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6-PHENYL-6-ALKYLAMIDO-5,6-DIHYDRO-2H-PYRAN-2-ONES: NOVEL HIV PROTEASE INHIBITORS

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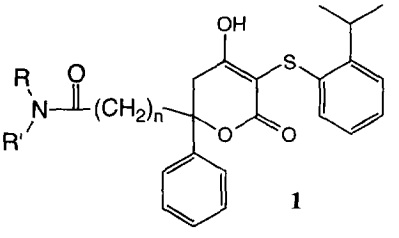
Abstract: Publications from our laboratories have recently described a series of 3-thioaryl substituted-4-hydroxy-pyrones[1] as HIV protease inhibitors. The current work examines the analogous 5,6-dihydro-2H-pyran-2-ones with 6,6-substitutions focusing on the use of 1°, 2°, and 3° alkyl amides of various chain lengths to fill the S1 through S3 enzyme pockets.

Inhibition of HIV protease is a target for drug design in a number of laboratories. This enzyme is essential for viral replication;[2] it processes the gag/pol polyproteins to generate structural proteins and viral enzymes within the budding virus. We have investigated 5,6-dihydro-2H-pyran-2-ones as HIV protease inhibitors.[3] This unique template differs from conventional protease inhibitors in that it is not peptidomimetic in nature, and thus offers potential advantages in bioavailability and viral resistance patterns. This paper focuses on the structure activity relationships of various amides at one of the 6 positions, holding the other 6 substituent as a phenyl, and keeping the rest of the molecule constant as shown in **1**.



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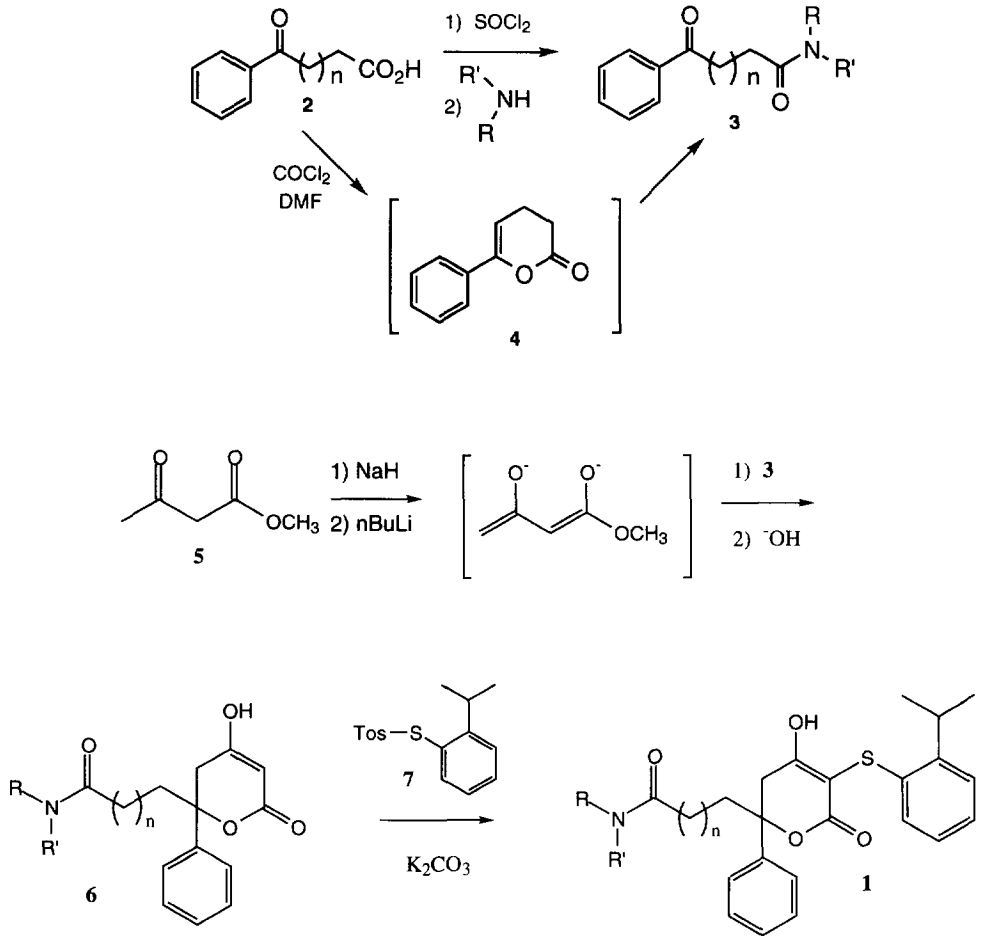
Chemistry.

The synthesis of the target compounds and the requisite ketones are shown in Scheme 1. The 2- and 4-carbon spaced ketoamides were made via treatment of the acid chloride with the appropriate amine; the 3-carbon analogs required opening of cyclic intermediate **4** with the amine. The dianion of methylacetoacetate was reacted with these ketones, followed by Aldol cyclization to afford dihydropyones **6**. Thio-tosylate **7**, used for introduction of the 3 thioaryl group, was made from phenylsulfonylhydrazide through the bromide.[4] This was refluxed with the penultimate dihydropyrone in DMF to give the target compounds **1 a-w**. In the case of BOC-protected piperazine derivatives, the final step was acidic removal of the protecting group to afford the final compound. All

compounds were synthesized and tested as racemic mixtures. Chiral resolution[5] of this 6,6-phenyl/alkylphenyl compound class has shown the advantage of a single enantiomer; however, both enantiomers have exhibit enzymatic activity.

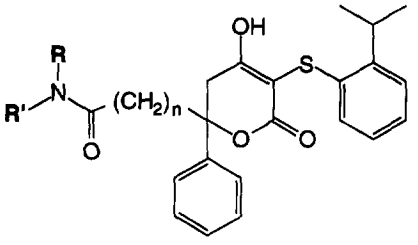
Scheme 1. Synthesis of Dihydropyrone Analogs



Biochemistry.

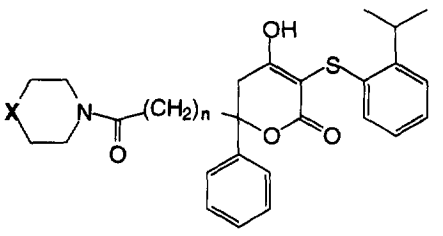
The in vitro HIV-1 protease inhibition assays were determined as previously described.[6] The compounds were tested against affinity-purified HIV-1 protease at a pH of 4.7 and a final enzyme concentration of 0.45-1.1 nM. Analyses were conducted in duplicate. Compound **XIV**[7] is included as a reference.

Table 1. Straight Chain Amides



Compound	n	R	R'	IC_{50}(nM)
1a	3	CH_3	Bz	676 ± 0.105
1b	3	CH_3	EtPh	216 ± 0.030
1c	3	CH_3	Ph	624 ± 0.096
1d	3	H	Bz	81 ± 0.005
1e	3	H	EtPh	146 ± 0.031
1f	3	H	Ph	429 ± 0.044
1g	4	H	Bz	26 ± 0.003
1h	4	H	EtPh	71 ± 0.011
1i	4	H	Ph	91 ± 0.012
1j	3	H	H	101 ± 0.012
1k	4	H	H	92 ± 0.012
XIV[7]				10 ± 0.0011

Table 2. Cyclic Amides



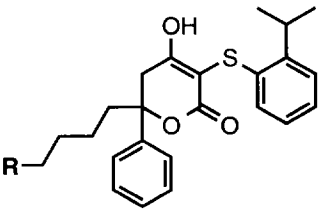
Compound	n	X	IC_{50}(nM)
1l	2	O	2440 ± 0.188
1m	2	S	1103 ± 0.168
1n	2	(BOC)N	1270 ± 0.148
1o	2	HN	1106 ± 0.074
1p	3	O	186 ± 0.024
1q	3	S	285 ± 0.046
1r	3	(BOC)N	124 ± 0.016
1s	3	HN	564 ± 0.091
1t	4	O	195 ± 0.030
1u	4	S	583 ± 0.106
1v	4	(BOC)N	127 ± 0.016
1w	4	NH	234 ± 0.025

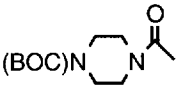
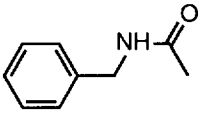
Results.

Results for acyclic amides are shown in Table 1. For compounds with a three carbon spacer, a secondary amide is preferred (**1 a-c** vs. **1 d-f**). Within the secondary amides, a potency order of benzyl > phenethyl > phenyl for enzyme inhibition was observed. Extending to a four methylene chain retained this order; all compounds with the additional carbon were more potent than their lower homolog. The most potent compound in this series was **1g**, which had an IC_{50} of 26 nM. The primary amides for the three and four carbon spacers (**1j** and **1k**) had IC_{50}'s of 101 and 92 nM, respectively. When the amide nitrogen was tied back into a cyclic system, a flatter SAR resulted (Table 2). The two carbon spacer analogs all exhibited an IC_{50} of greater than 1 mM (**1 l-o**). Extending to three carbons dropped the IC_{50} approximately 10-fold (**1 p-s**). No further reduction was seen by increasing the

chain length to four carbons (**1 t-w**). When compared to tertiary amides **1 a-c**, a slightly better affinity was seen with the cyclic system amides.

Table 3.



Compound	R	IC50 (nM)
8	HO_2C-	1.8 ± 0.00015
9	H_3C-	88 ± 0.014
1k	H_2NCO-	92 ± 0.012
1v		127 ± 0.025
1g		26 ± 0.011

Comparison of five 4-carbon chain length compounds is shown in Table 3. Replacing the amide with a carboxylic acid, as in compound **8**, produced a dramatic improvement in enzyme binding affinity (IC_{50} = 1.8 nM). When compared to the analogous primary amide **1k** (92 nM), and the straight alkyl chain analog **9** (88 nM), the importance of the functionality specific to the S2 pocket is illustrated.

Discussion.

The use of the dihydropyrone ring to interact simultaneously with the catalytic aspartic acids and the flap of HIV protease has offered a novel template from which to extend functionality to reach the S1 to S3 pockets (and the corresponding S1' and S3' pockets). Besides our work in this area, other laboratories have built on the pyrone structure to advance compounds as HIV protease inhibitors into clinical study.[8] The pseudo-tetrahedral geometry at the 6 position of the dihydropyrone ring allows the S1 pocket to be filled by one substituent (in this case predicted to be a phenyl ring for the more potent enantiomer based on X-ray and binding data[5]) and the S2 pocket by another. We sought to optimize placement of an amide functionality in one of these 6-positions to take advantage of hydrogen bonding as might occur in the natural peptide substrate.

Chain length and the nature of the amide used were examined. A carbon spacer of four methylene units was found to be best. Within the acyclic amides, a secondary amide was found to be preferable, an observation which is analogous to peptide amides. The best compound within these series was **1g**, the four methylene spacer benzyl amide, which had an IC_{50} of 26 nM. The relative importance of amide H-bonding in this series can be evaluated further by comparing compound **1g** with the straight-chain alkyl analog **9**. The benzyl amide is only about four times better than the corresponding alkyl compound, which suggests the binding energy that may be picked up by the amide, while beneficial, is not substantial.

The primary amides **1j** and **1k** were made as potential mimics of glutamine, and acid **8** as a glutamic acid surrogate. These substitutions would correspond to the amino acids found in the natural substrate in the P2 and P2' positions. In the case of the primary amides, the strategy did not prove to be beneficial; indeed, each compound was found to be less potent than the corresponding 2° benzyl amide. The second surrogate (for GLU) substituted an acid functionality in the terminal position (**8**), and this improved potency significantly. It is postulated this acid interacts with ASP 130 of the protease to pick up the additional enzyme affinity. This interaction is apparently independent of the backbone H-bonding interactions investigated with the amides, and serves as a distal anchor for the molecular side-chain. It is confirmed by an X-ray analysis of the compound bound in the enzyme (Figure 1).

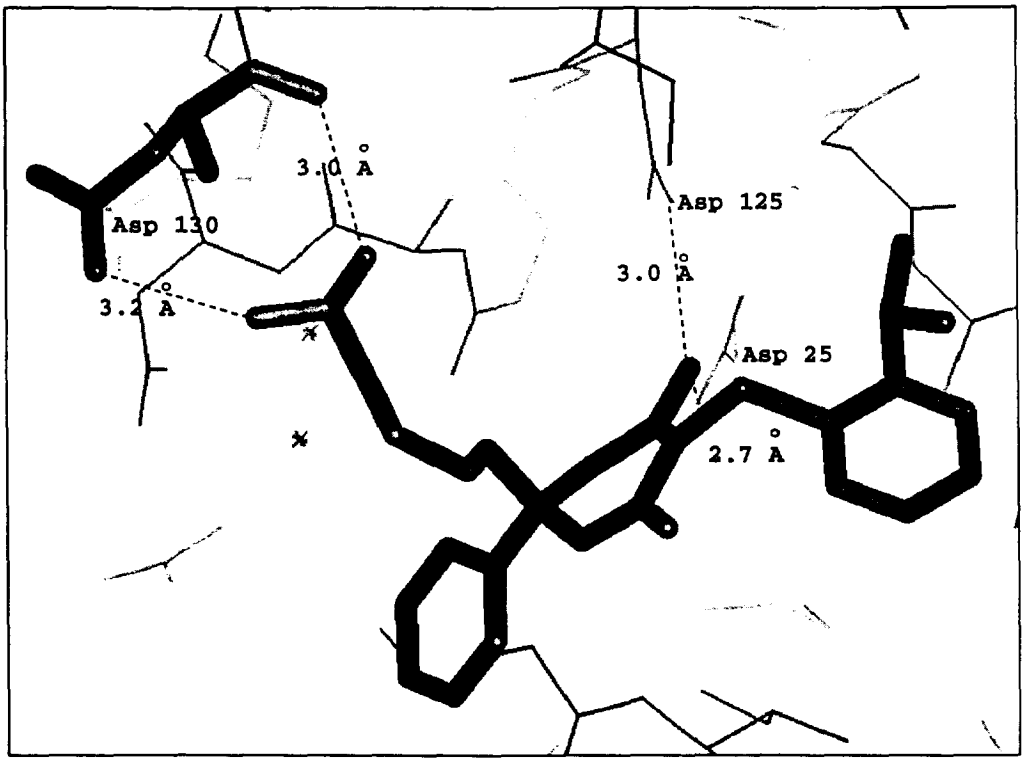


Figure 1. X-ray analysis of Compound **8** in Enzyme Active Site - Interaction with ASP 130

Conclusions.

The utility of amide functionality in a series dihydropyrone analogs designed as HIV protease inhibitors was examined and found to be of limited value. While predicted to serve as surrogate amino acids by hydrogen

bonding to the enzyme, amides were little improved over simple hydrocarbon chains when used in the 6-position of the dihydropyrone structure. The best compound in this series (**1g**) was a benzyl amide linked to the dihydropyrone with a four carbon chain. Investigation of alternative carbonyl functionality corresponding to GLN and GLU found in the natural substrate led to carboxylic acid derivative **8**. X-ray has shown this compound interacts with the protonated ASP 130 side chain; it has an IC_{50} of 1.8 nM. Analogs in this series are being tested in additional assays, and these data will be reported in future publications.

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