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NONPEPTIDIC HIV PROTEASE INHIBITORS: 6-ALKYL-5, 6-DIHYDROPYRAN-2-ONES POSSESSING ACHIRAL 3-(4-AMINO/CARBOXAMIDE-2-*t*-BUTYL, 5-METHYLPHENYL THIO) MOIETY: ANTIVIRAL ACTIVITIES AND PHARMACOKINETIC PROPERTIES

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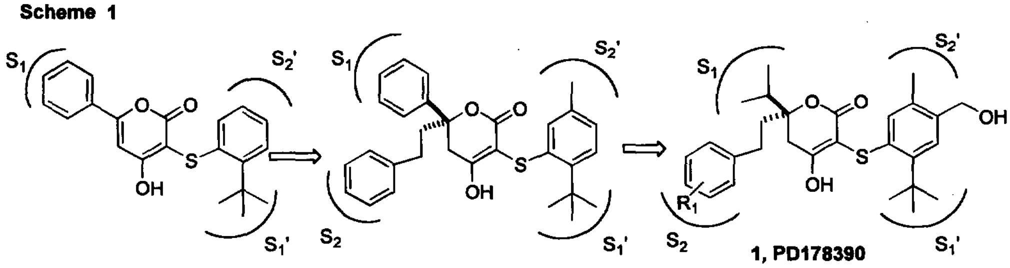
Abstract: Dihydropyran-2-ones possessing amino and carboxamide functionalities on 3-SPh (2-*tert*-butyl, 5-methyl) ring were synthesized and evaluated for their antiviral activities. Both the enantiomers of inhibitor **15** were synthesized. The in vitro resistance profile, inhibitory activities against cytochrome P450 isozymes and pharmacokinetic properties of inhibitor **15*S*** will be discussed.

Recently, four protease (PR, an enzyme essential for viral replication) inhibitors,[1] were approved for the treatment of human immunodeficiency virus (HIV) infection.[2] In view of the mutations that arise during monotherapy and the drug–drug interactions with these agents, there has been sustained interest to design structurally novel and small molecule leads for the inhibition of HIV replication.[3,4] Structure–activity studies directed at derivatization of an original mass screening lead[5] resulted in a core ring modification[6] to give 6-alkyl-5, 6-dihydropyran-2-ones (Scheme 1), which exhibited excellent antiviral activities.[7] An X-ray crystal structure of **1** (**PD 178390**, our lead inhibitor) bound to HIV PR showed that it occupies only the inner four pockets of the enzyme. Herein we describe our efforts to probe S_3'/S_4' pockets (Scheme 1) by replacement of the CH_2OH with amino and carboxamide functionalities[8] (inhibitor **1**), including antiviral activities and animal pharmacokinetics of selected inhibitors.

Scheme 1



IC_{50}: 17 nM at pH 4.7

IC_{50}: 10 nM at pH 4.7

R_1 = OH; K_i: 0.09 nM; EC_{50}: 0.46 μM; TI: >200
R_1 = NH_2; K_i: 0.11 nM; EC_{50}: 0.2 μM; TI: 1050

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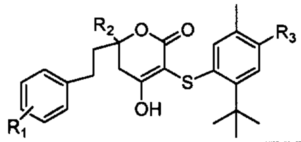
Table 1. 5,6-Dihydropyran-2-ones containing amine and carboxamide functionality and their HIV PR binding affinities (IC_{50}) tested in vitro, antiviral activities (EC_{50}), and toxicities (TC_{50}).[a]



Entry	R_1	R_2	R_3	IC_{50} (nM)[b]	EC_{50} (μM)[c]	TC_{50} (μM)[d]	Therapeutic Index
10[e]	4-OH	methyl	OH	6.5	>7	7	1
11	4-OH	methyl	NH_2	9.6	9.4	66	7
12	4-OH	*n*-propyl	NH_2	3.8	4.1	62	15
13	4-OH	*n*-butyl	NH_2	4.4	3.7	25	7
14	4-OH	*n*-pentyl	NH_2	7.7	>24	24	1
15	4-OH	iso-propyl	NH_2	2.7	1.0	92	92
16	4-OH	iso-butyl	NH_2	13.5	8.1	62	8
17	4-OH	cyclopropyl	NH_2	4.1	2.5	49	20
18	4-OH	cyclopentyl	NH_2	6	3.8	82	22
19	4-OH	cyclohexyl	NH_2	20	10	66	7
20	4-NH_2	iso-propyl	NH_2	7.5	0.9	30	33
21	H	iso-propyl	NH_2	3.3	1.9	69	36
22	H	phenyl	NH_2	11	>64	64	1
23	4-OH	iso-propyl	$NHCOCH_3$	3.4	4.8	>100	>21
24	4-OH	iso-propyl	NHCOPh	13.2	5.4	66	12
25	4-OH	iso-propyl	NHCOPh(4-CN)	5	4.1	66	16
26	4-OH	iso-propyl	NHCOPh(3-CN)	15	14	66	5
27	4-OH	iso-propyl	NHCOPh(4-CF_3)	12.1	5	27	5
28	4-OH	iso-propyl	NHCO(2-pyridyl)	6.1	1.5	66	44
29	4-OH	iso-propyl	NHCO(3-pyridyl)	5.1	4.9	>100	>20
30	4-OH	iso-propyl	NHCO(4-pyridyl)	5.08	5.1	>100	>20
31	H	iso-propyl	$NHCOCH_3$	3.5	1.8	83	46
32	H	iso-propyl	NHCOPh(4-CN)	14	8.3	46	6
33	4-OH	iso-propyl	$NHCOCH_2$*t*-Bu	60	16	67	4
34	4-OH	iso-propyl	NHCOO*t*-Bu	91	ND	ND	--
35	4-OH	iso-propyl	$N(CH_3)_2$	11	1.9	68	36

[a]All the compounds tested are racemic. [b]values are the average of at least two determinations. [c]EC_{50} indicates the concentration of the drug which provide 50% protection against HIV. [d]TC_{50} is the concentration of the drug, which elicits cytotoxicity in 50% of uninfected cells. [e]taken from ref 9.

Replacement of the *para*-hydroxyl group on the phenethyl moiety with a *para*-amino group in **15** to give **20** resulted in similar inhibitory activity against HIV PR and antiviral activity compared to **15**. However, this compound is more toxic compared to **15** resulting in a lower therapeutic index. Removal of this polar group in **15** and **20** to give **21** resulted in an inhibitor with similar enzymatic binding, but a two-fold reduction in antiviral activity. Replacement of isopropyl group with a phenyl ring led to **22**, which showed no antiviral activity. From these studies, it appears that an alkyl group at 6-position of the core ring (**21** vs **22**) is beneficial towards the antiviral activity in this series of protease inhibitors, whereas the polar substitution on the phenethyl moiety (**15**, **20** vs **21**) results in a slight enhancement (two-fold) in antiviral activity.

5,6-Dihydropyran-2-ones containing carboxamide moiety: In this series (**23–30**), the 6-position iso-propyl and the 4-hydroxylphenethyl groups were kept constant and various groups were placed on the carboxamide moiety to better interact at the S_3' pocket of the enzyme. Overall, all these carboxamides showed less antiviral activities as compared to the parent aniline **15**. The best inhibitor in this series **28**, showed EC_{50} of 1.5 μM with a therapeutic index of 44. In the case of N-acetyl derivative, polar group on 6-phenethyl group decreased antiviral activity by >two-fold (**23** vs **31**), though both exhibited similar binding affinities to HIV PR. However, in the case if 4-cyanophenyl amide analog removal of polar group reduced antiviral activity and enzymatic inhibitory activity by two-fold (**25** vs **32**). The analogs containing a *tert*-butylmethyl carboxamide, **33** or *tert*-butoxycarbonyl group (**34**) showed significantly less enzymatic binding affinity (6- to 20-fold) when compared to the aryl carboxamides. When the aniline moiety was dimethylated in compound **15** to derive **35**, it showed a four-fold decrease in enzymatic binding, and a two-fold decrease in antiviral activity, indicating the importance of hydrogens on the nitrogen.

Chiral 5,6-dihydropyran-2-ones: Since inhibitor **15** showed the best therapeutic index among the compounds described above, optically pure enantiomers of **15** were synthesized.[9] Their enzymatic binding affinities and antiviral activities are shown in Table 2. The *S*-enantiomer **15*S*** showed a K_i of 70 pM and is ten-fold more active compared to racemic compound, **15** (K_I = 670 pM). The other enantiomer, **15R** showed a K_i of 13 nM and is >180-fold and 19-fold less active compared to its **15S** and **15**, respectively. Not surprisingly, inhibitor **15S** also showed EC_{50} of 0.5 μM with a therapeutic index of 422. Molecular modeling studies showed that **15*S*** binds to HIV PR in a mode similar to that of **1** (Figure 1).

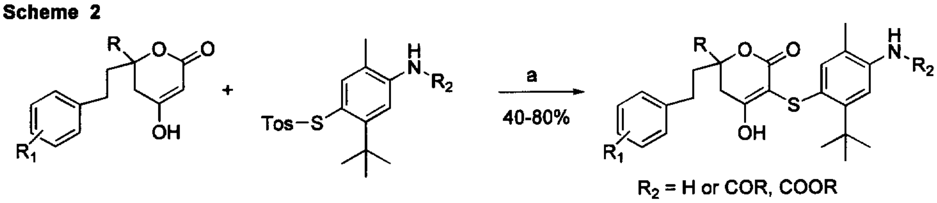
Table 2. Chiral 5,6-dihydropyran-2-one analogues and their HIV PR binding affinities and antiviral activities

Entry	K_i (nM)	Rotation in degrees (c, solvent)	EC_{50} (μM)	EC_{90} (μM)	TC_{50} (μM)	Therapeutic index
15	0.67	--	1.0	3.5	92	92
15*S*	0.07	+55.73 (1, MeOH)	0.5	1.0	211	422
15*R*	13	-56.8 (1, MeOH)	91	--	211	2

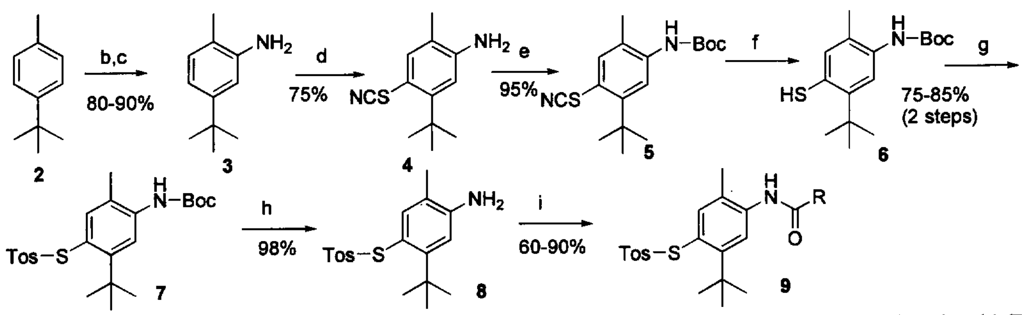
Since **15*S*** possessed an excellent therapeutic index, we further evaluated its inhibitory activity against mutant enzymes in vitro, enzymatic specificity, inhibitory activity against selected cytochrome P450 isozymes and pharmacokinetic properties. When tested against V32I, V82F, I84V, V32I/I84V and V82F/I84V mutant enzymes in vitro, compound **15*S*** showed a 6-, 25-, 49-, 46-, 24-fold increase in inhibitory activity, which is in contrast with our pre-clinical lead compound **PD178390**.[9] When **15*S*** was tested against other human aspartic proteases (renin, human cathepsin D, recombinant cathepsin E, native gastricsin and recombinant human pepsin) a selectivity index for HIV PR >5000 was observed. Inhibitor **15S** was also assessed for its effectiveness against various HIV strains employing HIV-1 infected PBMC cells, where it displayed EC_{50}s in the range of 0.2–0.74 μM. To determine the bioavailability, mice and dog were dosed PO with **15** and **15*S*** and their pharmacokinetic properties are shown in Table 3.

Synthesis: Inhibitors **11–35** were prepared by the sulfenylation of 6,6'-disubstituted-5,6-dihydropyran-2-ones with the corresponding thiotosylate in the presence of anhydrous K_2CO_3 (Scheme 2).[4,5] Thiotosylates possessing amine/carboxamide functionality (**8/9**) were synthesized as shown in Scheme 3. Commercially available *tert*-butyl-4-methylbenzene (**2**) was nitrated and further reduced to the corresponding aniline (**3**). Thiocyanation of the **3** with thiocyanate and bromine (**4**), followed by Boc protection of amine functionality furnished compound **5**. Reduction of thiocyanate to thiol (**6**) and tosylation with tosyl bromide gave thiotosylate **7**. Deprotection of Boc group furnished thiotosylate **8**. Compound **8** was further elaborated to **9**, via amide bond formation. All 5,6-dihydropyran-2-ones were synthesized as described previously.[7,9,10] All in vitro binding affinities were determined at pH 6.2.[7] Anti-HIV activities (EC_{50}, EC_{90}, TC_{50}) were assessed in a cell based assay with HIV-IIIB strain infected human lymphocyte derived CEM cells using XTT cytopathic method at Southern Research Institute and are shown in Table 1.[11]

Scheme 2



Scheme 3



(a) anhyd K_2CO_3, 6,6-disubstituted-2-one, DMF; (b) HNO_3, H_2SO_4; (c) 20% Pd/C, hydrogen (d) NaSCN, NaBr, bromine; (e) $(Boc)_2O$, 1 N NaOH; (f) DTT, ethanol, 0.02 M KH_2PO_4 buffer; (g) Tosyl bromide, Et_3N; (h) 4 N HCl, dioxane; (i) acid chloride, Et_3N.

Results and Discussion

5,6-Dihydropyran-2-ones possessing aniline functionality: Initial studies were focussed on probing the S_1 pocket of the enzyme, while maintaining the S_2 (4-hydroxyphenethyl group), S_1' and S_2' (3-(4-amino-2-*tert*-butyl-5-methylphenylthio) moiety) interactions constant. The 5,6-dihydropyran-2-one containing a 4-amino group on 3-(2-*tert*-butyl-5-methylphenylthio) moiety, **11** showed similar HIV PR binding affinity as phenolic derivative **10**, but displays less toxicity. Encouraged with this result, amines possessing straight chain as well as cyclic alkyl groups of varied steric bulk (**12–19**) were synthesized. It appears that either a small (**11**) or bulky (**14**, **16**, **19**) alkyl group at 6-position is detrimental to the binding affinity to the enzyme as well as antiviral activity. The best inhibitor in this series **15** showed an EC_{50} of 1 μM with a therapeutic index of 92.

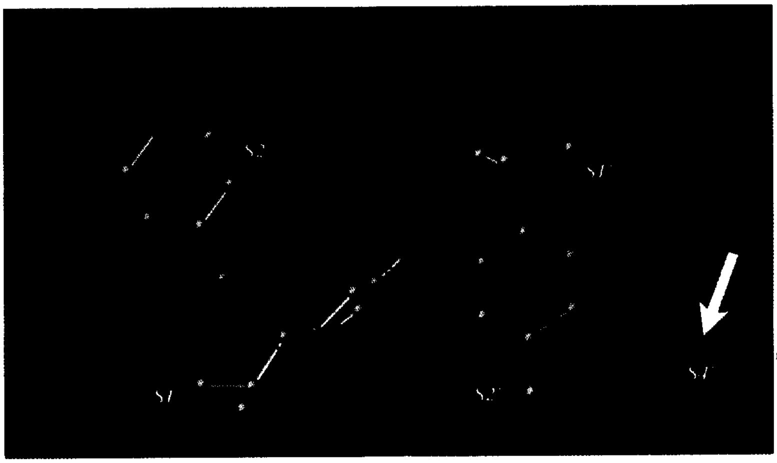


Figure 1. Model of **15*S*** bound in the HIV protease (green) X-ray crystal structure. The binding subsites are highlighted. The atoms of the inhibitor are colored by atom type (C-white, H-cyan, O-red, N-blue, S-yellow).

Table 3. Pharmacokinetics of **15** and **15*S***.

Animal	Dose	Inhibitor	$t_{1/2}$ (h)	C_{max} (μM)	AUC (μM*h)	Hours above EC_{90}	Bioavailability[c] (%)
Mouse[a]	25 mg/kg	**15**	2.1	21.0	44.82	3.2	59.2
Mouse[a]	25 mg/kg	**15*S***	1.2	21.7	30.03	4.0	100
Dog[b]	10 mg/kg	**15*S***	1.7	71	138	8.5	56

[a]vehicle: 20% 0.1 N NaOH/80% Methyl Cellulose [b]NaOH, buffered to pH 7.4 [c]mouse bioavailability is the comparison of AUC of subcutaneous dosing and AUC of PO dosing, whereas dog bioavailability is the comparison of AUC of IV and AUC of PO.

Inhibitor **15*S*** was tested against cytochrome P-450 isozymes, a study undertaken shed light on potential drug–drug interactions.[13] Inhibitor **15*S*** does not inhibit CYP3A4 or CYP2D6 isozymes when tested up to 100 μM concentration. However, **15*S*** do inhibit CYP2C9 with an IC_{50} around 100 μM. These results probably suggest that **15*S*** may show fewer drug interactions with drugs known to be metabolized by these three Cytochrome P-450 isozymes.

Conclusions. Overall, SAR studies showed that amine functionality is preferred when compared to carboxamide functionality to achieve better antiviral activities. The best inhibitor **15S** (also known as **PD 178392**) exhibited antiviral activity (EC_{50}: 0.5 μM) with an excellent therapeutic index (422), selectivity towards HIV PR, promising pharmacokinetic properties and favorable activity against Cytochrome P-450 isozymes. This inhibitor possesses low molecular weight and one chiral center.

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