ki was observed by adding the carbonyl. An even more dramatic improvement was seen when the Y-carbon of indole **5f** was replaced with an sp^2 hybridized nitrogen. Indazole **5b** (Ki = 0.018 nM) showed two orders of magnitude improvement in potency over the indole **5f**. Having a hydrogen bond donor at the X or Z position was beneficial but the most potent compound, benzimidazolone **5a** (Ki < 0.01 nM), had an N-H at both positions. Having an NH_2 group at position Y was detrimental except for aminobenzoxazole **5j**, which had about the same Ki as benzoxazolidinone **5i**. Sulfur at the X and Z positions proved detrimental as did replacing the carbonyl oxygen of benzoxazolidinone **5i** with sulfur (**5l**). Enlarging the ring from five to six atoms by insertion of carbon between position X and Y (**5u,v**) markedly decreased activity.

As mentioned above, the most potent inhibitor of HIV protease was benzimidazolone **5a**. In order to understand the binding motif responsible for this activity, an X-ray crystal structure of **5a** bound to HIV protease was obtained (Figure 1). As was observed with DMP 323, the inhibitor binds symmetrically to the enzyme and the same interactions with the cyclic urea core are observed.[6] In addition, two favorable H-bonds are formed between the benzimidazolone ring and the backbone of the enzyme. The N-H at the Z position forms

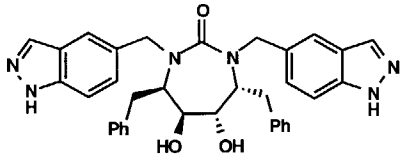
Table 1



3 R = Cl
4 R = Br



5



5b

Compound		3/4	Conditions	5	Ki (nM)[7]	IC_{90} (nM)[8]	-log Ki/IC_{90}	clogp[9]
	R	X-Y-Z		X-Y-Z				
a	Cl	N(P)-C(=O)-N(P)	a,b	NH-C(=O)-NH	<0.01	>65,000	>6.8	3.03 (2.72)
b	Br	CH=N-N(P)	c	CH=N-NH	0.018	8	2.6	4.43 (3.82)
c	Br	N(P)-N=CH	c	NH-N=CH	0.018	49	3.4	4.43
d	Br	$C(OMe)_2$-C(=O)-N(P)	a,b,d	CH_2-C(=O)-NH	0.14	370	3.4	3.09 (3.20)
e	Br	$C(OMe)_2$-C(=O)-N(P)	a,b	C(=O)-C(=O)-NH	0.81	8,500	4.0	1.91
f	Cl	CH=CH-N(P)	n	CH=CH-NH	1.3	190	2.2	5.07
g	Cl	N=CH=N(P)	c	N=CH-NH	11	19,000	3.2	3.56
h	Cl	N=N-N(P)	c	N=N-NH	0.09	530	3.8	3.47
i	Br	O-C(=O)-N(P)	c	O-C(=O)-NH	0.18	40	2.3	3.11 (3.54)
j	Br	O-C(=O)-N(P)	a,e,f	O-C(NH_2)=N	0.20	84	2.6	4.52
k	Br	O-C(=O)-N(P)	a,e,g	O-CH=N	3.4	440	2.1	3.97
l	Br	O-C(=O)-N(P)	a,e,h	O-C(=S)-NH	4.8	5,100	3.0	4.85
m	Br	X=NO_2	i,j	N=C(NH_2)-S	190	950	0.70	4.57
n	Br	X=NO_2	i,j,k	N=CH-S	28	3,200	2.1	4.81
o	Br	X=NO_2	i,j,l	NH-C(=O)-S	130	N.T.	-	4.34
p	Cl	S-C(DMP)=N	c	S-C(NH_2)=N	5.7	270	1.7	4.57
q	Cl	S-C(DMP)=N	c,l	S-C(=O)-NH	0.91	109	2.1	4.34 (4.22)
r	Cl	N=C(DMP)-N(P)	c	N=C(NH_2)-NH	5.8	8,100	3.1	2.91
s	Cl	N(P)-S$(O)_2$-N(P)	c	NH-S$(O)_2$-NH	0.19	32,000	5.2	2.31
t	Br	N(P)-C(=O)-O	a,b	NH-C(=O)-O	3.9	579	2.2	3.11
u	Cl	N=CH-C(=O)-N(P)	a,b	N=CH-C(=O)-NH	490	7,800	1.2	2.39
v	Cl	N=CH-C(=O)-N(P)	a,b,m	NH-CH_2-C(=O)-NH	6.1	7,700	2.1	1.42

P = SEM group; DMP = dimethylpyrrole group

Conditions: (a) TFA, $CHCl_3$, reflux (b) NaOH, MeOH (c) HCl, MeOH, reflux (d) H_2NNH_2, $HOCH_2CH_2OH$, reflux (e) NaOH, MeOH, reflux (f) BrCN, i-Pr_2EtN, CH_2Cl_2 (g) $(EtO)_3CH$, TsOH H_2O, EtOH (h) Im_2CS, THF (i) H_2, 10% Pd/C, EtOH (j) KSCN, $CuSO_4$, MeOH, reflux[10] (k) $NaNO_2$, H_2SO_4, H_2O, RT (l) $NaNO_2$, H_2SO_4, H_2O, 0 °C to room temperature (m) $NaBH_4$, MeOH (n) n-Bu_4NF, THF, ethylenediamine

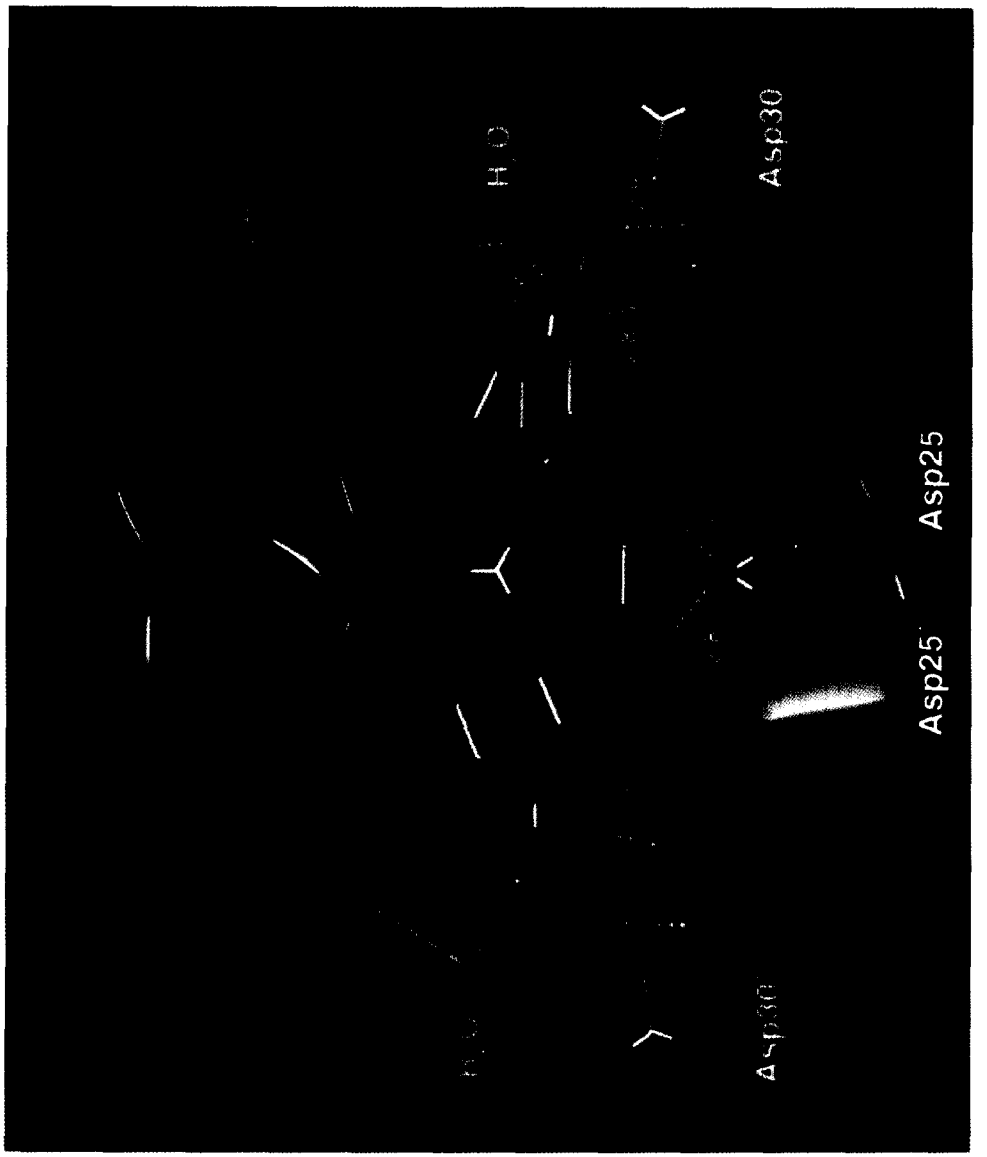


Figure 1. X-ray crystal structure of benzimidazolone **5a** bound to HIV protease. Only hydrogen bonded residues of the enzyme are shown for clarity. Hydrogen bonds are shown as dashed lines; distances are in Å.

a 2.8 Å hydrogen bond to the Asp30 carbonyl and the carbonyl oxygen at the Y position forms a 2.9 Å hydrogen bond to the Asp30 N-H. Unexpectedly, the benzimidazolone was much more potent than the benzoxazolone **5i** and the oxindole **5d**, which should be able to form the same H-bonds. A possible explanation came from further refinement of the X-ray, which revealed a bridging water molecule from the X nitrogen of **5a** to the enzyme flap Gly48 carbonyl. Although there is an analogous water at P2', it is not within hydrogen bonding distance of Gly48'. A bridging water may also account for the identical Ki's for the indazole isomers **5b** and **5c**. While the N-H of indazole **5b** may H-bond to Asp30 carbonyl, the N-H of indazole **5c** may form a water bridge to Gly48 carbonyl.

Although we were able to generate extremely potent inhibitors of HIV protease, this activity did not always translate to antiviral activity in the cell-based assay. In Table 1 we define translation as the negative log of the ratio of the Ki to the IC_{90} (i.e., the smaller the number the better the translation). While benzimidazolone **5a** was the most potent compound against HIV protease, it had very weak antiviral activity. Poor translation from the enzyme assay to the cell-based assay is likely due to problems associated with cell penetration and is commonly observed with polar compounds. For example, in comparing the series **5a**, **5d**, **5i**, and **5q**, the translation improved as the log P increased in going from NH, to CH_2, to O, to S at position X. Balancing potency, which comes from having polar H-bond donor/acceptor groups at P2/P2', with lipophilicity for good translation is thus a significant issue encountered in the design of HIV protease inhibitors. Fortunately, indazole **5b** strikes a good balance and the superb potency (Ki = 0.018 nM) translated well to antiviral activity (IC_{90} = 8 nM).

Encouraged by the improvement in activity over DMP 323, indazole **5b** was selected for in vitro metabolism and pharmacokinetic studies. Incubation with rat liver homogenates revealed no significant metabolic liability with indazole **5b**.[11] In addition, rat IV studies showed very low clearance (CL = 0.2 L/h/kg) and improved half life ($t_{1/2}$ = 2.7 h) over DMP 323 (CL = 7.1 L/h/kg; $t_{1/2}$ = 0.95 h).[5] Unfortunately, very low oral bioavailability was observed in rat (C_{max} = 0.03 ug/mL with 10 mg/kg dose). Since metabolism and clearance were likely not the problem, it is believed that low aqueous solubility (3 ng/mL in water)[12] resulted in poor absorption.

Further bioavailability studies with indazole **5b** in dog were somewhat more encouraging. Although clearance was higher in dog (1.1 L/h/kg) and the half life was slightly shorter ($t_{1/2}$ = 2.0 h), the C_{max} (0.6 ug/mL with 10 mg/kg dose) and oral bioavailability (F% = 13%) were both higher than for rat. Unfortunately, this low level of bioavailability is probably not sufficient for a sustained antiviral effect in man.

In conclusion, incorporating P2/P2' hydrogen bond donor/acceptor functionality into a benzofused heterocyclic ring has circumvented problems with metabolism associated with DMP 323. In addition, dramatic increases in both enzyme and antiviral activity were achieved. Although the indazole **5b** proved metabolically stable, low oral bioavailability was observed most likely due to poor aqueous solubility. Future work will focus on increasing solubility characteristics for better oral bioavailability.

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