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NON-PEPTIDIC HIV PROTEASE INHIBITORS : C_2-SYMMETRY-BASED DESIGN OF BIS-SULFONAMIDE DIHYDROPYRONES

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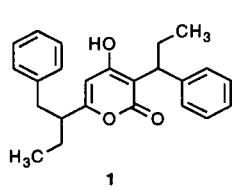
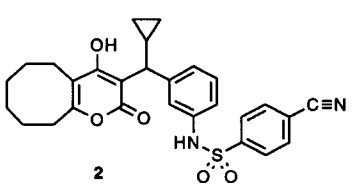
Abstract: Potent, non-peptidic, dihydropyrone sulfonamide HIV protease inhibitors have been previously described. Crystallographic analysis of dihydropyrone sulfonamide inhibitor/HIV protease complexes suggested incorporation of a second, C_2 symmetry-related sulfonamide group. Selected bis-sulfonamide dihydropyrone analogues display high HIV protease inhibitory activity.

HIV protease inhibitors have become important therapeutic agents in the treatment of AIDS.[1] We have previously described a series of structure-based drug design efforts that afforded pyrone **1** (K_i = 38 nM, IC_{50} = 3 μM) as our first generation clinical agent.[2] Additional studies led to the identification of cyclooctylpyranone sulfonamide **2** (K_i = <1 nM, IC_{50} = 1-2 μM) as a second generation compound.[3] Recently, sulfonamide dihydropyrones closely related to compound **3c** (K_i = 1 nM, IC_{50} = 0.5 μM) have shown significant therapeutic potential.[4]



1



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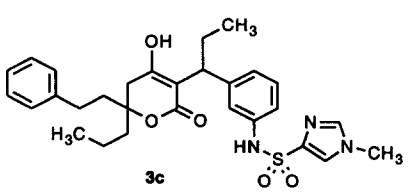
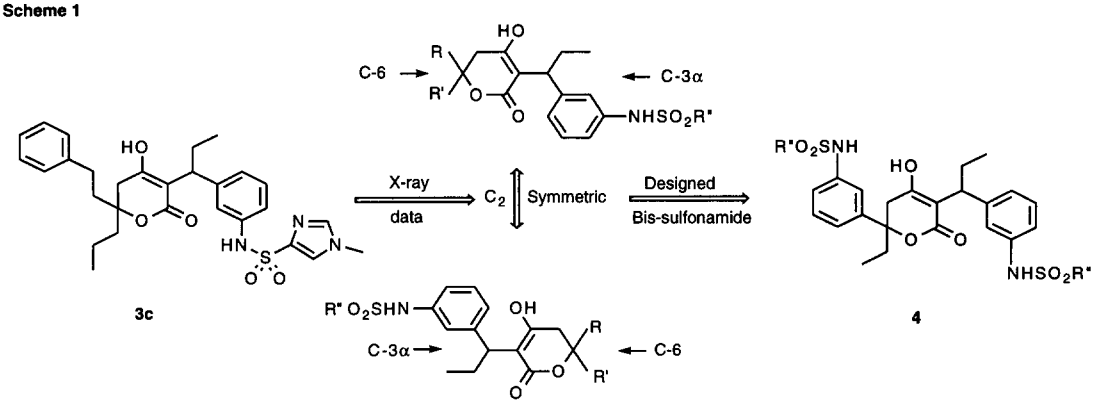


3c

PII: S0960-894X(98)00197-8

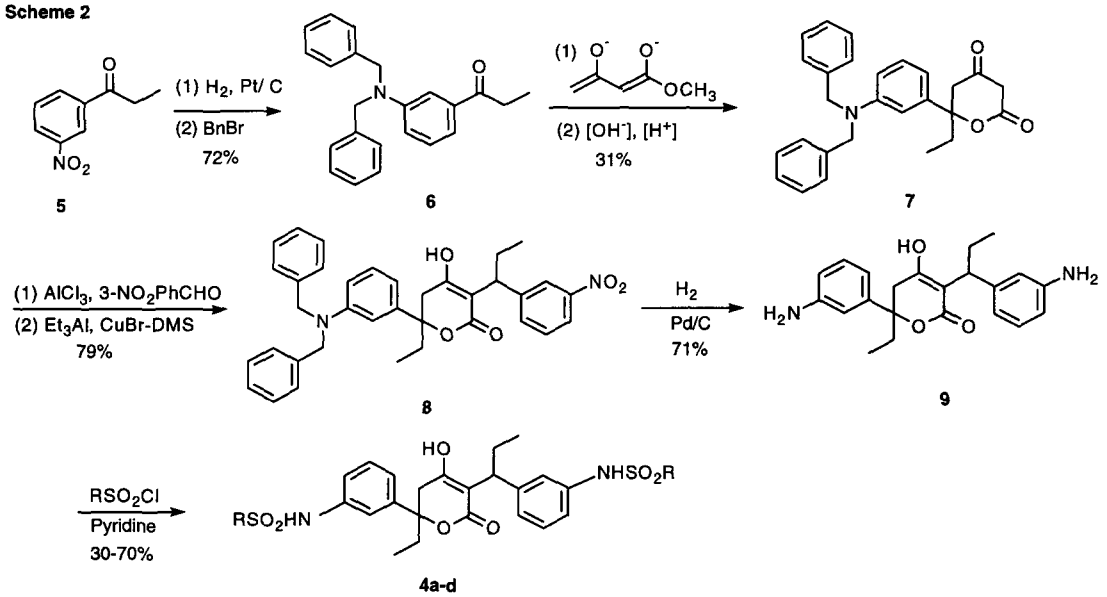
Due to the C_2 symmetrical nature of the dimeric HIV protease, many inhibitor/enzyme crystallographic structures that have been determined result in two orientations of the ligands related by a 180° rotation in the active site.[2] Analysis of crystallographic data containing sulfonamide dihydropyrone **3c** complexed with HIV protease[4] suggested that a C_2 symmetry operation would nearly superimpose the C-3α carbon of one conformer upon the C-6 carbon of the C_2-symmetry related conformer (Figure 1). This observation led to the design, synthesis and biological and crystallographic evaluation of a cognate class of dihydropyrone HIV protease inhibitors in which the C-6 substituents (propyl and phenylethyl sidechains) are replaced by the C-3α substitution (ethyl sidechain and phenyl sulfonamide group) shown as generic inhibitor **4** (Scheme 1).

Scheme 1



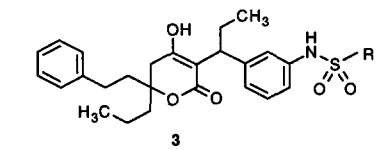
Bis-sulfonamide dihydropyrones[5] were prepared as shown in Scheme 2 following chemistry similar to that previously described.[6] Reduction of commercially available 3-nitropropiophenone (**5**) with platinum on carbon and hydrogen gas afforded crude 3-aminopropiophenone. The aniline nitrogen was subsequently bis *N,N*-protected with excess benzyl bromide using diisopropylethylamine to give the dibenzylated amine **6**. The dianion of methyl acetoacetate was condensed with propiophenone **6**, and the intermediate hydroxy-ester was cyclized with dilute aqueous hydroxide to give the dione **7**. Cyclic β-keto ester **7** was condensed with 3-nitrobenzaldehyde using aluminum trichloride. The resulting labile benzylidene adduct was used directly in the next step without further purification. Conjugate addition with triethylaluminum, in the presence of copper bromide-dimethyl sulfide complex, provided the C-3α ethylated product **8**. Treatment with palladium on carbon and hydrogen gas removed the benzyl protecting groups and reduced the aromatic nitro group to afford the intermediate diamine **9**. Reaction of compound **9** with various sulfonyl chlorides using pyridine in dichloromethane provided the desired bis-sulfonamide dihydropyrone analogues **4a–d**.

Scheme 2

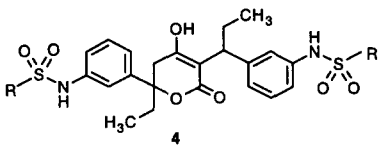


The resulting inhibitors displayed a wide range of enzymatic binding affinities[7] depending on the nature of the sulfonamide group (Table 1). Surprisingly, simple aryl or heteroaryl analogues **4a** and **4b** failed to show significant activity in the binding assay when the corresponding mono-sulfonamides **3a** and **3b** showed nanomolar K_i values; while the "designed" inhibitor **4c**, the direct bis-sulfonamide analog of the potent mono-sulfonamide **3c**, maintained high binding affinity. In the mono-functionalized dihydropyrone series, the sulfonamide moiety has been shown[4] to substantially influence the inhibitor's binding affinity. A progressive increase in enzymatic activity can be seen by changing the phenyl ring to a mono-cyclic, nitrogen-containing system. This effect of the sulfonamide substituent to modulate the protein-substrate interaction appears to have been profoundly enhanced in the bis-sulfonamide class. Analogues such as **4d** were also found that exhibited subnanomolar binding affinity against the enzyme. In an attempt to confirm the expected mode of binding for these inhibitors, X-ray crystallographic data were collected on the HIV protease complex of bis-sulfonamide dihydropyrone **4d**.

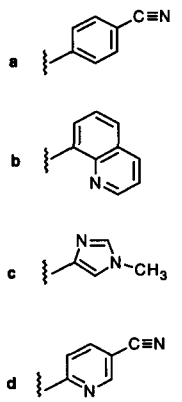
Table 1



3



4



R	Compound	K_I (nM)
a	3a	8
	4a	19% Inhibition at 1 μM
b	3b	3
	4b	35% Inhibition at 1 μM
c	3c	1
	4c	4.7
d	3d	0.1*
	4d	0.1*

*determined in tandem assay (ref. 4)

Analysis of the structural data strongly supports the molecular modeling prediction for the location and orientation of the bis-sulfonamide sidechains based on the earlier mono-sulfonamide structures. Figure 2 is the crystallographically determined structure **4d** as found in the HIV-1 protease complex. The hydroxy group of the central ring is positioned to interact with the two catalytically essential aspartic acid residues. The C-3α and C-6 ethyl sidechains are directed into the S_1/S_1 subsites, respectively. The sulfonamide group attached to the *m*-carbon of the phenyl ring located in the $S_{2'}$ subsite places the pyridyl substituent into the $S_{3'}$ subsite. This positioning of the sulfonamide moiety allows for additional favorable interactions between the substrate and the enzyme. Hydrogen bonding relationships between the sulfonamide linkage and the active site residues can be observed. These additional interactions may contribute to the improved enzymatic binding affinities for selected inhibitors. The sulfonamide group tethered to the C-6 carbon extends through the S_2 subsite to position the pyridyl ring in the S_3 subsite.

While this structure-based design approach afforded selected C_2-symmetry-based bis-sulfonamide dihydropyrones which displayed potent enzymatic activity, they surprisingly showed no antiviral activity in a whole cell assay at 3 μM.[8] The precise cause for this lack of *in vitro* activity is currently unclear. However, closely related mono-sulfonamides are currently undergoing clinical evaluation for the treatment of AIDS.[4]

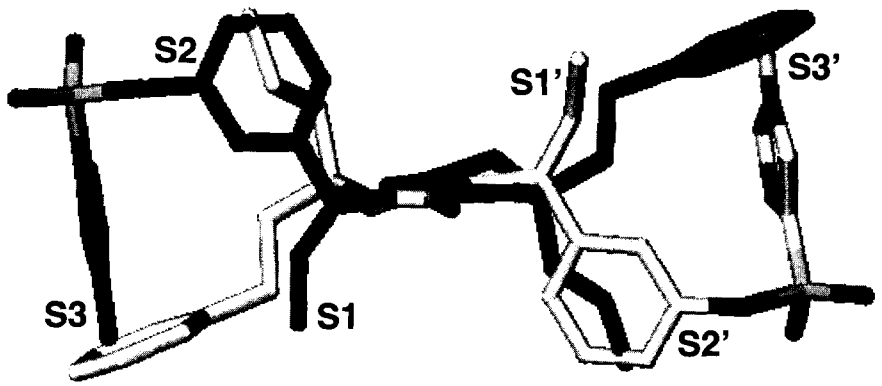


Figure 1. Superposition of the two C_2 related orientations of **3c** from the crystal structure of HIV–1 protease/**3c** complex. The two nearly C_2 symmetry orientations of the dimeric protein were superimposed by a least–squares fit. The protein is not shown in the figure but the subsites S3–S3' are indicated. One orientation has more lightly shaded bonds.

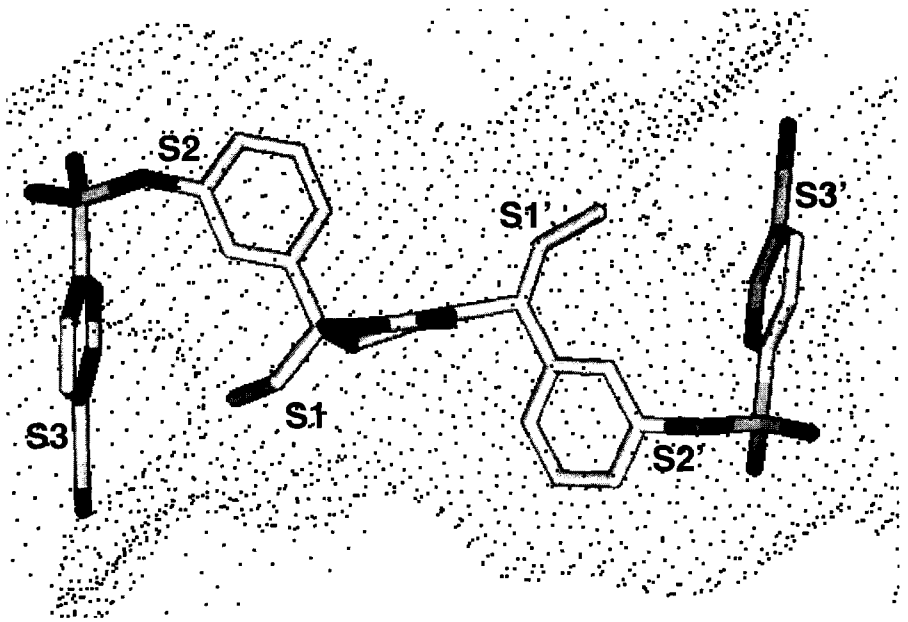


Figure 2. Conformation of **4d** from the HIV–1 protease/inhibitor crystal structure. The protein surface is shown with dots.

References and Notes:

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5. All new compounds were consistent with spectral data. Selected spectroscopic data for **4a** : MS (FAB) *m/z* (rel int) 697 (M+H, 100), HRMS (FAB) calcd for $C_{36}H_{33}N_4O_7S_2+H_1$ = 697.1791, found 697.1779; **4b** : MS (FAB) *m/z* (rel int) 749 (M+H, 100), HRMS (FAB) calcd for $C_{40}H_{36}N_4O_7S_2+H_1$ = 749.2103, found 749.2110; **4c** : MS (FAB) *m/z* (rel int) 655 (M+H, 100), HRMS (FAB) calcd for $C_{30}H_{34}N_6O_7S_2+H_1$ = 655.2008, found 655.1995; **4d** : MS (FAB) *m/z* (rel int) 699 (M+H, 100), HRMS (FAB) calcd for $C_{34}H_{30}N_6O_7S_2+H_1$ = 699.1695, found 699.1679.
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7. The biological assay used to measure in vitro inhibition of the HIV-1 protease has been previously described in reference 2.
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