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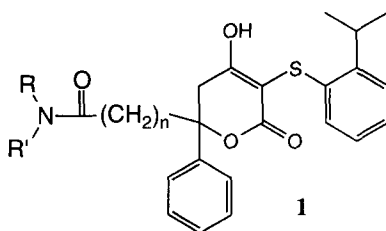
## 6-PHENYL-6-ALKYLAMIDO-5,6-DIHYDRO-2H-PYRAN-2-ONES: NOVEL HIV PROTEASE INHIBITORS

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**Abstract:** Publications from our laboratories have recently described a series of 3-thioaryl substituted-4-hydroxy-pyrones<sup>1</sup> 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;<sup>2</sup> 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.<sup>3</sup> 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**.

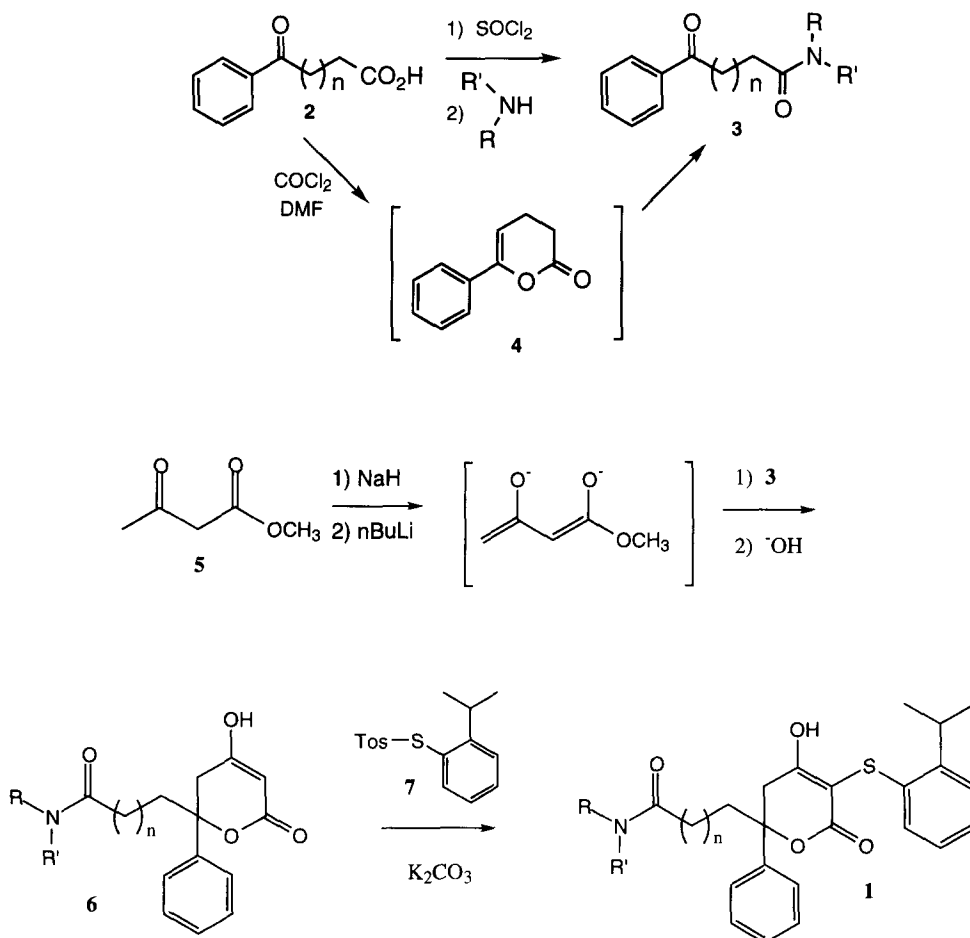


### Chemistry.

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 dihydropyrones **6**. Thio-tosylate **7**, used for introduction of the 3 thioaryl group, was made from phenylsulfonylhydrazide through the bromide.<sup>4</sup> This was refluxed with the penultimate dihydropyrene 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

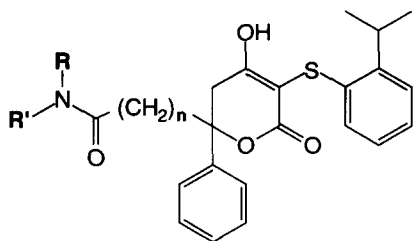
compounds were synthesized and tested as racemic mixtures. Chiral resolution<sup>5</sup> of this 6,6-phenyl/alkylphenyl compound class has shown the advantage of a single enantiomer; however, both enantiomers have exhibit enzymatic activity.

**Scheme 1.** Synthesis of Dihydropyrone Analogs

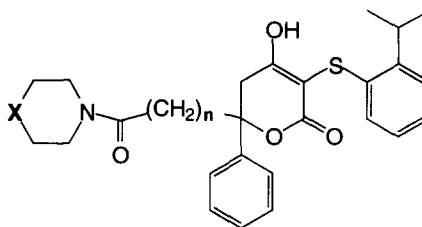


**Biochemistry.**

The *in vitro* HIV-1 protease inhibition assays were determined as previously described.<sup>6</sup> 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**<sup>7</sup> is included as a reference.

**Table 1.** Straight Chain Amides

Compound	n	R	R'	IC <sub>50</sub> (nM)
<b>1a</b>	3	CH <sub>3</sub>	Bz	676 ± 0.105
<b>1b</b>	3	CH <sub>3</sub>	EtPh	216 ± 0.030
<b>1c</b>	3	CH <sub>3</sub>	Ph	624 ± 0.096
<b>1d</b>	3	H	Bz	81 ± 0.005
<b>1e</b>	3	H	EtPh	146 ± 0.031
<b>1f</b>	3	H	Ph	429 ± 0.044
<b>1g</b>	4	H	Bz	26 ± 0.003
<b>1h</b>	4	H	EtPh	71 ± 0.011
<b>1i</b>	4	H	Ph	91 ± 0.012
<b>1j</b>	3	H	H	101 ± 0.012
<b>1k</b>	4	H	H	92 ± 0.012
<b>XIV</b> <sup>7</sup>				10 ± 0.0011

**Table 2.** Cyclic Amides

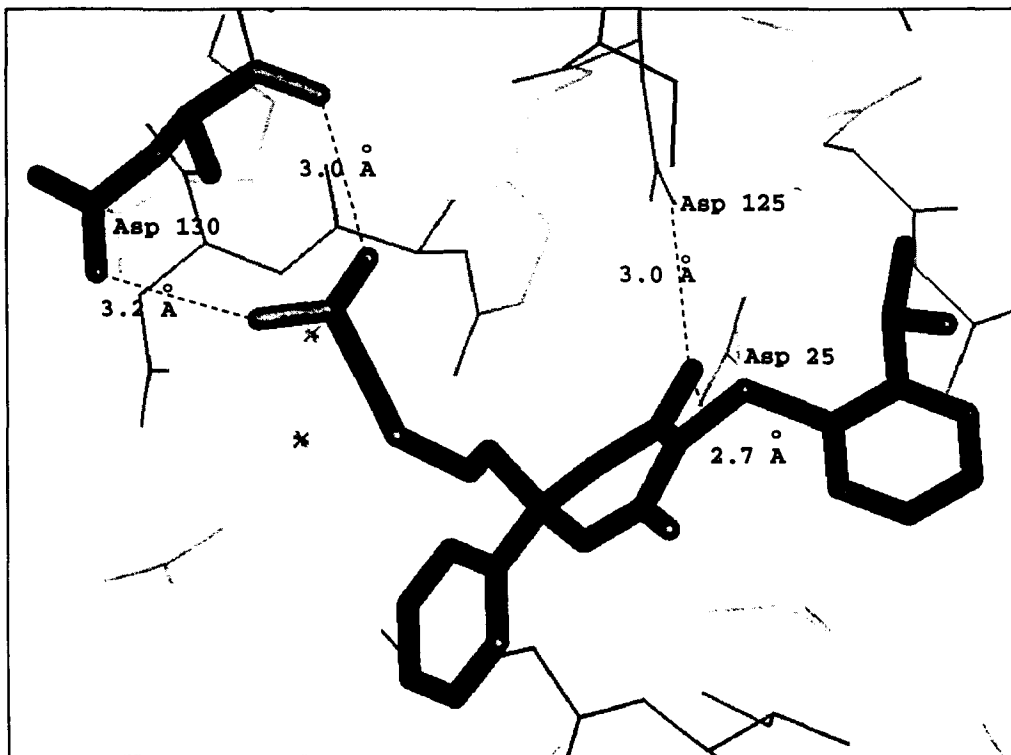
Compound	n	X	IC <sub>50</sub> (nM)
<b>1l</b>	2	O	2440 ± 0.188
<b>1m</b>	2	S	1103 ± 0.168
<b>1n</b>	2	(BOC)N	1270 ± 0.148
<b>1o</b>	2	HN	1106 ± 0.074
<b>1p</b>	3	O	186 ± 0.024
<b>1q</b>	3	S	285 ± 0.046
<b>1r</b>	3	(BOC)N	124 ± 0.016
<b>1s</b>	3	HN	564 ± 0.091
<b>1t</b>	4	O	195 ± 0.030
<b>1u</b>	4	S	583 ± 0.106
<b>1v</b>	4	(BOC)N	127 ± 0.016
<b>1w</b>	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<sub>50</sub> of 26 nM. The primary amides for the three and four carbon spacers (**1j** and **1k**) had IC<sub>50</sub>'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<sub>50</sub> of greater than 1 mM (**1 l-o**). Extending to three carbons dropped the IC<sub>50</sub> approximately 10-fold (**1 p-s**). No further reduction was seen by increasing the

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<sub>50</sub> 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 dihydropyrene 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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