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NONPEPTIDIC HIV PROTEASE INHIBITORS: 6-ALKYL-5,6-DIHYDROPYRAN-2-ONES POSSESSING A NOVEL AND ACHIRAL 3-(2-*t*-BUTYL-5-METHYL-4-SULFAMATE)PHENYLTHIO MOIETY.

J.V.N. Vara Prasad,[a]* Larry J. Markoski,[a] Fred E. Boyer,[a] John M. Domagala,[a] Edmund L. Ellsworth,[a] Christopher Gajda,[a] Susan E. Hagen,[a] Bradley D. Tait,[a] Elizabeth A. Lunney,[a] Peter J. Tummino,[b] Donna Ferguson,[b] Tod Holler,[b] Donald Hupe,[b] Carolyn Nouhan,[b] Stephen J. Gracheck,[c] Steven VanderRoest,[c] James Saunders,[c] K. Iyer,[d] M. Sinz[d]

Departments of Chemistry,[a] Biochemistry,[b] Infectious Diseases[c] and PDM,[d] Parke-Davis Pharmaceutical Research , Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48106, U.S.A.

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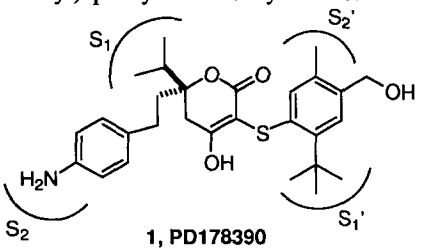
Abstract: Dihydropyran-2-ones possessing a sulfamate moiety at the 4-position of the thiophenyl ring were designed to reach S_3' pocket of the HIV protease. Synthetic routes for the preparation of thiotosylates possessing 3-(2-*t*-butyl-5-methyl-4-sulfamate) phenylthio moiety were established. SAR of various sulfamate analogs including HIV protease binding affinities, antiviral activities and therapeutic indices will be described.

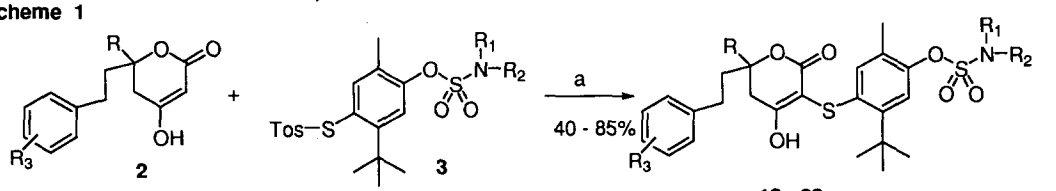
Recently we reported nonpeptidic human immunodeficiency virus (HIV) protease (PR) inhibitors,[1] namely, 6-alkyl-5,6-dihydropyran-2-ones exhibiting excellent antiviral activities; among them PD 178390 (**1**) was selected as a preclinical lead inhibitor.[2,3] X-ray crystal structure of **1** bound to HIV PR showed that the 4-hydroxyl group was hydrogen bonded to catalytic aspartic acid residues and the lactone moiety was hydrogen bonded with Ile50 and Ile150 residues in the homodimeric enzyme. This lead inhibitor **1** occupies only the inner four pockets of the enzyme. We replaced the 4-hydroxymethyl functionality from the 3-(2-*tert*-butyl-4-hydroxymethyl-5-methyl) phenylthio moiety with sulfamates in order to reach an additional pocket of the



1, PD178390

K_i = 0.11 nM; IC_{50} = 1 nM
EC_{50} = 0.2 μM; TI = 1050

Scheme 1



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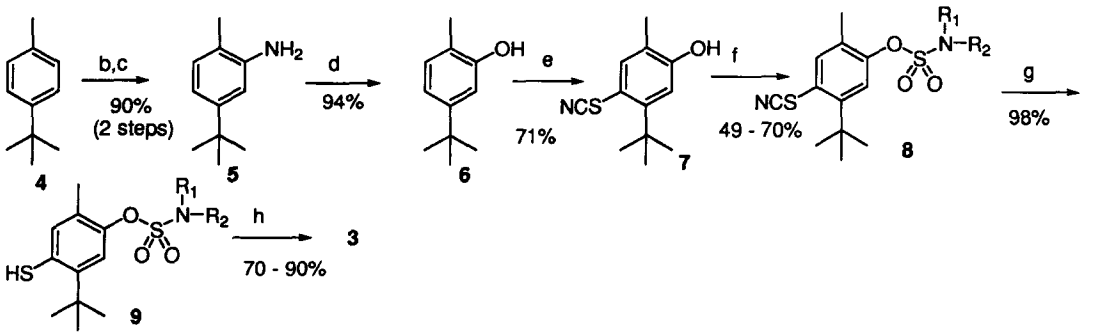
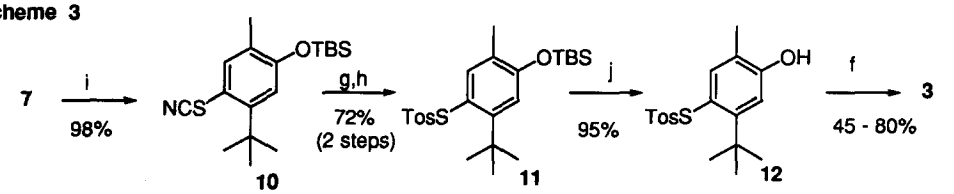
enzyme.[4,5] Herein we report SAR of these 5,6-dihydropyran-2-one analogs including HIV PR binding affinities, antiviral activities and therapeutic indices. Also included are cytochrome P450 isozymes inhibition data and pharmacokinetics for selected inhibitors.

Synthesis: Inhibitors[6] **13–33** were prepared by the sulfenylation of 6,6-disubstituted-5,6-dihydropyran-2-ones with the corresponding thiosulfonates in the presence of anhydrous K_2CO_3 (Scheme 1).[3] Thiosulfonates possessing sulfamate functionality were synthesized as shown in Schemes 2 and 3. Commercially available *tert*-butyl-4-methylbenzene (**4**) was nitrated and further reduced to the corresponding aniline (**5**). Diazotization of **5** followed by hydrolysis afforded the corresponding phenol (**6**). Thiocyanation of **6** with sodium thiocyanate and bromine furnished compound **7**, which upon further treatment with sulfamyl chloride afforded sulfamates (**8**). Reduction of thiocyanate to thiol (**9**) and tosylation with tosyl bromide gave thiotosylate **3**. Alternatively, compound **7** on treatment with TBS chloride in the presence of a base furnished the corresponding TBS ether (**10**). Further conversion of thiocyanate to thiol and tosylation afforded thiotosylate **11**. Deprotection of the TBS group afforded **12**, which upon treatment with sulfamyl chloride[7] in the presence of base furnished thiotosylate **3**. All 5,6-dihydropyran-2-ones were synthesized as described previously.[3] All in vitro binding affinities were determined at pH 6.2[2]. Anti-HIV activities were assessed in an in vitro cell based assay[8] with HIV-IIIB strain infected human lymphocyte derived CEM cells using XTT cytopathic method and are shown in Table 1.

Scheme 2



Scheme 3



Reaction conditions. (a) anhydrous K_2CO_3, 6,6-disubstituted-5,6-dihydropyran-2-one, DMF, RT, 16 h (b) Nitric acid, sulfuric acid, 60 °C for 30 min, RT, 18 h (c) Pd/C, hydrogen, 50 psi, RT (d) sodium nitrite, aqueous H_2SO_4, 70 °C e) NaSCN, NaBr, bromine, MeOH, 0 °C to RT (f) corresponding sulfamyl chloride, triethylamine, 0 °C to RT, 1h (g) DTT, ethanol, 0.02 K_2HPO_4 buffer, 80 °C, 16 h (h) Tosyl bromide, triethylamine, CCl_4, 0 °C, 16h (i) TBS chloride, imidazole, DMF, 24 h (j) TBAF, THF, RT, 15 min.

Results and Discussion

Molecular modeling studies[9] showed that the S_3' pocket of the enzyme is accessible *via* substitution on 3-thiophenyl ring. Alternately, it could reach to the S_4' pocket or the solvent surrounding the enzyme (Fig. 1).

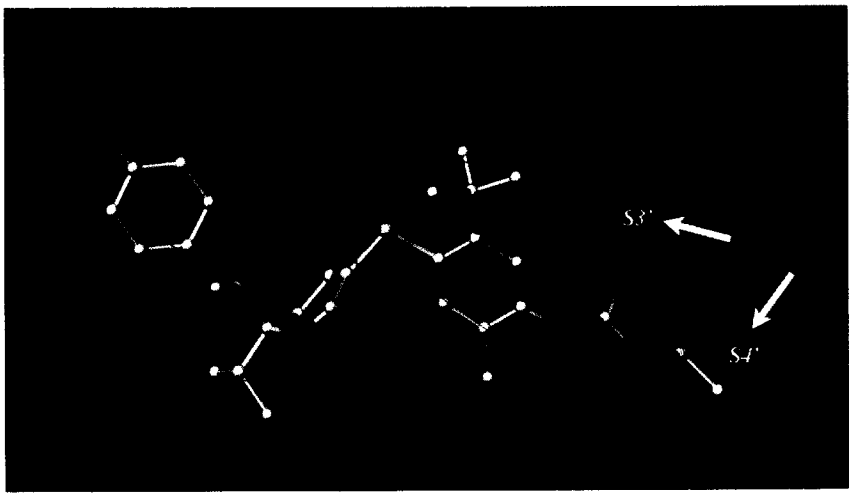


Fig. 1. Molecular model of **29** docked in the HIV PR (green) X-ray structure. The S_3' and S_4' subsites are highlighted. The atoms of the inhibitor are colored by atom type (C-white, H-cyan, O-red, N-blue, and S-yellow).

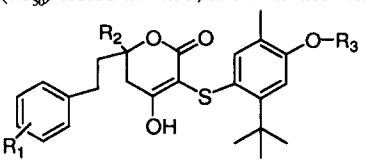
I. Variation of groups on the nitrogen of the sulfamate moiety: Initially, the 4-hydroxyphenethyl group (occupying S_2 pocket) was kept constant and substituents on the sulfamate moiety were varied to probe S_3'/S_4' interactions with the enzyme. Three alkyl groups representing various steric bulk, namely, methyl, isopropyl and cyclohexyl were used at the 6-position (occupying the S_1 pocket of the enzyme) of the 5,6-dihydropyran-2-one ring.

a) 6-Methyl Series: Inhibitor **14**, containing hydrogens on the sulfamate nitrogen showed a slight enhancement in enzymatic binding and more significantly, less toxicity compared to parent compound **13** (possessing no sulfamate group). Sulfamate analogs containing alkyl groups of varying steric bulk on the nitrogen of the sulfamate moiety, *e.g.;* ethyl (**15**), n-propyl (**16**), dimethyl (**17**) and N-methylpiperazine (**18**) showed binding affinities in 1.6 to 7.1 nM range and antiviral activities in 1.5 to 8.2 μM range. There is little correlation between enzymatic binding affinity and antiviral activities. Interestingly, inhibitors **17** and **18** exhibited no toxicity when measured at 100 μM concentration.

b) 6-Isopropyl Series: Among the 6-iso-propyl series (**19–24**), all the inhibitors showed similar binding affinities (within 2-3 fold) to HIV PR, though the steric bulk of the alkyl group on the nitrogen of the sulfamate moiety varies, *e.g.;* methyl (**19**), ethyl (**20**), n-propyl (**21**), dimethyl (**22**), N-methylpiperazine (**23**)

and morpholine (**24**). The antiviral activities of these analogs (0.5–1.6 μM) were consistently improved compared to the methyl series. The best inhibitor in this series, the compound containing a N-methlpiperazine (**23**) exhibited a therapeutic index of >200, similar to the 6-methyl series.

Table 1. 5,6-Dihydropyran-2-ones containing sulfamate 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
13	4-OH	methyl	H	6.5	>7	7	1
14	4-OH	methyl	SO_2NH_2	1.8	5.2	69	13
15	4-OH	methyl	SO_2NHEt	2.3	1.5	66	44
16	4-OH	methyl	SO_2NH(n-Pr)	6.2	4.3	66	15
17	4-OH	methyl	$SO_2N(Me)_2$	1.6	8.2	>100	>12
18	4-OH	methyl	SO_2(N-methylpiperazine)	7.1	2.3	>100	>44
19	4-OH	isopropyl	SO_2NHMe	1.2	1.1	>100	>91
20	4-OH	isopropyl	SO_2NHEt	4.9	1.1	>100	>91
21	4-OH	isopropyl	SO_2NH(n-Pr)	3	1.2	>100	>83
22	4-OH	isopropyl	$SO_2N(Me)_2$	3.4	0.7	70	100
23	4-OH	isopropyl	SO_2(N-methylpiperazine)	3.1	0.5	>100	>200
24	4-OH	isopropyl	SO_2(morpholine)	2.6	0.77	84	109
25	4-OH	cyclohexyl	SO_2NHEt	4.8	1.4	67	48
26	4-OH	cyclohexyl	$SO_2N(Me)_2$	20	5	60	12
27	4-OH	cyclohexyl	SO_2(N-methylpiperazine)	9.7	5.1	>100	20
28	4-OH	phenyl	SO_2NHEt	26	4.3	66	15
29	4-NH_2	isopropyl	SO_2NHEt	4.3	0.63	74	117
30	4-NH_2	isopropyl	$SO_2N(Me)_2$	3.9	0.74	64	86
31	4-NH_2	isopropyl	SO_2(N-methylpiperazine)	1.0	0.56	71	127
32	H	isopropyl	SO_2NHEt	8.9	0.77	67	87
33	H	isopropyl	SO_2(N-methylpiperazine)	3.9	1.65	>100	>61

[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. 3.

*c) **6-Cyclohexyl Series:*** 5,6-dihydropyran-2-ones containing a 6-position cyclohexyl group (**25–27**), showed a different SAR compared to the 6-methyl and 6-iso-propyl series. Thus, as the bulk of the alkyl group on nitrogen is increased there is reduction in enzymatic binding. Surprisingly, the best inhibitor in this series is the ethyl sulfamate analog (**25**) with a therapeutic index of 48. Over all, the sulfamates containing a 6-position cyclohexyl group showed lower antiviral activities compared to the 6-iso-propyl series.

In this sulfamate series, 6-position alkyl groups showed superior HIV PR binding affinities and antiviral activities compared to 6-phenyl analogs (**15, 20, 25** vs. **28**), similar to our lead compound (**1**) series.[3]

II. Substitutions on the phenyl ring of 6-phenethyl moiety: In this series, the 4-hydroxyl group on the phenethyl moiety at C-6 was replaced with 4-amino group, while keeping the iso-propyl group constant. The resulting inhibitors (**29–31**) showed somewhat similar inhibitory activities against HIV PR as well as antiviral activities when compared to 4-hydroxy analogs (**29** vs. **20**), (**30** vs. **22**) and (**31** vs. **23**) indicating that the 4-amino group is also well tolerated in these sulfamate inhibitors. These unsubstituted analogs (**32** and **33**), lacking 4-hydroxyl or 4-amino group on the phenethyl moiety, showed similar inhibitory activity against HIV PR. The inhibitor containing the ethyl sulfamate (**32**) showed similar antiviral activity when compared to 4-hydroxyl (**20**) or 4-amino (**29**) analogs. However, N-methylpiperazine (**33**) analog showed a 2-fold reduction in antiviral activity when compared to polar substituted analogs (**33** *vs.* **23, 31**).

The SAR indicates that out attempts to improve the potency (binding affinity) of **1** (our lead structure) by extending to the S_3' or S_4' site was unsuccessful. Possible factors involved might be a reduced interaction with Asp129(NH) and the balance of enthalpic gain with the unfavorable entropic effect of any additional flexibility. However, when compared to **13**, the parent compound from which these sulfamates were derived, a significant improvement in antiviral activity and toxicity was achieved. This could be due to various physical properties e.g.; aqueous solubility, cell penetration, etc. of the sulfamate inhibitors.

Inhibitors **15** and **23** were tested against Cytochrome P450 isozymes (Table 2), a study undertaken to evaluate the potential drug-drug interactions, which is one of the drawbacks with the marketed protease inhibitors.[10] Similar to our lead inhibitor (**1**), these compounds inhibit CYP3A4 and CYP2C9 isozyme at ~100 μM concentration. However, unlike **1**, these compounds inhibit CYP2D6 isozyme at 1-10 μM concentration.

Table 2. P450 Inhibition of Selected HIV PR Inhibitors (% inhibition of individual isozymes)

Entry	3A4			2D6			2C9		
	1μM	10μM	100μM	1μM	10μM	100μM	1μM	10μM	100μM
15	1	10	58	62	89	100	0	6	46
23	0	22	81	39	86	100	ND[a]	ND[a]	ND[a]
1[b]	4	16	30	3	16	33	2	12	59

[a]ND: not determined; [b]taken from ref. 3.

Pharmacokinetics: Selected inhibitors were dosed in mice to determine the pharmacokinetic properties of this class of compounds. Mice were dosed 25 mg/kg PO using 20% 0.1N sodium hydroxide/80% of 0.5% methyl cellulose. The inhibitors, **20, 23** and **24** showed plasma concentrations (C_{max}) of less than 1 μM. However, inhibitor **33** showed C_{max} of 5.3 μM. Though the actual reasons for poor pharmacokinetics are not known, it could be due to the increase in molecular weight of these inhibitors compared to **1**.

Conclusions. The above SAR studies clearly showed that potent non-peptidic inhibitors of HIV PR could be derived from 5,6-dihydropyran-2-one template containing a sulfamate moiety. Moreover, these 5,6-dihydropyran-2-ones possess a novel achiral template (i.e., 3-(2-*t*-butyl-5-methyl-4-sulfamate)phenylthio) occupying the S_1', S_2' and S_3'/S_4' pockets of the HIV PR. An alkyl group at the 6-position of 5,6-dihydropyran-2-one ring is essential for superior antiviral activities compared to 6-phenyl analogs. Inhibitors containing a polar group at the 4-position of the 6-phenthyl moiety gave overall better inhibitors. These sulfamate analogs did not exhibit any gross toxicity. In conclusion, starting from inhibitor **13**, structure-activity studies led to **23** (racemic analog) with an EC_{50} of 0.5 μM.

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*Corresponding author

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