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Novel arylsulfonamides possessing sub-picomolar HIV protease activities and potent anti-HIV activity against wild-type and drug-resistant viral strains

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Abstract—A novel series of P1′ chain-extended arylsufonamides was synthesiszed and evaluated for wild-type HIV protease inhibitory activity and in vitro antiviral activity against wild type virus and two protease inhibitor-resistant mutant viruses. All of the compounds showed dramatic increases in enzyme activity as compared to the currently marketed HIV protease inhibitors amprenavir, indinavir, and nelfinavir. In addition, significant improvements in antiviral potencies against wild type and the two mutant viruses were also realized.
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The introduction of protease inhibitors into the HIV therapeutic arsenal in the mid-1990's dramatically improved the treatment of HIV disease. Therapies that combine HIV protease inhibitors (PIs) with agents that target other aspects of the viral replication cycle (such as nucleoside reverse transcriptase inhibitors) yield dramatic and sustained reductions in viral load in HIV positive patients.[1] Such combination therapies, known as highly active antiretroviral therapy or 'HAART', have transformed HIV into a much more manageable disease and have therefore been adopted as standard clinical practice. In spite of their success, the currently marketed HIV-PIs are not without problems. Included among their shortcomings are significant pill burdens, gastrointestinal side effects, and long term metabolic disturbances.[2] Of particular concern is the emergence of strains of HIV that are resistant to the current generation of PIs.[3] As a result of these limitations, a number of pharmaceutical companies are currently engaged in a search for newer generation HIV-PIs with improved side effect profiles and increased potency against both wild-type and resistant viral strains.

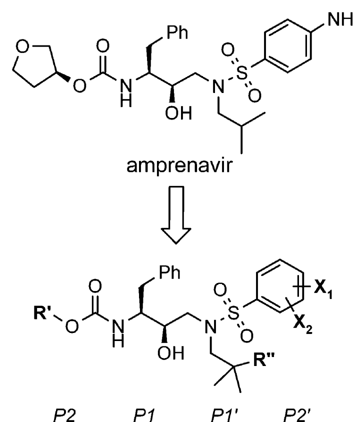

Figure 1.

Amprenavir is a potent, well tolerated HIV-PI that was introduced in 1999.[4] Recent efforts in our laboratories have been focused on identifying an improved, next generation HIV-PI by systematically modifying the side chains attached to the amprenavir arylsulfonamide scaffold. Toward this end, we have explored a number of modifications of the P2, P1′, and P2′ positions (Fig. 1). These efforts led to the identification of the

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Scheme 1. (a) 1.1 equiv 4-nitrophenylchloroformate, pyridine, CH_2Cl_2, 89%; (b) *t*-butylamine, $MgSO_4$, Et_2O, 3 days, 60%; (c) for **5a**: LDA, *N*-(2-chloroethyl)dibenzylamine, THF, −20 °C to 0 °C, 20%; for **5b**: LDA, 1-bromo-4-chlorobutane, THF, −20 °C to 0 °C, 95%; (d) NaN_3, 1:1 DMF/H_2O, 100 °C, 75%; (e) 1:1 TFA/CH_2Cl_2, then 1N aqueous NaOH, 86%; (f) 1.0 equiv **3**, DIEA, MeCN, 77%; (g) H_2 (40 psi), 5% Pd(C), MeOH, 98%; (h) 1.1 equiv 4-cyano-2,2-dimethylbutanal, $NaBH(OAc)_3$, 9:1 THF/AcOH, 75%; (i) 1.1 equiv $ArSO_2Cl$, 10 mol% DMAP, DIEA, THF, 81–89%; for **10a** Ar = 4-nitrophenyl, for **10b** Ar = 3-nitrophenyl, for **10c** Ar = 3,4-methylenedioxyphenyl; (j) H_2 (40 psi), Raney Ni, 2 M NH_3 in MeOH, 79–91%; (k) 1.1 equiv methyl chloroformate, DIEA, THF, 82–98%; (l) 1.2 equiv **5a**, $NaBH(OAc)_3$, 3:1 THF/AcOH, 70%; (m) 1.1 equiv 3,4-methylenedioxybenzenesulfonyl chloride, 10 mol% DMAP, DIEA, THF, 89%; (n) 5% HCOOH/MeOH, 10% Pd(C), 68%; (o) 1.1 equiv methyl chloroformate, DIEA, THF, 95%; (p) 1.2 equiv **5b**, $NaBH(OAc)_3$, 4:1 THF/AcOH, 61%; (q) 1.1 equiv 3,4-methylenedioxybenzenesulfonyl chloride, 10 mol% DMAP, DIEA, THF, 88%; (r) 1.2 equiv KCN, DMSO, 60 °C, 42%; (s) H_2 (40 psi), Raney Ni, 2 M NH_3 in MeOH, 89%; (t) 1.0 equiv methyl chloroformate, DIEA, THF, 93%.

stereochemically defined bis-THF subunit shown in Figure 2 as the optimal fragment at the P2 position. This moiety, which was originally reported by Ghosh and co-workers, yields dramatic improvements in both enzyme inhibitory activity and in vitro antiviral potency.[5] The improved enzyme activity is attributed to two additional hydrogen bonds between the bis-THF ring oxygens and Asp 29 and Asp 30 backbone NH's of the enzyme.

More recently, we have attempted further optimization of this series by exploring alternate substitution patterns on the P2′ phenylsulfonyl group while simultaneously elaborating the P1′ moiety. Specifically, we were interested in tethering polar substituents to the P1′ isobutyl sidechain. Our rationale here was the possibility of introducing additional enzyme–inhibitor binding interactions and the potential to favorably modulate physiochemical properties such as aqueous solubility. Examination of HIV protease X-ray crystal structures

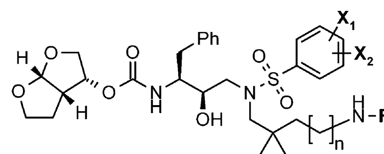
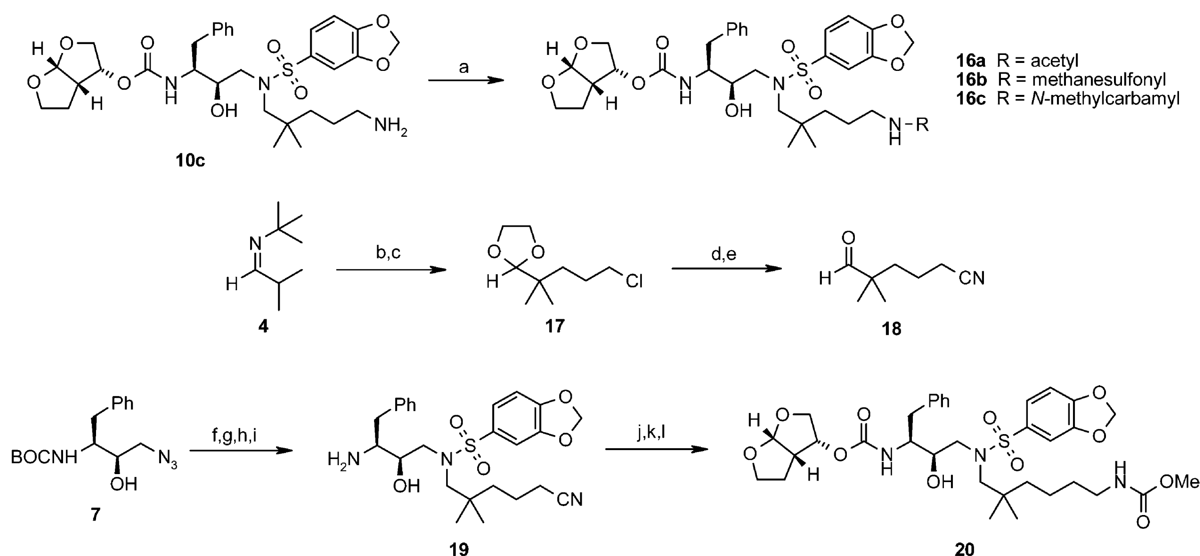


Figure 2.

with bound inhibitors suggests the possibility of achieving hydrogen bond interactions between a distal P1′ polar group and the guanidine side-chain of Arg 8. A viable synthetic plan would allow for the introduction of several different P1′ terminal groups as well as variation of the tether chain-length in order to optimize any potential new interactions. This paper describes the synthesis of a series of P1′ amino derivatives that have the bis-THF group in the P2 position. We have addressed 3 structural variables: the P2′ phenylsulfonyl substitution pattern, the nature of the P1′ amino capping



Scheme 2. (a) For **16a**: 1.1 equiv acetyl chloride, DIEA, THF, 86%; for **16b**: 1.1 equiv methanesulfonyl chloride, DIEA, THF, 97%; for **16c**: 5.0 equiv methyl isocyanate, THF, 77%; (b) LDA, 1-bromo-3-chloropropane, −20 °C to 0 °C, 70%; (c) ethylene glycol, PTSA toluene, reflux, 75%; (d) KCN, DMSO, 100 °C, 18 h, 85%; (e) HCl, H_2O/THF, 70 °C, 4.5 h; (f) H_2 (40 psi), 10% Pd(C), MeOH, 98%; (g) 1.2 equiv **18**, $NaBH(OAc)_3$, DMF/THF/AcOH, 55%; (h) 1.10 equiv 3,4-methylenedioxybenzenesulfonyl chloride, 10 mol% DMAP, DIEA, THF, 86%; (i) 1:1 TFA/CH_2Cl_2, then 1 N aqueous NaOH, 100%; (j) 1.1 equiv **3**, DIEA, MeCN, 76%; (k) H_2 (40 psi), Raney Ni, 2 M NH_3 in MeOH, 89%; (l) 1.1 equiv methyl chloroformate, DIEA, THF, 89%.

group, and the length of the P1′ tether (Fig. 2). In addition, we present enzyme inhibition data for wild type HIV protease and in vitro antiviral data for wild type virus and two drug-resistant mutant viruses.

Syntheses of compounds containing 4, 5, and 7 carbons in the P1′ chain all proceed through primary amine intermediate **9** (Scheme 1). The route begins with commercially available epoxide **6** which was converted to amino azide **8** by ring opening with NaN_3 followed by removal of the BOC protecting group. The bis-THF subunit was introduced by acylation with the PNP-carbonate derivative of optically pure alcohol **2**.[5b] Compound **8** was subjected to catalytic reduction to afford intermediate **9**. Reductive alkylation of **9** with 4-cyano-2,2-dimethylbutanal[6] followed by sulfonylation with the appropriate sulfonyl chloride and then Raney nickel reduction afforded primary amines **10a–c**. Acylation of **10a–c** with methyl chloroformate gave carbamate derivatives **11a–c**.

The preparation of 4 and 7 carbon derivatives **13** and **15** required aldehyde building blocks **5a** and **5b** which were prepared by alkylation of aldimine **4** with the appropriate electrophile. Conversion of **9** to dibenzyl protected amine **12** was achieved by reductive alkylation with aldehyde **5a** followed by sulfonylation with 3,4-methylenedioxybenzenesulfonyl chloride.[7] Deprotection of **12** by catalytic transfer hydrogenation gave the primary amine which was then acylated with methyl chloroformate to afford **13**. Reductive alkylation of **9** with chloroaldehyde **5b** followed by sulfonylation gave a primary chloride which was reacted with KCN to afford cyano derivative **14**. Raney nickel reduction followed by acylation with methyl chloroformate gave **15**. The preparation of 5-carbon derivatives with 3 alternate amino capping groups from amine **10c** is shown at the top of Scheme 2.

The synthesis of the 6-carbon methyl carbamate **20** was achieved using an alternate approach in which the bis-THF group was introduced at a later stage in the route (Scheme 2). The requisite aldehyde building block **18** was prepared using a straightforward 4-step sequence. Aldimine **4** was subjected to LDA alkylation with 1-bromo-3-chloropropane followed by treatment with ethylene glycol under acidic conditions to afford acetal **17**. Intermediate **17** was converted to cyanoaldehyde **18** by reaction with KCN at elevated temperature and then cleavage of the acetal protecting group. The BOC-protected azide **7** was subjected to catalytic reduction of the azide group followed by reductive alkylation with aldehyde **18**. The resulting secondary amine was sulfonylated with 3,4-methylenedioxybenzenesulfonyl chloride and then treated with TFA to give amine **19**. Acylation of **19** with PNP carbonate **3** followed by Raney nickel reduction of the cyano group and acylation with methyl chloroformate afforded compound **20**.

Table 1 shows enzyme activities and in vitro antiviral data for 10 new P1′ amino arylsulfonamides. Also shown are analogous data for the currently marketed PIs amprenavir, indinavir, and nelfinavir for comparison. The wild-type enzyme inhibition data is based on a radioligand binding competition assay.[8] The antiviral IC_{50}s for wild-type HIV virus (HXB2)[9] and two multi-PI resistant viruses (EP13 and D545701)[10] were determined in an MT4 cell line.[11] The most obvious observation is that all of the compounds are dramatically more potent in the enzyme assay than any of the 3 currently marketed PIs shown in the table. The inhibitor binding constants range from 100 to 4000 times lower than the K_i for amprenavir. With the exception of **10c**, all of the compounds show significant improvements in antiviral potency against the wild type and both PI-resistant viruses. Another general observation is that the enzyme SAR does not necessarily parallel the antiviral

SAR. This is likely due to variations in physiochemical properties that influence the efficiency of cell penetration. This effect is particularly noticeable for primary amine **10c** which is over 900 times as potent as amprenavir against the enzyme but shows a 3-fold drop in wild-type antiviral potency.

A comparison of compounds **11a**, **11b**, and **11c** indicates a modest increase in enzyme potency for the *para* and *meta* amino derivatives as compared to the methylenedioxy compound. On the other hand, **11c** is significantly more potent in the wild-type antiviral assay. The effect of the P1′ amino capping group can be inferred by comparing compounds **11c**, **16a**, **16b**, and **16c**. Acetamide **16a**, urea **16b**, and sulfonamide **16c** have similar enzyme activities and are collectively 10-fold more potent than methyl carbamate **11c**. However, **11c** shows generally improved antiviral activity versus the other three derivatives. A comparison of compounds **11c**, **13**, **20**, and **15** indicates a pronounced effect of the P1′ chainlength on both enzyme and antiviral activity. With an enzyme K_i of 14 fM, compound **20** is over 4000 times as potent as amprenavir. It also shows very impressive antiviral activities against wild-type and both PI-resistant viruses. These data are certainly consistent with a new enzyme–inhibitor binding interaction which is optimal for the $n=3$ chainlength of compound **20**. In addition, compound **20** was subjected to pharmacokinetic analysis in the rat and showed an oral bioavailability of 20% and a half-life of 1.3 h.

In summary, we have discovered a novel series of arylsulfonamide HIV-PIs that show remarkable enzyme inhibitory activity and very potent in vitro antiviral activity against wild-type virus and two multi-PI resistant viruses. Structural studies aimed at characterizing any new P1′ enzyme–inhibitor interactions are currently in progress. In addition, further SAR work in the arylsulfonamide series is presently being pursued. The results of these efforts will be reported in future accounts.

Table 1. Wild-type enzyme inhibitory activity and in vitro antiviral activity for P1′ amino arylsulfonamides

Entry	n	Ar	R	WT Enzyme K_i (pM)	HXB2 IC_{50} (nM)	EP13 IC_{50} (nM)	D545701 IC_{50} (nM)
10c	2	3,4-methylenedioxyphenyl	H	0.061	370	540	730
11a	2	4-NH_2-phenyl	C(O)OMe	0.26	31	46	95
11b	2	3-NH_2-phenyl	C(O)OMe	0.21	18	36	100
11c	2	3,4-methylenedioxyphenyl	C(O)OMe	0.52	4.7	38	66
13	1	3,4-methylenedioxyphenyl	C(O)OMe	0.16	1.4	22	40
20	3	3,4-methylenedioxyphenyl	C(O)OMe	0.014	1.6	15	9.2
15	4	3,4-methylenedioxyphenyl	C(O)OMe	0.12	5.5	46	65
16a	2	3,4-methylenedioxyphenyl	C(O)Me	0.054	23	30	120
16b	2	3,4-methylenedioxyphenyl	C(O)NHMe	0.048	38	110	200
16c	2	3,4-methylenedioxyphenyl	SO_2Me	0.058	33	47	150
Amprenavir	—	—	—	57[a]	130	440	>1000
Indinavir	—	—	—	70[a]	50	330	440
Nelfinavir	—	—	—	170[a]	320	450	>1000

[a] Determined using an activity assay based on a fluorescently labeled peptide substrate.[12]

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