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NONPEPTIDIC HIV PROTEASE INHIBITORS: 3-(S-BENZYL SUBSTITUTED)-4-HYDROXY-6-(PHENYL SUBSTITUTED)-2H-PYRAN-2-ONE WITH AN INVERSE MODE OF BINDING

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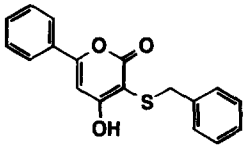
Abstract: Systematic substitutions on 6-phenyl and 3-SCH_2Ph rings of inhibitor **1**, were carried out to optimize the inhibitory activity against HIV PR. These studies lead to 3-Sbenzyl esters with enhanced potency. The X-ray crystal structure of **32** bound to HIV PR revealed that the 3-SCH_2phenyl group is occupying the P_2' pocket, which is contrary to the binding mode of **1** (derivative lacking ortho isopropyl ester group). In the latter case, benzyl group occupies the P_1' pocket.

Human immunodeficiency virus (HIV) protease (PR) is one of the three crucial key enzymes, viz.; reverse transcriptase (RT), PR and integrase (IN) essential for the replication of HIV.[1] Since the discovery that HIV PR is an aspartic PR, many groups have reported various inhibitors by modifying either the peptide substrates, i.e., peptidomimetics, or renin inhibitors.[2] Growing evidence indicates that HIV mutates at the PR level.[3] Therefore, interest has developed to design structurally novel and small molecule leads, which occupy minimum number of pockets, for the inhibition of HIV. [4] Recently we have reported nonpeptidic HIV PR inhibitors viz., various analogs of 4-hydroxy-pyran-2-ones, arising from a mass screening lead, as conformationally restricted P_1-P_1' peptidomimetics.[5] Unique resistance pattern of an inhibitor, derived from 4-hydroxypyran-2-one template, against eight HIV PR mutants was also described.[6] An X-ray crystallographic structure of 3-benzylsulfanyl-4-hydroxy-6-phenylpyran-2-one, **1**, bound to HIV PR showed that the groups attached to 6- and 3-position occupied S_1 and S_1' pockets of the enzyme. 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. Substitution of the 6-phenyl ring with *para*-hydroxyphenyl (viewed as tyrosine mimic) showed a 2-fold increment in inhibition of HIV PR.[5] Encouraged with the results, we undertook a systematic study, varying substitutions on the aryl rings of the pyran-2-one.

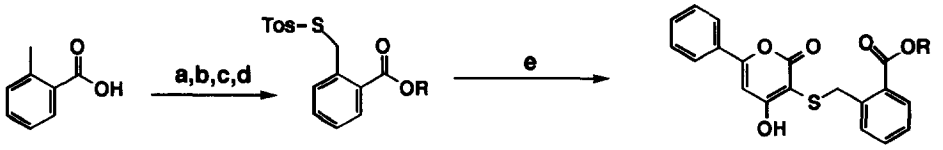


1 IC_{50} : **1.7 μM**

Synthesis: Pyran-2-one analogs **2** - **22** were prepared by the thermal condensation of trimethylsilyl enol ether of corresponding acetophenones, with 2-(S-substituted)propane-1,3-dioates.[7] Compounds **23** - **37** were

synthesized by sulfenylation of 6-aryl-pyran-2-one with the corresponding thiosulfonates in the presence of 1N sodium hydroxide using ethanol as solvent.[8] The starting materials 6-aryl pyran-2-ones were prepared by the condensation of the trimethylsilyl enol ether of the corresponding acetophenones with malonyl dichloride.[9] Thiosulfonates used in the preparation of **32 - 35** were synthesized according to Scheme I. The esters of *ortho*-toluic acid were prepared by treating *ortho*-toluyl chloride with the corresponding alcohol. The esters obtained were brominated with NBS in the presence of benzoyl peroxide to obtain the corresponding benzyl bromides.[10] The benzyl bromides on treatment with potassium thiotosylate in ethanol afforded the corresponding *para*-toluene thiosulfonates in good yields.

Scheme I



(a) thionyl chloride reflux, 16 hours; (b) isopropyl alcohol, 18 hours (or magnesium t-butoxide, THF, rt, 3 hours); (c) NBS, carbon tetrachloride, benzoyl peroxide, 2.5 hours; (d) potassium thiotosylate, ethanol reflux, 18 hours; (e) 4-hydroxy-6-phenylpyran-2-one, 1N sodium hydroxide, ethanol reflux, 16 - 18 hours.

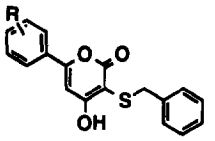
Results and Discussion

I. Substitutions on 6-phenyl ring of 3-Benzylsulfanyl-4-hydroxy-6-phenylpyran-2-one, 1: Various substituted pyran-2-one analogs were synthesized in order to probe the size of the S_1 pocket, as well as to vary the electronic nature of the pyran-2-one ring. Hydroxy, methoxy, methyl and chloro groups representing hydrophilic as well as hydrophobic, and electron withdrawing as well as electron donating properties, were chosen to substitute on the 6-phenyl ring at *para*, *meta* and *ortho* positions.

Among the various *para* substituted pyran-2-one analogs synthesized, hydroxyl (**2**) or carboxyl (**3**) substitutions enahanced the binding affinity by 2- and 5-fold, respectively (Table 1). Methoxy (**4**) or carboxymethyl (**5**) substitution, maintained the binding affinity, whereas methyl substitution (**6**) decreased the binding affinity by 7-fold. The *meta* position of the 6-phenyl ring appeared to tolerate most of the substitutions. Thus, *meta*-hydroxy (**7**) and *meta*-methoxy (**8**), *meta*-methyl (**9**) and *meta*-chloro (**10**) substitutions showed a modest enhancement in binding affinity to HIV PR relative to **1**. Analog with *meta*-ethyl (**11**) showed a 2-fold reduction in binding affinity to HIV PR relative to **9**. *Meta*-trifluoromethyl substituted analog (**12**) showed a similar activity as **9**, whereas the *meta*-trifluoromethoxy substituted derivative (**13**) showed a 2-fold reduction in binding affinity compared to **8**. To explore multiple substitutions, *meta,meta*-disubstituted pyran-2-one analogs were also synthesized. The *meta,meta*-dimethyl pyran-2-one analog (**14**) showed a 6-fold enhancement in binding affinity compared to the unsubstituted parent pyran-2-one, **1**. Similarly, *meta,meta*-ditrifluoromethyl substitution to yield compound **15**, increased inhibitory activities against HIV PR by 3-fold. Ortho substitution on 6-phenyl ring of **1**, by hydroxy , methyl and chloro to give compounds **16**, **17**, and **18**, respectively,

showed a 3-, 2- and 8-fold reduction in binding affinity towards HIV PR.

Table 1: 6-Phenyl substituted pyran-2-ones and Their IC_{50} values tested in vitro .[a]



Entry	R	IC_{50} μM	Entry	R	IC_{50} μM
2	*para*-hydroxy	0.69	**13**	*meta*-OCF_3	2.14
3	*para*-COOH	0.36	**14**	*meta,meta'*-dimethyl	0.26
4	*para*-OCH3	1.63	**15**	*meta,meta'*-ditrifluoromethyl	0.63
5	*para*-$COOCH_3$	>100	**16**	*ortho*-OH	6.0
6	*para*-CH_3	12.26	**17**	*ortho*-CH_3	14.16
7	*meta*-hydroxy	1.19	**18**	*ortho*-chloro	2.54
8	*meta*-OCH_3	0.99	**19**	*meta-methyl,para*-methoxy	0.74
9	*meta*-CH_3	0.86	**20**	*meta,meta'*-dimethyl,*para*-OH	0.25
10	*meta*-Cl	0.69	**21**	[3,4-dioxane]	0.79
11	*meta*-CH_2CH_3	2.04	**22**	[3,4-dioxolane]	0.49
12	*meta*-CF_3	0.60			

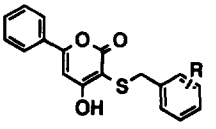
[a] Values are the average of at least two determination. For the details of the assay see ref. 11.

Mixed di- and tri-substitutions on the 6-phenyl ring of **1** was also performed to further explore any additive nature of these substitutions. Compound **19** containing *meta*-methyl and *para*-methoxy substitutions on the 6-phenyl ring of compound **1**, showed activity similar to the *meta*-methyl derivative **9**. An analog containing *meta,meta*-dimethyl and *para*-hydroxy substitutions (**20**), which individually enhanced the potency, showed a 7-fold increase in binding affinity and is as active as the *meta,meta*-dimethyl substituted derivative, **14**. Varying the 6-phenyl ring of compound **1** to benzo[1,4]dioxane (**21**) or benzo[1,3]dioxolane (**22**) rings (can be viewed as restricting the *meta* and *para* substitutions) enhanced the potency by 2- and 3-fold respectively compared to **1**. In summary, the above results indicated that *para* position prefers a polar group, *viz.*, hydroxyl or carboxyl, whereas *meta* position prefers a small hydrophobic group *viz.*, methyl.

II. Substituions on the 3-S-CH_2phenyl ring of 3-Benzylsulfanyl-4-hydroxy-6-phenylpyran-2-one, 1: Similarly, to the 6-phenyl ring substitutions, 3-SCH_2phenyl ring substitutions were also performed. Varying the substitutions either at the *para* or *meta* position from methyl (**23** and **26**), to carboxyl (**24** and **27**) to carboxomethoxy (**25** and **28**) groups did not increase inhibitory activity against HIV PR (Table 2). Having methyl (**29**) or carboxyl (**30**) substitutions at *ortho* position of 3-SCH_2phenyl ring did not enhance the binding affinity to HIV PR. However, an analog containing *ortho*-carbomethoxy group to give inhibitor (**31**) slightly

enhanced the binding potency. We next planned to synthesize various pyran-2-one analogs varying the size of the ester group. Interestingly, analog (**32**) containing an *ortho*-isopropyl ester on 3-SCH_2Ph ring showed a 50-fold increased binding affinity to HIV PR compared to **1**. Thus, it appears that the *iso*-propyl substituent indeed reaches to an additional pocket of the enzyme when compared to **1**. Further extension to an *iso*-butyl ester (**33**), in the the place of iso-propyl ester, resulted in an 18-fold reduction in binding affinity. Upon increasing the size of the group from *iso*-propyl to cyclopentyl (**34**) and cyclohexyl (**35**), a 2-fold decrease in binding affinity compared to **32** was observed.

Table 2: 3-SCH_2Phenyl substituted pyran-2-ones and Their IC_{50} values tested in vitro .[a]



Entry	R	IC_{50} μM	Entry	R	IC_{50} μM
23	*para*-CH_3	4.58	**31**	*ortho*-$COOCH_3$	1.0
24	*para*-COOH	10.21	**32**	*ortho*-COO(*iso*-propyl)	0.034
25	*para*-$COOCH_3$	>10	**33**	*ortho*-COO(*iso*-butyl)	0.619
26	*meta*-CH_3	8.54	**34**	*ortho*-COO(cyclopentyl)	0.050
27	*meta*-COOH	6.15	**35**	*ortho*-COO(cyclohexyl)	1.2
28	*meta*-$COOCH_3$	19.33	**36**	*ortho*-CONH(*tert*-butyl)	0.19
29	*ortho*-CH_3	5.37	**37**	[3,4-dioxane]	3.42
30	*ortho*-COOH	24.76			

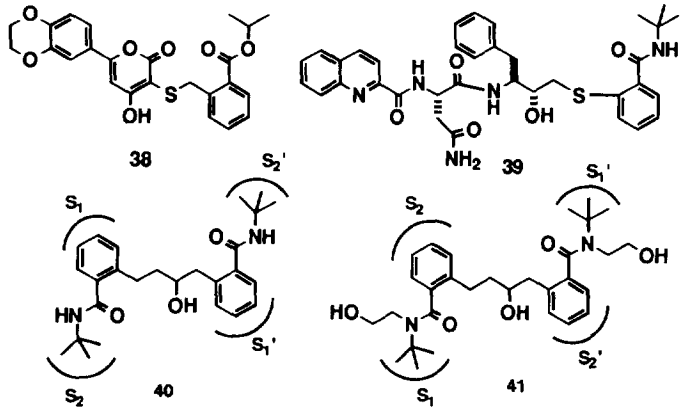
[a] Values are the average of at least two determination. For the details of the assay see ref. 11.

An analog containing substitutions, which individually showed an increase in activity when substituted at the 6- and 3-positions of the pyran-2-one ring was also synthesized. Inhibitor **38** had an IC_{50} of 66 nM, indicating no additive effect with these substitutions. Antiviral activity of these inhibitors in cellular assays correlated poorly with enzymatic inhibition. Due to the lack of clear differentiation between antiviral activities (low to high uM) and cytotoxicities, within ~3 fold, problems in interpreting true antiviral activity were introduced. Optimization of antiviral activity is is progress.

X-ray Crystal Structure of 32 Bound to HIV PR and its Comparison with 1 bound to HIV PR: The X-ray crystallographic structure of inhibitor **32** bound to HIV PR showed similar key interactions of the pyran-2-one ring with the enzyme relative to that observed with **1** complexed with the PR, i.e., the displacement of water-301 by the lactone moiety and the hydrogen bond formation of enolic hydroxyl group with the catalytic aspartates. (Figure 1).[4-6,12-15] However, the pyran-2-one rings in these two structures bind at different angles in the enzyme, such that upon aligning the structures, the sulfur atoms are oriented 1.3 A apart. The 6-phenyl ring, 3-SCH_2Ph moiety and isopropyl group in **32** occupied S_1, S_2' and S_1' pockets respectively. Conversely, the 3-

SCH_2Ph group of **1** bound in the S_1' site in the crystal complex. Thus, by a single substitution on the 3-SCH_2Ph ring in **1** resulted in an inverse mode of binding i.e., 3-SCH_2Ph moiety occupying S_1' pocket in **1** vs. 3-SCH_2Ph moiety occupying S_2' pocket in **32**. Upon modeling the ester group into the *ortho* position of the SCH_2Ph ring in the bound structure of **1**, the *iso*-propyl moiety is presented in the upper region of the S_2' pocket. The more favorable positioning of this group in S_1' as observed in the complex with **32**, may dictate the mode of binding.

Figure 1 : An overlay of the X-ray crystal structure of **32** bound to HIV PR (red) and inhibitor **1** (orange). The amino acid residues are from the X-ray crystal structure of **32**.



Recently Cho *etal.* reported peptide-derived β-hydroxy sulfides (**39**, where the benzamide group occupies S_1'/S_2' pockets of the enzyme), as HIV PR inhibitors, however, they exhibited less potency compared to the pyran-2-ones desrcribed above.[16] Probably, these pyran-2-ones being cyclic with restricted conformations

are providing positive entropy relative to flexible, linear structures. [6,15,17] An inverse in binding mode was also observed by Reich *et al.* in a different series of nonpeptidic HIV PR inhibitors (**40**, **41**).[18] It would be useful to obtain multiple X-ray crystal structures, especially during iterative structure-based drug design.

In conclusion, starting from **1** (IC_{50}:1.7 μM), by systematic substitutions on both the phenyl rings, potency was increased by 50-fold (**32**, IC_{50}:34 nM). These inhibitors described are of low molecular weight, readily synthesizable and possess no chiral centers. The X-ray crystal structure showed an inverse binding mode for **32** relative to **1**.

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