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4-HYDROXY-5,6-DIHYDRO-2*H*-PYRAN-2-ONES. 3. BICYCLIC AND HETERO-AROMATIC RING SYSTEMS AS 3-POSITION SCAFFOLDS TO BIND TO S_1' AND S_2' OF THE HIV-1 PROTEASE ENZYME.

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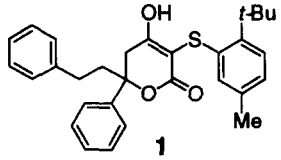
Abstract: 5,6-Dihydro-2*H*-pyran-2-ones are potent inhibitors of HIV-1 protease, which bind to the S_1, S_2, S_1', and S_2' pockets and have a unique binding mode with the catalytic aspartyl groups and the flap region of the enzyme. Efforts to explore 3-position heterocyclic scaffolds that bind to the S_1' and S_2' pockets have provided a number of selected analogs that display high HIV-1 protease inhibitory activity.

Introduction

The elucidation of the cellular events culminating in viral replication of the human immunodeficiency virus (HIV-1) has led to the identification of HIV-1 protease as a target for therapeutic intervention in the treatment of AIDS.[1] In this area, we have recently reported 4-hydroxy-5,6-dihydro-2*H*-pyran-2-ones such as **1**[2] and **2**[3,4] as inhibitors of HIV-1 protease. Compound **1** is a potent inhibitor of HIV-1 protease yet demonstrates little cellular activity. Appropriate modifications to add polarity to the molecule while also adding hydrogen



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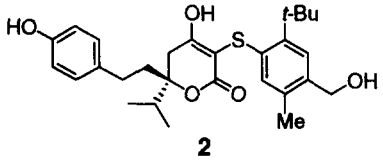

2

bonding interactions with the protein provides compounds such as **2** which exhibit subnanomolar potency against the protease enzyme and demonstrate good antiviral and pharmacologic activities.

X-ray crystallographic studies of these compounds[2] bound to the enzyme reveal that these molecules have a unique binding mode. The 4-hydroxyl group binds at the catalytic diad aspartate residues (Asp-25 and Asp-125) and the lactone moiety displaces H_2O-301 in the flap region, a water molecule that is typically conserved in the X-ray structures of peptidic inhibitors. These inhibitors hydrogen bond directly to NH's in the flap (Ile 50/150). The more potent *S*-enantiomer[2,3] binds the 6-position isopropyl or phenyl group in the S_1 site and orients the phenethyl chain in the S_2 pocket. The 3-position substituent, which occupies the S_1' site via the *t*-

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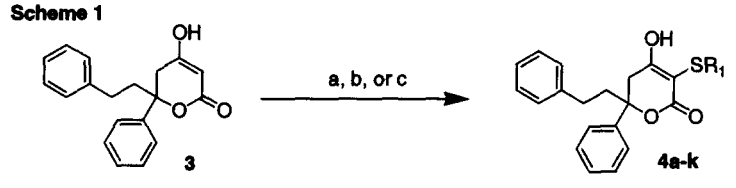
butyl group and the S_2' site via the 5-methyl substituent is achiral, thus avoiding additional complexity of synthesis.

This paper will focus on our initial efforts to explore alternative scaffolds for the 3-position of the dihydropyrone ring through the introduction of heteroaromatic systems. It was hoped that the polarity associated with these systems might provide an alternative to the peripheral functionality as used in **2**. Therefore, a series of compounds substituted with bicyclics and heteroaromatics at C-3 was designed and optimized.

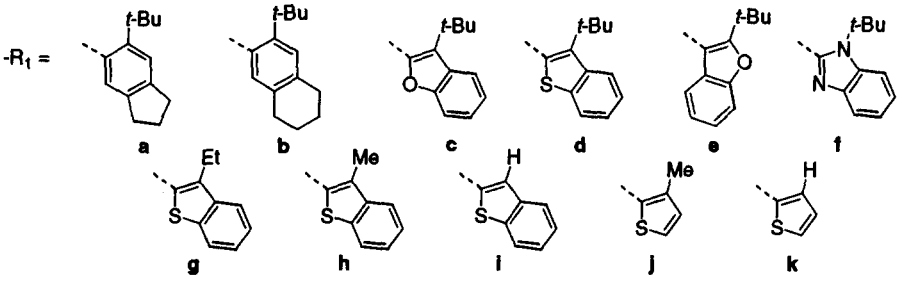
Chemistry

The synthetic pathway for the preparation of the 4-hydroxy-5,6-diydropyran-2-one protease inhibitors (**1** and **2**) has been previously described.[2,3] The preparations of compounds **4a-k** are shown in Scheme 1. Using

Scheme 1



[a]$TsSR_1$ (**10a,b**), K_2CO_3, DMF. [b]R_1SSCO_2Et (**14c-e,g-k**), $NaHCO_3$, EtOH. [c]R_1SCl (**18**), Et_3N, THF.

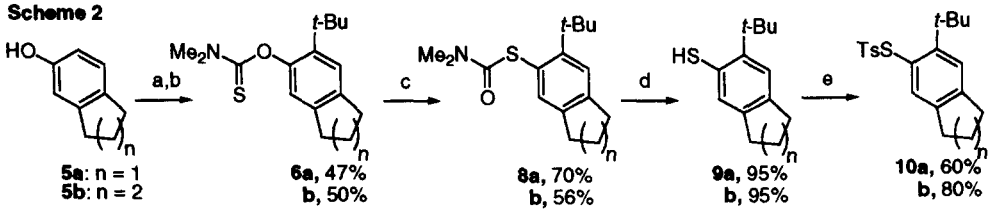


the previously described 5,6-diydropyran-2-one **3**[2], the 3-position sulfur side chains were attached using an appropriately activated sulfur coupling reagents: **10a** and **10b** (tosylthiolates)[3], **14c-e, g-k** (activated disulfides) and **19** (sulfenyl chloride). The activated sulfur electrophiles were prepared as shown in Schemes 2, 3 and 4. Tosylthiolates **10a** and **10b** were prepared from phenols **5a** and **5b** (Scheme 2).[5] Derivatization with dimethylthiocarbamoyl chloride provides **6a** and **6b**, which are then thermally rearranged to **8a** and **8b**.[6] Reduction of the thiocarbamoyl groups provides **9a** and **9b**, which when treated with tosylbromide yields **10a** and **10b**.[2]

Activated sulfur derivatives **14c-e, g-k** were prepared as shown in Scheme 3 from **11c**[7a], **d**[7b], **g, h, I, k** and bromides **12e** (from **11e**[7c]), **j** by metallation of each with n-BuLi and then quenching the reaction mixtures with elemental sulfur to provide thiols **13c-e, g-k**. The thiols were used crude and treated with chlorocarbonylsulfenylchloride in ethanol to provide **14c-e, h-k** which was used without purification.

Sulfenyl halide **18** was prepared as shown in Scheme 4 starting from **15**. Flouro displacement with *t*-butylamine followed by reduction of the nitro group provides **16** in high yield. Treatment of **16** with carbon

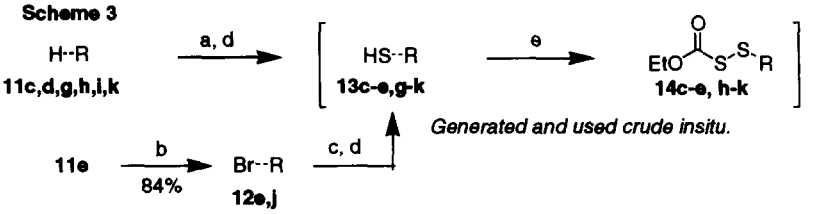
Scheme 2



[a]t-BuOH, H_3PO_4, 90°C. [b]dimethylthiocarbamoyl chloride, NaH, DMF [c]300°C. [d]LAH, Et_2O. [e]Tosyl bromide, CCl_4, Et_3N.

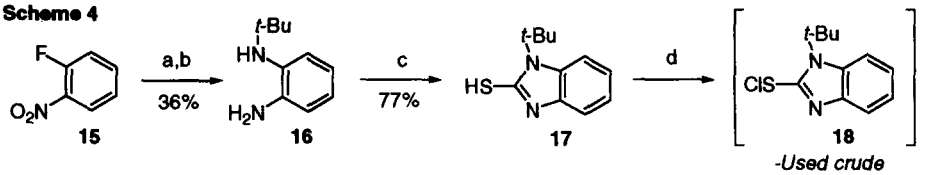
disulfide at elevated temperature generates **17**. Chlorination with *n*-chlorosuccinamide provides **18** which is used without purification.

Scheme 3



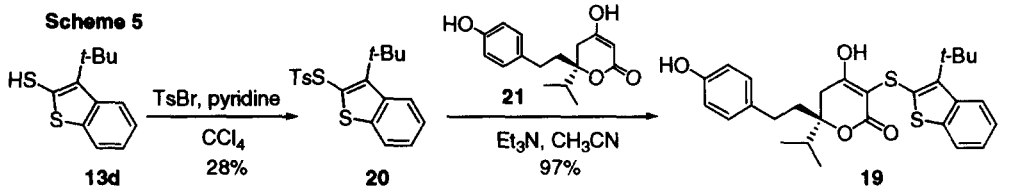
[a]n-BuLi, THF, -40 °C. [b]Br_2, Et_2O [c]n-BuLi, THF, -78 °C. [d]S_8, -78 °C to RT. [e]chlorocarbonylsulfenyl chloride, $NaHCO_3$, EtOH.

Scheme 4



[a]NaH, DMSO, t-$BuNH_2$ [b]Ra-Ni, H_2, MeOH [c]CS_2, DMF, Δ [d]NCS, CH_2Cl_2

Scheme 5



The synthetic pathway for the preparation of **19** is shown in Scheme 5. Thiol **13d**, prepared as described in Scheme 3, was treated with tosyl bromide to provide **20** which was then coupled with **21**[3] to provide **19**.

Results and Discussion

The binding site of the protease enzyme is actually not a series of distinct pockets conventionally described as S_1, S_2, etc.; rather a continuum from one end of the cleft to the other. As ligands bind, they not only fill the various defined binding pockets but the regions in between them. Compound **1** fills in a more conventional manner S_1 and S_2. At S_1' and S_2', a 3-position scaffold is provided that is well complemented in size and shape to fill the pockets yet quite different than that typically observed with most peptide-like inhibitors. Molecular modeling has shown that larger bicyclic ring systems can be accommodated by the protease enzyme in

this region. In order to explore this proposal, two nonheterocyclic analogs of compound **1** (**4a** and **4b**) have been prepared. Compound **4a** is equipotent with **1** and approximately threefold more potent than **4b** (Table 1) using pH 4.7 data.[2b] The 5-membered ring of **4a** is envelope-shaped and less sterically demanding than the twist-boat conformation of **4b**, yet each demonstrates that a larger bicyclic system is compatible in this region. Holding the *t*-butyl constant, a number of heteroaromatic systems were next examined as alternative 3-position scaffolds (**4c-f**) proposed to fill a similar region to that of the 3-positions of **4a** and **4b** and to increase the polarity of the system. Compounds **4c**, **4d** and **4e** are approximately equipotent with **1**. The most polar heterocyclic replacement (**4f**) drops off precipitously demonstrating a lack of tolerance for some types of polar substitution near the core of the enzyme.

It has been reported[2] that the protease activities of some 5,6-dihydropyran-2-ones demonstrate significant pH dependence on the conditions in which the assay is run, due to the pKa of the 4-position hydroxyl group (pK_a = 4.5 - 6.5). This sensitivity to pH, however, has been found to be variable and appears to be

Table 1. Melting Points and Inhibition Data of the HIV Protease Inhibitors Reported in this Study.

	Yield %	mp, °C	IC_{50} (nM)[b]	EC_{50}[d] (uM)		Yield %	mp, °C	IC_{50} (nM)[b]	EC_{50}[d] (uM)
1		ref 2a	10 (35)[c]	46	**4g**	62	62 - 64	28	>65
4a	60	140 - 141	9 (130)[c]	22	**4h**	88	70 - 72	74	>100
4b	83	68 - 70	28	33	**4i**	68	76 - 78	363	>100
4c	34	66 - 68	11 (210)[c]	31	**4j**	81	51 - 53	195	>100
4d	22	75 - 77	7 (17)[c]	>66	**4k**	89	79-81	290	>73
4e	70	79 - 81	18 (211)[c]	10	**2**		ref. 3	(1.0)[c] Ki = 0.09 nM	0.5
4f	92	135 - 137	505	61	**19**	97	207	(< 1.0)[c] Ki = < 0.1 nM	1.0

[a]Yields are those obtained from the coupling step to provide the final product. [b]Data obtained at pH 4.7 and are averages of at least 2 runs (refs 2 and 7). [c]Data obtained at pH 6.2 and are averages of at least 2 runs (refs 2 and 7). [d]Ref 3.

subject more to substitution than to pKa.[2] Thus, compounds **1**, **4a**, **4c**, **4d** and **4e** were retested for inhibition of protease activity at pH 6.2 (Table 1). Compound **4d** demonstrates little sensitivity to pH (twofold) relative to the other bicyclic systems tested (>tenfold). It is also 2-fold more potent than **1** at pH 6.2.

Compound **4d** was docked in the binding site of the HIV protease structure using the genetic algorithm docking program GOLD.[8,9] Five docking runs were carried out. As expected, all results generally orient the dihydropyrone ring system as has been observed in the X-ray structures of HIV protease bound with members of the dihydropyrone series.[10] The highest scoring result is shown in Figure 1. The phenylethyl group is oriented in S_2, the phenyl ring in S_1 and the *t*-butyl moiety in S_1'. Figure 1 also shows how, relative to the peptidomimetic inhibitor, A74704[10], the benzothiophene ring binds in the region occupied by the backbone and side chain of the

A74704 P_2' Val and further extends into what would be considered the P_3' region. The benzothiophene lies within 4 Å of several residues in the protease cleft, and forms hydrophobic contacts with Ile50, Val132 and Ala128. Substituted with the *t*-butyl substituent ortho to the sulfur, the benzothiophene template is a steric complement of the prime region of the enzyme.

Figure 1. Compound **4d** docked in the HIV protease binding site using the program GOLD[8,9] is overlaid with the peptidomimetic inhibitor, A74704, in its bound conformation in the X-ray crystal structure. The protein is from the docking experiment and is colored greenblue. The inhibitors are shown in ball-and-stick rendering and are colored by atom type (nitrogen - blue, hydrogen - cyan, oxygen - red, sulfur - yellow, carbon for compound 4 - magenta, carbon for A74704 white). The binding sites are labeled.

In order to examine the importance of the predicted binding mode of **4d** and the enzyme, a number of additional derivatives were prepared (compounds **4g - k**). It is apparent that as one replaces the *t*-butyl group with smaller substituents that the activity drops off (**4g, 4h** and **4i**), thereby demonstrating the significance of the large *t*-butyl as a P_1' substituent and its requirement[2] to maintain high potency.

Understanding the degree of interactions between the phenyl of the benzothiophene ring and the S_2' pocket necessitated the synthesis of **4j** and **4k**. Compound **4h** demonstrates a small enhancement in activity over **4j** (2.5 to 3-fold). Compounds **4i** and **4k** are equipotent. However, a fivefold enhancement in activity is observed going from the unsubstituted benzothiophene **4i** to substituted **4h**. This trend is not observed with **4k** to **4j** (<twofold). This observation may be explained by the benefit conferred by additional hydrophobic interactions between the inhibitor and the residues in the cleft in S_2'. It appears, however, that in order to take advantage of these interactions, an appropriate P_1' substituent must also be present (**4h** vs **4j** and **4i** vs **4k**).

The 3-position benzothiophene ring system of **4d** coupled to the more optimal dihydropyran-2-one of **2** provides **19**, which is an extraordinary inhibitor of the protease enzyme with an IC_{50} of < 1.0 nM at pH 6.2 more than 15-fold more potent than **4d** and equipotent with **2**.

The antiviral activity of **4d** is similar to that of **1**.[2a] However, the more potent compound **19**, containing the 6-isopropyl substituted dihydropyran-2-one, exhibits an antiviral EC_{50} of 1.0 uM which is similar to that observed for **2** (EC_{50} = 0.5 uM).[2a]

In conclusion, a study of a variety of heterocycles as replacements for the substituted 3-postion phenyl used in compound **1** was undertaken. The benzothiophene ring system of **4d** was found to be one of the best heterocycles examined to date. This substituent when combined with the dihydropyrone **21** provides **19** which demonstrates significant enzymatic and antiviral activity. Indeed, the use heterocycles may prove to be an effective alternative to that which has been previously employed[3] to enhance the cellular activity of dihydropyrones as inhibitors of HIV-1 protease. More research taking this concept forward is underway.

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