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Synthesis and antiviral activity of P1′ arylsulfonamide azacyclic urea HIV protease inhibitors

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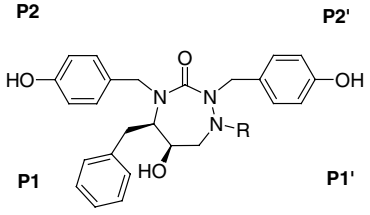
Abstract—A series of novel azacyclic urea HIV protease inhibitors bearing a benzenesulfonamide group at P1′ were synthesized utilizing a parallel synthesis method. Structural studies of early analogs bound in the enzyme active site were used to design more potent inhibitors. The effects of substituting the P1′ benzenesulfonyl group on antiviral activity and protein binding are described.

Inhibitors of the human immunodeficiency virus (HIV) protease are important therapeutic agents used in the treatment of AIDS infection.[1] We have previously reported azacyclic urea **1a** as a potent inhibitor of HIV protease.[2,3] Recent efforts to replace the benzyl substituent at P1′ with a variety of acyl groups resulted in compounds, which were found to exhibit similar or greater potency as HIV protease inhibitors.[4] For example, the benzoyl analog **1b** had excellent activity when compared to that of **1a**, with a K_i of 0.01 nM (Fig. 1).



	K_i (nM)	EC_{50} (μM)
1a R = CH_2Ph	0.07	0.01
1b R = COPh	0.01	---
1c R = SO_2Ph	1.37	0.459

Figure 1. Azacyclic urea HIV protease inhibitors.

However, the benzenesulfonyl analog **1c**, was found to be >10-fold less active than **1a**, suggesting that the sulfonyl analog **1c** might have a significantly different binding mode than **1a** and **1b**. Molecular modeling studies encouraged us to further investigate the P1′ benzenesulfonyl series in an effort to take advantage of novel vectors available for substituents on the benzene ring, thereby obtaining new interactions with the enzyme that might improve potency in the series. Taking advantage of the wide variety of substituted benzenesulfonyl chlorides that are commercially available, we were able to develop a parallel synthesis method to quickly prepare these compounds for testing.

The synthesis of P1′ benzenesulfonyl azacyclic ureas is outlined in Scheme 1. Compound **2** was prepared as previously reported.[2] Debenzylation of **2** by hydrogenolysis using Pearlman's catalyst, followed by reprotection of the exposed nitrogen with a Cbz group, gave **3**. Bis-alkylation of the urea nitrogens was accomplished by deprotonation with sodium hydride in DMF followed by the addition of trimethylsilylethoxymethyl (SEM) protected 4-hydroxybenzyl chloride (**4**). Subsequent removal of the Cbz protecting group by hydrogenolysis gave **5**, a common intermediate for the synthesis of P1′ acyl and sulfonyl derivatives. P1′ sulfonyl analogs were synthesized using a simple, rapid parallel approach, involving sulfonylation and subsequent removal of the SEM protecting groups. First, the appropriate benzenesulfonyl chloride was added to

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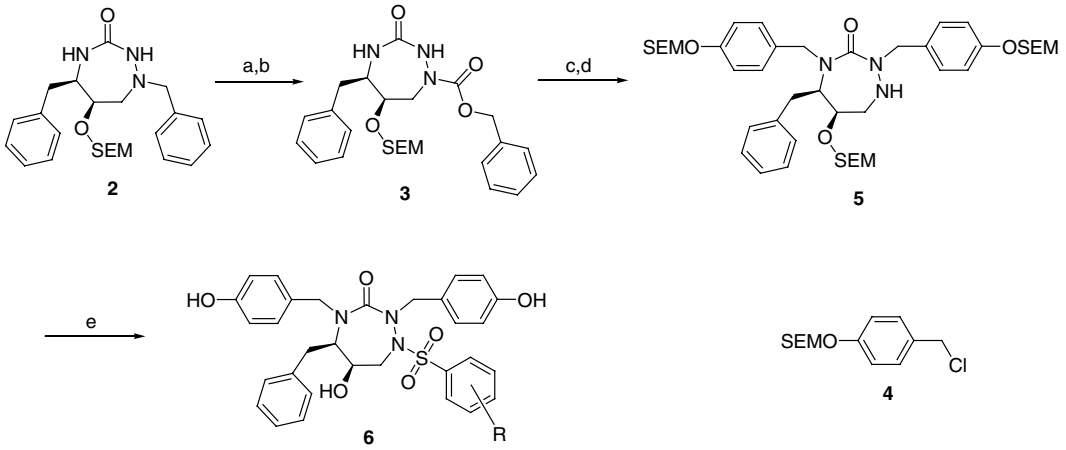
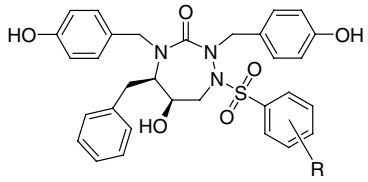


Scheme 1. Reagents and conditions: (a) $Pd(OH)_2/C$, H_2, MeOH, 50 °C, 77%; (b) BnOCOCl, Hunig's base, CH_2Cl_2, 77%; (c) **4**, NaH, DMF, 75%; (d) 10% Pd/C, H_2, EtOAc, 97%; (e) (i) $ArSO_2Cl$, NEt_3, DMAP, $ClCH_2CH_2Cl$, 40–50 °C, 18–90 h, (ii) polyamine resin, rt, 1 h, (iii) 4 N HCl, MeOH, 1 h, 16–78% (three steps).

5, followed by addition of a polyamine resin[5] to scavenge excess benzenesulfonyl chloride. Upon filtration of resin and evaporation of solvent, removal of the SEM ether protecting groups using anhydrous HCl in methanol provided the target compounds **6**.

Table 1 shows a summary of SAR for a series of substituted benzenesulfonyl azacyclic ureas prepared using the described method. The *ortho*-methyl substituted compound **6a**, showed a 2-fold improvement in EC_{50} over the parent compound **1c**. Of the numerous compounds prepared with a single substituent in the *meta* position (see, as examples, **6b**–**g**), only **6b**, the *meta*-cyano analog, showed an improvement in activity over **1c**. Monosubstitution at the *para*-position resulted in a greater than 2-fold loss in activity relative to the unsubstituted analog (for examples, see **6h**–**k**). Further, disubstitution resulted in compounds that were less active than the unsubstituted benzene analog **1c** (for examples, see dichloro derivatives **6l**–**o**). In general, monosubstitution in either the *ortho*- or *meta*-position was better tolerated. Also, the electron withdrawing/donating nature of the substituents did not seem to have a significant effect on antiviral activity.

Table 1. SAR for P1′ benzenesulfonyl azacyclic ureas bearing 4-hydroxybenzyl substituents at P2 and P2′



Compd	R	EC_{50} (μM)[a]
1c	H	0.459
6a	*o*-CH_3	0.238
6b	*m*-CN	0.2
6c	*m*-Br	0.428
6d	*m*-OCH_3	0.469
6e	*m*-F	0.598
6f	*m*-NO_2	0.817
6g	*m*-CH_3	0.927
6h	*p*-Methoxy	0.758
6i	*p*-CH_3	0.879
6j	*p*-Cl	1.1
6k	*p*-CF_3	1.1
6l	2,3-Dichloro	0.562
6m	3,5-Dichloro	0.67
6n	3,4-Dichloro	0.946
6o	2,4-Dichloro	3.2

[a] For assay procedures, see Ref. 6.

An X-ray crystal structure of compound **6b** bound in the HIV protease active site revealed that the P1′ *meta*-cyano group is projected into a water pocket on the side of the S1′ site and displaces one of two water molecules typically observed in this pocket (see Fig. 2a).[7] An analogous water pocket is also found in many other aspartyl proteases.[8] However, to date, we are unaware of a report in which this pocket has been exploited by an inhibitor for any aspartyl protease. As shown in Figure 2a, a vector into this novel S1′ subsite is provided by the *meta*-position of the benzenesulfonamide. Although activity data in Table 1 indicates no significant advantage for initial analogs bearing a *meta*-substituent over the unsubstituted analog **1c**, further exploration of this site with novel substituents in the *meta*-position of the benzene ring could result in compounds with improved binding. In order to achieve an improvement in potency, it was believed that the new *meta*-substituents would need to displace water from the site and effectively stack against the planar surface formed by the interaction of Arg8 with Asp29 (see Fig. 2b).

The X-ray crystal structure of **6b** was used as a model to design a new series of inhibitors to explore this novel S1′ subsite. For this new series, the alkyl substituents on the urea nitrogens were modified to give optimal binding at the P2/P2′ positions. The substitution pattern used was based in part on literature reports, in which the amidoxime group[9] and the *n*-butyl group[10] were both shown to give compounds with improved antiviral activity. Thus, a series of compounds was prepared bearing an

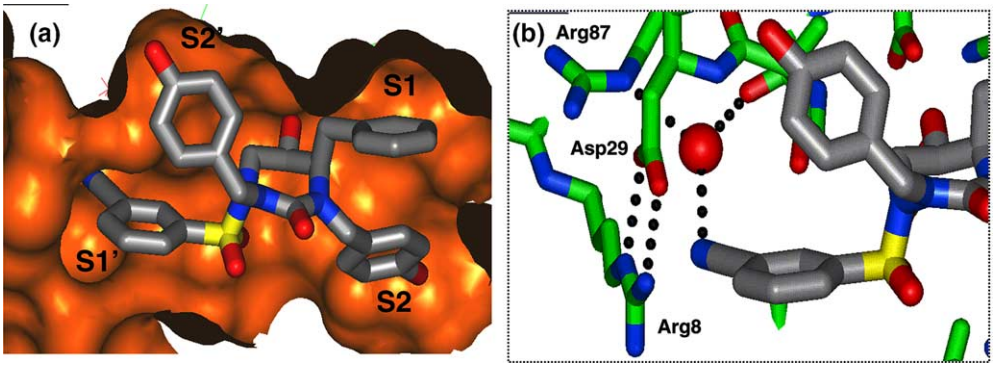
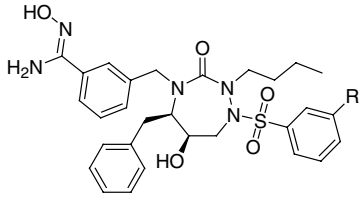


Figure 2. (a) Crystal structure of **6b** bound to HIV protease indicating a cavity on the side of the S1′ pocket to be probed with new substituents in this series. (b) Crystal structure of **6b** bound to HIV protease showing a well known aspartyl protease water pocket on the side of the S1′ pocket formed by Arg8 and Asp29. In the presence of **6b**, there are two water molecules remaining in this pocket, one of which is H-bonded to the *meta*-CN group.

amidoxime group at P2 and an *n*-butyl group at P2′, a substitution pattern that was found to give optimal activity in our assay. From a chemical perspective, our initial goals were to prepare compounds bearing reactive functionality in the *meta*-position of the benzenesulfonamide, thereby allowing for late-stage derivatization at this position of the molecule.

Table 2 shows a series of *meta*-substituted benzenesulfonyl analogs prepared with optimized substituents at P2 and P2′.[11] The antiviral activities of these compounds was determined both in the absence of human serum (0% HS) and in the presence of 50% human serum (50% HS) in order to estimate the effect of protein binding on in vivo potency. In general, these compounds showed a considerable improvement in antiviral activity over the 4-hydroxybenzyl series (compare the 0% HS activities for compounds in Table 2 with those in Table 1). For example, the *meta*-methoxy analog **7b** was 15-fold more active than **6d**, and the *meta*-nitro analog **7e**, showed a 20-fold improvement in activity over **6f**. The most active compound in this series was the ketoxime analog **7a**, with an EC_{50} of 0.024 μM in absence of human serum. In the presence of human serum, activity was attenuated some 17-fold (0.40 μM), which was in line with serum effects for other compounds in the series (typically 10–15-fold). The amino derivative **7c**, which was very active in the absence of serum (0.037 μM), displayed a very large serum effect (>60-fold), indicating this compound is highly bound to serum proteins. The *meta*-cyano derivative is not reported in this series due to difficulties encountered in synthesis of this analog using our synthetic method. Overall, the improvement in activity with the series of compounds in Table 2 (relative to compounds in Table 1) may be attributed primarily to the modification of P2/P2′ groups rather than the substituents at the *meta*-position of the benzenesulfonamide.

Table 2. SAR for *meta*-substituted benzenesulfonyl analogs designed to optimize potency



Compd	R	EC_{50} (μM)[a] (0%HS)[b]	EC_{50} (μM)[a] (50%HS)[c]
7a	C(=NOH)CH_3	0.024	0.401
7b	OCH_3	0.033	0.563
7c	NH_2	0.037	2.38
7d	CH(OH)CH_3	0.04	0.852
7e	NO_2	0.041	0.375
7f	OH	0.156	2.56
7g	$COCH_3$	0.157	1.09
7h	Br	0.162	2.49
7i	CH=CH_2	0.221	1.86

[a] For assay procedures, see Ref. 4.
[b] Assay performed in the absence of human serum.
[c] Assay performed in the presence of 50% human serum.

In summary, we have synthesized a series of novel P1′ benzenesulfonyl azacyclic urea HIV protease inhibitors using a parallel synthesis approach. Attempts to increase antiviral potency by making modifications on the phenyl ring of the benzenesulfonyl group resulted in very limited success in this series (none of the substituted analogs was more than 2-fold more active than the unsubstituted analog **1c**). Although the compounds presented in Tables 1 and 2 represent a fairly diverse set of substituted benzenesulfonamides, both series of compounds show a lack of dynamic range in activity. X-ray crystallographic study of compound **6b** bound in the enzyme active site revealed that the *meta*-cyano group of this inhibitor was projecting into a novel binding pocket in the S1′ site. This structural data was used as the basis to design new analogs for synthesis that were aimed at exploring this novel binding interaction. Initial analogs in this series (see Table 2) did not result in improvements in activity, suggesting that displacing water from this site is insufficient for potency gains. Further derivatization with substituents that replace water molecules in the S1′ subsite with appropriate hydrogen bond coordination and/or stack more effectively against the residues that define the pocket may result in better inhibitors.

Acknowledgements

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7. HIV protease was purified and crystallized in the presence of compound **6b** according to the procedures described by Stoll et al.[12] Data were collected at the Argonne National Laboratory synchrotron on the IMCA ID17 beamline using a Mar 165 CCD detector. Data were processed using HKL2000.[13] The co-crystals of HIV protease and compound **6b** belong to the orthorhombic space group $P2_12_12$ with unit cell dimensions $a = 57.326$ Å, $b = 86.001$ Å, $c = 46.077$ Å. Calculation of initial electron density maps and refinement were done using CNX[14,15] and refined to a final $R_{work} = 25.35$ and $R_{free} = 27.69$–2.2 Å resolution. Coordinates for the crystal structure of HIV protease complexed with **6b** have been deposited in the RCSB protein data bank, PDB ID 1T7K.
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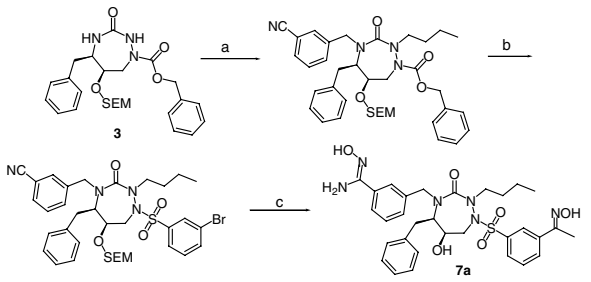
11. Compounds in Table 2 were prepared via an analogous procedure to that described below for **7a**:



Reagents and conditions: (a) (i) butyl iodide, potassium *t*-butoxide, THF, 45 °C, (ii) 3-cyanobenzyl bromide, NaH, DMF; (b) (i) 10% Pd/C, H_2, MeOH, (ii) 3-bromobenzenesulfonyl chloride, NEt_3, DMAP, $ClCH_2CH_2Cl$, 40–50 °C, (iii) polyamine resin, rt, 1 h; (c) (i) tributyl(1-ethoxyvinyl)tin, $Pd(Ph_3P)_4$, toluene, 100 °C, (ii) 4 N HCl, MeOH, 1 h, (iii) $NH_2OH{\cdot}HCl$, NEt_3, EtOH, 50 °C.
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