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Synthesis and antiviral activities of novel *N*-alkoxy-arylsulfonamide-based HIV protease inhibitors

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Abstract—A series of novel *N*-alkoxy-arylsulfonamide HIV protease inhibitors with low picomolar enzyme activity and single digit nanomolar antiviral activity is disclosed.

1. Introduction

HIV protease inhibitors are the only antivirals that have been employed in HAART (highly active anti-retroviral therapy) that do not target the reverse transcriptase during viral replication. These agents, which prevent production of infectious virions, have become the mainstay in an ever-increasing milieu of drug combinations that successfully suppress viral replication in infected patients. However, these agents are not without side effects, owing in part to the relatively high doses necessary to completely suppress viral replication. Patient compliance coupled with the development of viral resistance can ultimately lead to virologic breakthrough and antiretroviral failure.[1] Therefore, the need for agents with increased potency and activity against resistant strains of HIV is well recognized. This in turn has to be accommodated against a complex competitive landscape of extensive prior art directed against this aspartyl protease.

Amprenavir (Fig. 1) and its prodrug fosamprenavir are prototypical HIV protease inhibitors (PI) which are generally well tolerated by patients and exhibit a favorable clinical resistance profile.[2] As such, the arylsulfonamide scaffold coupled to an ethanolamine backbone, which is found in most inhibitors from this class, might serve as a logical starting point for further improvements. We reasoned that incorporation of an *N*-alkoxysulfonamide to occupy the P1′/P2′ pocket of the active site of the viral protease might provide a novel pharmacophore from which to generate agents with increased antiretroviral potency (Fig. 1). The results of this investigation are detailed below.

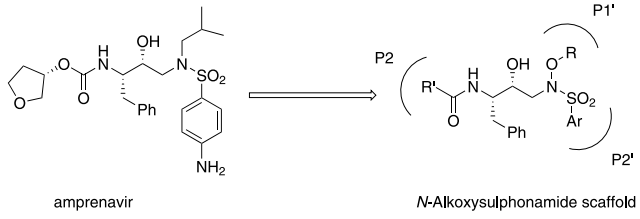

Figure 1.

2. Chemistry

The general synthetic strategy for construction of these analogs involved either addition of suitably substituted *N*-alkylhydroxylamines **1** or *N*-alkoxy-arylsulfonamides **2** into known phenyl alanine-derived epoxide **3**[3] (Scheme 1). Hydroxylamines were prepared through known methodology of either alkylation or Mitsonobu coupling of *N*-hydroxyphthalimide, followed by hydrazine deprotection to yield the free hydroxylamine **1**.[4]

Addition of these substituted hydroxylamines into epoxide **3** was often sluggish (Method A). However, addition of lithium triflate to a THF solution provided reasonable

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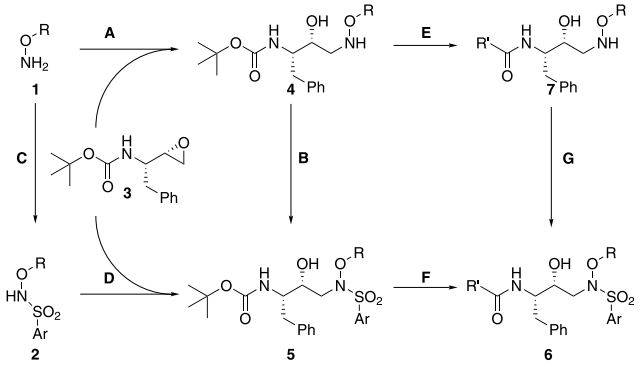


Scheme 1. Reagents. Method A: $LiSO_3CF_3$, THF, epoxide **3**. Method B: $ArSO_2Cl$,[5] DIEA, cat. DMAP, THF. Method C: $ArSO_2Cl$,[5] DIEA, CH_2Cl_2. Method D: (1) P4-phosphazene, epoxide **3**, THF; or (2) LiHMDS, epoxide **3**, THF; or (3) (a) H_2, Pd on $BaSO_4$, EtOH; (b) P4-phosphazene, epoxide **3**, THF. Method E: (a) TFA; (b) DIEA, (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-yl 4-nitrophenyl carbonate,[6] THF. Method F: (1) (a) TFA; (b) (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-yl 4-nitrophenyl carbonate,[6] DIEA, CH_2Cl_2 or CH_3CN; or (2) HATU, DIEA, 3-methyl-*N*-[(methyloxy)carbonyl]-L-valine,[7] DMF; or (3) EDC, HOBT, DIEA, N^2-(2-quinolinylcarbonyl)-L-asparagine;[7] or (4) (a) TFA; (b) (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-yl 4-nitrophenyl carbonate,[6] DIEA, CH_2Cl_2; (c) H_2, 10% Pd on C, 2 M NH_3 in MeOH. Method G: (1) (a) $ArSO_2Cl$,[5] DIEA, cat. DMAP, THF; (b) H_2, 10%Pd on C, EtOH; or (2) $ArSO_2Cl$,[5] DIEA, cat. DMAP, THF.

yields of intermediate **4** without requiring large excesses of the hydroxylamine. Treatment of **4** with an arylsulfonyl chloride (Method B) then provided inhibitors **5** with all of the key functionalities in place at P1′, P2′, and P1, albeit with the suboptimal BOC-amine group occupying the P2 pocket. Arylsulfonyl chlorides were either available commercially or prepared through literature methods.[5] This approach allowed rapid optimization of the hydroxylamine at P1 and provided a useful intermediate for further elaboration. Alternatively, intermediate **5** could also be generated by initial sulfonylation of hydroxylamine **1** (Method C), followed by addition of the *N*-alkoxysulfonamide anion, generated with a suitably strong base (e.g., lithium bis(trimethylsilyl)amide or P4-phosphazene), into epoxide **3** (Method D).

Analogs with optimized substituents at P2 could be prepared in two separate ways. Boc-deprotection of **5** with TFA followed by acylation of the free amine provided analogs **6**, which allowed diversification at the P2 position subsequent to fixing the P1′ substituent (Method F). Acylation protocols involved either reaction with known activated carbonates[6] or coupling of known carboxylic acids[7] with EDC or HATU in DMF.

A final synthetic strategy involved Boc-deprotection of intermediate **4**, providing a diamine intermediate where the primary amine could be selectively acylated over the less reactive *N*-alkoxyamine (Method E) to provide intermediates **7**. Final analogs **6** were then provided through sulfonylation of **7** with the appropriate arylsulfonyl chloride (Method G). This sequence provided our strategy for optimizing P2′ after fixing substituents at P2.

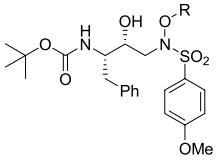
All aminobenzenesulfonamides were derived from their corresponding nitrophenylsulfonyl chloride. We did observe that nitro-substituted *N*-alkoxy-arylsulfonamides were incompatible with P4-phosphazene and therefore were reduced to the aniline prior to addition to epoxide **3**. Otherwise, reduction of the nitro groups, if present in final analogs, was performed at the end of the sequence. Similarly, the *O*-benzyl-protecting group on the phenolic sulfonamides **n** and **t** was removed at the last stage via hydrogenolysis.

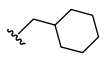
3. Results and discussion

Our SAR strategy was to initially optimize the hydroxylamine at P1′ since this functionality was unprecedented in HIV PIs. We felt that with this accomplished, we could then complement these results with known SAR at P2 from published[1] and internal data and finally obtain completely optimal antiviral activity through substituents on the arylsulfonamide group. We elected to keep the benzyl group at P1 fixed on the ethanolamine backbone due to the arduous synthetic investment necessary for these analogs.

Optimization of the P1′ position of our inhibitors was focused on lipophilic groups due to known elements of the active site for this position of the protease.[8] Table 1 summarizes a subset of prepared analogs that address the intrinsic enzyme inhibition[9] activity of the core hydroxylamine substituent. The *O*-isopropyl analog **a**, isosteric to the isobutyl group in amprenavir, was surprisingly a relatively weak inhibitor with a K_i of

Table 1. Optimization of P1′ *N*-alkoxysulfonamide substituent



Entry[a]	R	K_i (nM)[b]	Synthetic sequence (yields)[c]
a		14.0	C, D1 (69%, 60%)
b		3.2	A, B (51%, 70%)
c		4.0	C, D2 (92%, 100%)
d		2.8	C, D2 (83%, 22%)
e		1.6	C, D1 (79%, 89%)
f		12.0	C, D2 (80%, 99%)
g		22.0	C, D1 (90%, 99%)

[a] All compounds were >95% pure by ^1H NMR and HPLC.
[b] K_i, enzyme inhibition constant.[9]
[c] See Figure 1 for details.

14 nM. However, the analogous carbon analog has been reported in the literature as having a K_i of 34 nM.[10] Extension with a single methylene or two methyl groups (analogs **b** and **c**, respectively) increased enzyme activity 3-fold. Direct substitution of cyclohexyl (**d**) and a cyclopentyl (**e**) on the oxygen provided an almost 10-fold increase in inhibitory potency relative to isopropyl. However, activity was attenuated by either extending the cycloalkyl moiety (**f**) or by substituting a phenyl (**g**) for the cycloalkyl group. From these data, we were able to conclude that *O*-cyclopentyl and *O*-cyclohexyl alkoxysulfonamides would serve as preferred P1′ substituents.

Next, we addressed a small group of substituents at P2 that had historically given potent enzyme binding inhibition and antiviral potency, as measured in an MT4 cell line (Table 2).[11] The hydroxy tetrahydrofuran carbamate found in amprenavir led to subnanomolar enzyme inhibitor **h**, although with an observed enzyme binding constant roughly five times higher than that of the parent drug. However, we were encouraged that the enzyme potency translated into similar cellular antiviral activity between the two scaffolds. The antiviral potency of **h** was also roughly 5-fold less active than that of the marketed drug. *t*-Butylglycine methylcarbamate analog **i**, derived from atazanavir, displayed enzyme and antiviral activities roughly comparable to those of amprenavir.

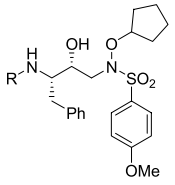
Given sufficient enzyme potency, it was clear that we could surpass the antiviral activity displayed by prototypical HIV PIs. Accordingly, the asparagine quinoline carboxamide found in saquinavir and hexahydrofuro[2,3-*b*]furan-3-ol carbamate P2 substituents generated inhibitor analogs **j** and **k**, respectively, with an inhibitory concentration of less than 5 pM against the enzyme. An impressive antiviral activity of 24 nM was recorded for the saquinavir-derived analog, whereas the fused furan carbamate provided even further improvements, with a single digit antiviral potency of 6 nM.

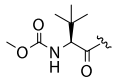
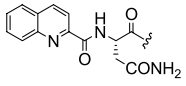
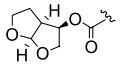
Finally, we directed our efforts toward optimization of the P2′ group. Given that the hexahydrofuro[2,3-*b*]furan-3-ol carbamate at P2 displayed the optimal antiviral potency, we elected to focus on this preferred substituent at this position. Similarly, the *O*-cyclopentyl and the *O*-cyclohexyl substituents at P1′ displayed the most favorable enzyme potency in our study. We therefore focused on these two substituents at P1′ in our P2′ study (Table 3).

Without exception, the substituents that we chose at P2′ displayed exceptional enzyme and antiviral activities when combined with optimized P2 and P1′ groups (Table 3). Notably, only the combination of the P1′ cyclohexyl group and the P2′ 4-aminophenylsulfonyl group, analog **s**, displayed slightly attenuated enzyme potency. However, a corresponding decrease in antiviral potency was not observed. Each of the analogs in this series displayed $\leqslant$20 nM antiviral activity in our cell-based assay, which corresponded to a 7.5- to 50-fold increase in antiviral potency relative to amprenavir.

Given the exceptional antiviral activity displayed by these analogs, we screened a subset of compounds against two multi-PI resistant viral strains[12] (EP13 and D545701) in MT4 cell lines in order to assess their potential against resistant viral strains (Table 4). Our assay cutoff of >240 nM reflected our estimation of a clinically relevant acceptable antiviral activity. Notably, analogs **p** and **t** displayed acceptable antiviral potency against both mutant strains of virus.

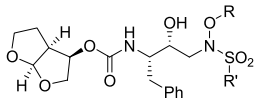
Table 2. Optimization of P2



Entry[a]	R	K_i (nM)[b]	IC_{50} (nM)[c]	Synthetic sequence (yields)[d]
h		0.19	738	C, D1, F1 (69%, 60%, 87%)
i		0.090	203	C, D1, F2 (69%, 60%, 35%)
j		<0.005	24	C, D1, F3 (69%, 60%, 41%)
k		<0.005	6	C, D1, F1 (69%, 60%, 82%)
Amprenavir		0.04	150	

[a] All compounds were >95% pure by ^{1}H NMR and HPLC.
[b] K_i, enzyme inhibition constant.[9]
[c] IC_{50}, antiviral inhibition against HIV-1 in MT-4 cell culture.[11]
[d] See Figure 1 for details.

Table 3. Optimization of P2′



Entry[a]	R	R′	K_i (nM)[b]	IC_{50} (nM)[c]	Synthetic sequence (yields)[d]
l			<0.005	17	C, D3, F1 (87%, 77%, 63%)
m			<0.005	9	A, B, F4 (86%, 88%, 41%)
n			<0.005	12	A, B, F4 (86%, 50%, 77%)
o			<0.005	7	A, B, F1 (86%, 79%, 47%)
p			<0.005	7	A, B, F1 (86%, 89%, 68%)
q			<0.005	20	C, D2, F1 (83%, 22%, 27%)
r			0.005	11	A, E, G1 (36%, 72%, 82%)
s			0.018	8	A, E, G1 (36%, 72%, 74%)
t			<0.005	5	A, E, G1 (36%, 72%, 79%)
u			<0.005	13	A, E, G1 (36%, 72%, 87%)
v			<0.005	3	A, E, G1 (36%, 72%, 86%)
Amprenavir			0.04	150	

[a] All compounds were >95% pure by ^{1}H NMR and HPLC.
[b] K_i, enzyme inhibition constant.[9]
[c] IC_{50}, antiviral inhibition against HIV-1 in MT-4 cell culture.[11]
[d] See Figure 1 for details.

Table 4. Resistance profile of selected analogs (IC_{50} nM in MT4 cells)

Entry	EP13 (fold change)	D545701 (fold change)	HIV-1
m	212 (24)	>240 (>27)	9
p	184 (26)	194 (28)	7
t	44 (9)	117 (23)	5
u	107 (8)	>240 (>18)	13
v	100 (33)	>240 (>80)	3
Amprenavir	440 (3)	>1000 (>7)	150

Our final challenge remained achieving acceptable pharmacokinetic profiles. As has been observed with most HIV PIs, bioavailability of these analogs was uniformly poor. However, one notable exception was found: analog **l** demonstrated 11% oral bioavailability after dosing intravenously (iv) and by gavage at 5 mg/kg in HW rats. Encouraged by this modest level of plasma exposure, we examined an identical oral dose and an iv dose of 1 mg/kg in beagle dogs which produced a 16% bioavailability. We were especially encouraged by these results in the context that the second-generation HIV protease inhibitor lopinavir provides a modest 25% bioavailability in rat and no intrinsic bioavailability in either dog or monkey. In this case, bioavailability was enhanced in all species via coadministration with the CYP 3A4 inhibitor, ritonavir.[13]

In summary, we have disclosed a novel series of *N*-alkoxy-arylsulfonamide-based HIV protease inhibitors that display both exceptional enzyme inhibitory activity and antiviral potency. Members of this series also demonstrate antiviral activity against mutant viral strains. In addition, one analog also demonstrated modest bioavailability in both rat and dog without the need for the added pharmacoenhancing properties of ritonavir.

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