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Synthesis and SAR Studies of Potent HIV Protease Inhibitors Containing Novel Dimethylphenoxyl Acetates as P_2 Ligands

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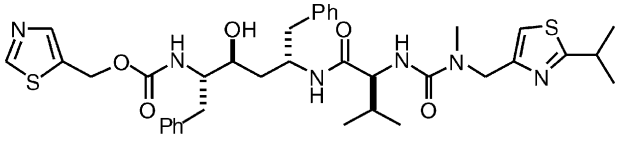
Abstract—Isopropyl substituted 4-thioazolyl valine side chains are highly optimized P_2–P_3 ligands for *C*2 symmetry-based HIV protease inhibitors, as exemplified by the drug ritonavir. Replacement of the side chain with the conformationally constrained hexahydrofurofuranyloxy P_2 ligand in combination with a dimethylphenoxyacetate on the other end of the ritonavir core diamine yielded highly potent HIV protease inhibitors. The in vitro antiviral activity in MT4 cells increased by 10- and 20-fold, respectively, in the absence and presence of 50% human serum compared to ritonavir. The structure–activity relationships of inhibitor series with this combination of ligands were investigated. Preliminary pharmacokinetic studies in rats indicated rapid elimination of the inhibitors from the blood, and the plasma levels were not significantly enhanced by coadministration with ritonavir. However, the novel structural features and the high intrinsic antiviral potency of this series provides potential for the future exploration of prodrug strategies.

Human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS). HIV protease is a virally encoded homodimeric aspartyl protease that is responsible for processing the *gag* and *gag/pol* gene products, allowing for the organization of the core structural proteins and release of the viral enzymes. Inhibition of this viral enzyme prevents the maturation and replication of the virus in cell culture. Thus, inhibition of HIV protease becomes one of the most important approaches for the therapeutic intervention of HIV infection. Recent clinical results have demonstrated that HIV protease inhibitors significantly reduce viral replication and substantially elevates CD4 cell levels in patients. Subsequent to the introduction of protease inhibitor-based combination therapy, the death rate due to HIV infection decreased markedly in western countries. Over the last several years, a wide variety of classes of peptidomimetic and nonpeptidic inhibitors have been reported based upon HIV protease substrate sequences and on the three-dimensional structure of the C_2-symmetric, homodimeric enzyme active site.[1] Despite the success of these agents, most members of the current generation of the protease inhibitors have limitations. Some are high protein bound to plasma proteins, which greatly reduce in vivo antiviral activity. Relatively frequent dosing is needed in order to achieve viral suppression at plasma trough levels. Poor adherence to therapy as a result of high pill burden and/or adverse events may compromise treatment efficacy and allow the emergence of resistant mutants. Consequently, new structurally diverse HIV protease inhibitors that can maintain high blood concentrations and possess high activity against resistant viruses are still needed.



Ritonavir

We previously reported the discovery of ritonavir, a potent HIV protease inhibitor with high oral bioavailability and long plasma half-life.[2] The in vitro antiviral activity of ritonavir is attenuated by 10- to 20-fold due to the high protein binding, mainly to human serum albumin and the α-acid glycoprotein.[3] Although ritonavir has

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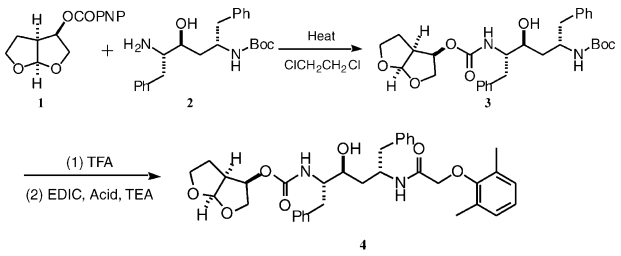
high blood concentration in human, monotherapy with ritonavir ultimately selects resistant virus in many patients. Analyzing the patterns of resistance emergence revealed that an initial change at valine-82 in HIV protease in majority of patients.[4] Due to the V82 mutation to alanine, threonine, phenylalanine or serine, the hydrophobic interaction between the thiazolyl isopropyl group of ritonavir and the residue 82 side chain is no longer optimal, and isolates containing these amino acid changes display reduced phenotypic susceptibility to ritonavir. As part of our program to continue to explore inhibitors structurally related to ritonavir, we investigated protease inhibitors that lacked the aforementioned hydrophobic interaction under the assumption that such inhibitors would be less likely to select V82 mutants and to be cross-resistant to ritonavir-selected mutants. In addition, we were interested in identifying inhibitors with improved protein binding compared to ritonavir. Here, we report a novel class of HIV protease inhibitors that are 10- and 20-fold more potent than ritonavir in vitro in the absence and presence 50% human serum, respectively.

Initially, we hypothesized that inhibitors with side chains that primarily interact with the backbone amide linkages of the protease would provide for improved binding affinities and a profile with less cross-resistance, since backbone amides would be expected to undergo minimal change in position during mutation. The hexahydrofurofuranyloxy group has previously been shown to function as a conformationally constrained P_2 ligand in which each of the two ether oxygen atoms of the furofuran hydrogen bonds to the NH groups of Asp29 and Asp30, respectively, of the viral protease.[5] This additional hydrogen binding is strong enough to compensate the loss of binding interactions at the S3 site; thus, truncated, lower molecular weight inhibitors display highly potent (lower nM) inhibition.

As a further modification of the structure of ritonavir, we chose to replace the P2′-thiazolyl group. This group has been shown to exhibit type-II binding to the heme of cytochrome p450 (CYP) 3A4 active site resulting in potent inhibition of this hepatic enzyme,[6] and its replacement was deemed advantageous for avoiding strong CYP inhibition. Since HIV-PR is a C_2-symmetric, homodimeric enzyme, we initially investigated pseudo-C_2-symmetric inhibitors containing the furofuran ligands attached to the symmetry-based core diamine of ritonavir (**2**). Although these inhibitors displayed lower nM level activity against HIV in vitro, initial pharmacokinetic studies produced very low bioavailability due to limited aqueous solubility. Researchers from Boehringer Ingelheim have reported inhibitors with a dimethylphenoxyl acetate as a P_2 ligand.[7] The simplicity of this P_2 ligand was appealing, furthermore, the potential of this ligand for symmetry-based inhibitors (e.g., compound **4**) was further supported by the computer assisted modeling studies indicating a suitable fit into the S2 site of the protease.

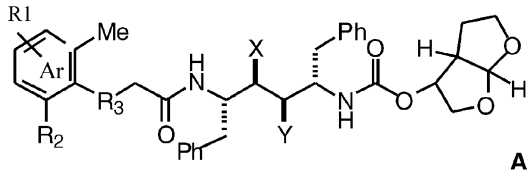
The enantiomeric bis-tetrahydrofuranes were synthesized and characterized according to literature precedent[5] and converted to the corresponding activated carbonates **1** by treatment with *p*-nitrophenyl chloroformate and *N*-methyl morpholine (Scheme 1). The activated carbonates were heated with the Boc protected diamine core **2**[8] to furnish the key intermediate **3**. After removal of the Boc protecting group of **3**, treatment with a 2-substituted acetic acid under standard peptide coupling conditions provided the desired inhibitors **4**. All final compounds showed satisfactory purity by ^{1}H NMR and mass spectral analysis. The IC_{50} values for members of this series against HIV protease and the anti-HIV activity (EC_{50}) and cytotoxicity ($CCIC_{50}$) of each inhibitor in MT4 cells using a cytopathicity assay were measured according to reported methods.[9] We also measured the EC_{50} in MT4 cells in the presence of 50% human serum as an empirical estimate of the effect of protein binding on the activity of the inhibitors.[3] The results are shown in Table 1.

In a previous study,[10] we observed different binding affinities to HIV protease depending upon the proximal or distal relationship of the furofuran to the central hydroxyl group of the core diamine because of unsymmetric binding of the core to the active site. As has been observed in other series, the 3*R*-furofuran was found to be a better P_2 ligand than the 3*S*-furofuran in this series. However, protease inhibition activity was not especially sensitive to the position of the hydroxyl group relative to the 3R-furofuran moiety, as demonstrated by the similar inhibitory potency of compounds **4** and **5**. Dimethyl substitutions for the phenyloxy acetate group were found to be optimal for potent activity. Compounds **6**, **7** and **5**, which contained 0–2 methyl groups on the 2- and 6-position of the phenyl ring, respectively, displayed a corresponding increase in potency from 61% inhibition to 87% inhibition at 0.5 nM. Replacement of one of the methyl groups on the phenyl ring with a hydroxymethyl group was tolerated without loss of protease inhibitory potency (compound **8**). Replace methyl group with an *O*-methyl group (compound **9**) resulted in significant loss of antiviral activity. The phenyl group was also highly preferred to a pyridine or pyrimidine group, and analogues **10** and **11** displayed substantially diminished anti-HIV activity. Replacement of the oxygen linker with a NH (compound **12**) or NMe linker (compound **13**) produced a modest decline in inhibitory activity from 87% for the parent compound **5** to 69 and 50% inhibition at 0.5 and 2.4nM, respectively. This is in agreement with a much weaker EC_{50} of **13**. Replacement with a sulfide, sulfoxide or sulfone group dramatically lowered potency (compounds **14**–**16**) comparing with parent oxygen linker compound



Scheme 1. Synthesis furofuran containing HIV protease inhibitors.

Table 1. Inhibition of HIV protease by furofuran containing analogues of **A**



A

No.	R1	R2	R3	Ar	X	Y	FF	% In[a]	nM	EC_{50} (0%)μM[b]	EC_{50} (50%)μM[c]
Ritonavir	—	—	—	—	—	—	—	78	0.5	0.07	0.81
4	H	Me	O	Ph	OH	H	*R*	91	0.5	0.061	0.272
5	H	Me	O	Ph	H	OH	*R*	87	0.5	0.041	0.3
6[d]	H	H, H	O	Ph	H	OH	*R*	61	0.5	ND	ND
7	H	H	O	Ph	H	OH	*R*	84	0.5	ND	ND
8	H	CH_2OH	O	Ph	H	OH	*R*	88	0.5	0.037	0.197
9	H	OMe	O	Ph	H	OH	*R*	74	0.5	0.353	1.38
10	H	Me	O	3-Py	H	OH	*R*	49	0.5	0.184	0.438
11	H	Me	O	Primidine	H	OH	*R*	56	0.5	0.815	1.39
12	H	Me	NH	Ph	H	OH	*R*	69	0.5	0.253	2.146
13	H	Me	NMe	Ph	H	OH	*R*	50	2.4	2.914	30.22
14	4-NH2	Me	S	Ph	H	OH	*R*	56	1	5.793	1.04
15	4-NH2	Me	SO2	Ph	H	OH	*R*	50	12	10.33	61.82
16	4-NH2	Me	SO	Ph	H	OH	*R*	50	31	37.47	98.44
17	4-NH2	Me	O	Ph	H	OH	*R,S*	70	0.5	0.012	0.067
18	4-NH2	Me	O	Ph	H	OH	*R*	78	0.5	0.009	0.031
19	4-NH2	Me	O	Ph	H	OH	*S*	60	0.5	0.047	0.129
20	3-NH2	Me	O	Ph	H	OH	*R*	87	0.5	0.005	0.035
21	3-OH	Me	O	Ph	H	OH	*R*	92	0.5	0.007	0.098
22	4-OH	Me	O	Ph	H	OH	*R*	82	0.5	0.032	0.076
23	4-NHMe	Me	O	Ph	H	OH	*R,S*	64	0.5	0.112	0.507
24	4-NMe2	Me	O	Ph	H	OH	*R*	43	0.5	0.16	2.071
25	4-NHMs	Me	O	Ph	OH	H	*R*	50	12	ND	ND
26	4-Me	Me	O	Ph	H	OH	*R*	82	0.5	0.205	0.479
27	3-NH2,4-Me	Me	O	Ph	H	OH	*R*	74	0.5	0.023	0.117
28	3-NH2-4-Cl	Me	O	Ph	H	OH	*R*	86	0.5	0.062	0.659
29	3,4-Diamine	Me	O	Ph	H	OH	*R*	76	0.5	0.644	1.476
30	3-NHCO-4-NH	Me	O	Ph	H	OH	*R*	85	0.5	0.972	3.109
31	4-OCO-3-NH	Me	O	Ph	H	OH	*R*	61	0.5	0.16	0.501
32	4-OCH2CH2Mop	Me	O	Ph	H	OH	*R*	77	0.5	0.024	0.34
33	4-F	Me	O	Ph	H	OH	*R*	69	0.5	0.268	0.866
34	4-NO2	Me	O	Ph	H	OH	*R,S*	53	0.5	0.927	8.111
35	4-CONH2	Me	O	Ph	H	OH	*R*	ND	0.5	0.255	0.987
36	4-CONHMe	Me	O	Ph	H	OH	*R*	ND	0.5	0.22	0.533
37	4-CONMe2	Me	O	Ph	H	OH	*R*	ND	0.5	2.307	11.89
38	COOEt	Me	O	Ph	H	OH	*R*	84	0.5	0.106	3.215
39	4-N-Oxazolinone	Me	O	Ph	H	OH	*R*	74	0.5	0.214	0.93
40	NSN	Me	O	Ph	H	OH	*R*	71	0.5	0.621	5.647
41	Triazole	Me	O	Ph	H	OH	*R*	67	0.5	0.188	1.879

[a]Percentage of inhibition of HIV protease was measured in the presence of inhibitor at indicated concentration.
[b]Antiviral activity was tested in the presence of 0% human serum.
[c]Antiviral activity was tested in the presence of 50% human serum.
[d]Phenyl without any methyl substitutions.
ND, no data; FF, hexahydrofurofuranyl; 5-Pym, 5-pyrimidine; 3-Py, 3-pyridine; 3,4-BIMD, benzimidazole; 3-NHCO-4-NH, 3,4-benzoimidazolone; 4-OCO-3-NH, benzoxazolone; 3,4-NSN, benzo-thiadiazole; 3,4-triazole, benzotriazole.

18. The preference (*R*)-furofuran and (*S*)-furofuran was not particularly apparent when the furofuran occupied a position distal to the hydroxyl group. Compound **18** inhibited 78% HIV protease activity at 0.5 nM compared to 60% inhibition by compound **19** at the same concentration.

The effect of substitutions on the phenyl ring upon the antiviral activity was extensively explored. Electron-withdrawing groups (F, NO_2, COOEt, amides) on the phenyl ring generally resulted in weak inhibitory potency (compounds **33**–**38**), while electron-donating groups enhanced the antiviral activity. Thus, compounds bearing an amino and a hydroxyl substituted phenoxyl acetate group (compounds **18**–**22**) were the most active compounds in this series. Substitution on the 3-position of the phenyl ring was preferred to the substitution on the 4-position. For example, compound **20** with a 3-amino group on the phenyl ring displayed higher inhibitory potency than 4-amino compound **18**. Similarly, 3-hydroxyl substituted analogue **21** was also more potent than the 4-hydroxy substituted inhibitor **22**. Although the hydroxyl substitution yielded more potent compounds than the corresponding amino substituted analogues in the protease inhibition assay, the amino substituted analogues showed slightly higher antiviral activity in the antiviral assay, both in the absence and presence of 50% human serum. Substituents at the 3- and 4-position did not produce additive activity. Thus, 3,4-diamino analogue **29** was

relatively weakly active, with an EC_{50} of 0.6μM. Alkylated amino analogues (compounds **23**–**24**) as well as mesylation analogue **25**, displayed disappointing potency. Compound **30**, which connected 3,4-diamino groups with a carbonyl group, was a potent enzyme inhibitor but displayed weak antiviral activity, possibly due to poor cellular penetration. Compound **32**, with a 4-hydroxy group further substituted with a relatively large morpholino ethyl group, inhibited 77% of protease activity at 0.5 nM and produced an EC_{50} of 24 nM. Molecular modeling of compound **32** in the protease active site revealed that the morpholine ethyl side chain did not participate in the interaction with the enzyme, but rather extended out of the active pocket into the solvent. It is possible that the 4-position of the phenyl ring could be used as an anchor for making various prodrugs to modify the physicochemical properties of the inhibitors in order to achieve the desired pharmacokinetic profile. Additional dimethyl substituted heterocyclic analogues (**39**–**41**) were explored without achieving compounds with increased antiviral activity.

Compounds **18** and **20** were dosed in rats via intravenous and oral dosing. Both compounds were characterized by high clearance, low plasma exposure and negligible oral bioavailability. Interestingly, the oral pharmakinetic profile of compound **18** was not enhanced by coadministration with ritonavir, suggesting that its major metabolic pathway in rats is not due to CYP450 3A4 oxidative elimination. However, the exact in vitro and in vivo metabolic fate of compound **18** has not been fully elucidated.

We discovered a new series of highly potent HIV protease inhibitors by replacing the side chains of ritonavir with a hexahydrofurofuranyloxy group as a conformationally constrained P_2 ligand and a dimethylphenoxyl acetate as a P_2' ligand on the other end of the core diamine **2**. The in vitro antiviral activity (EC_{50}) in MT4 cells increased by 10- and 20-fold, respectively, in the absence and presence of 50% human serum by comparing with ritonavir. Although the most potent compound did not give satisfactory oral bioavailability and high plasma exposure, the novel structural features and the high intrinsic antiviral potency of this series provides the basis for future analogues or orally bioavailable prodrugs.

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