Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3496–3500
Bioorganic & Medicinal Chemistry Letters

Novel P1 chain-extended HIV protease inhibitors possessing potent anti-HIV activity and remarkable inverse antiviral resistance profiles

John F. Miller,* Michael Brieger,† Eric S. Furfine,‡ Richard J. Hazen, Istvan Kaldor, David Reynolds,§ Ronald G. Sherrill and Andrew Spaltenstein

GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709, USA

Received 12 April 2005; revised 26 May 2005; accepted 31 May 2005

Abstract—A novel series of tyrosine-derived HIV protease inhibitors was synthesized and evaluated for in vitro antiviral activity against wild-type virus and two protease inhibitor-resistant viruses. All of the compounds had wild-type antiviral activities that were similar to or greater than several currently marketed HIV protease inhibitors. In addition, a number of compounds in this series were more potent against the drug-resistant mutant viruses than they were against wild-type virus.

The advent of highly active antiretroviral therapy or 'HAART' in the mid-1990s resulted in dramatic improvements in the treatment of HIV disease. Prior to this time, treatment consisted of mono- or combination therapy with various nucleoside reverse transcriptase inhibitors. The inclusion of protease inhibitors (PIs) as a component of combination regimens led to significantly improved clinical outcomes.[1] In spite of their success, the presently marketed HIV-PIs have a number of shortcomings. Of particular concern is the emergence of strains of HIV that are resistant to the current generation of PIs.[2] Therefore, the discovery of newer agents in the PI class that have activity against PI-resistant viral strains is becoming increasingly important.

We recently described a novel series of arylsulfonamide HIV-PIs that possessed sub-picomolar HIV protease activities and potent antiviral activity against wild-type and two drug-resistant viruses.[3] This series, exemplified by compound **1** (Fig. 1), was obtained by systematically

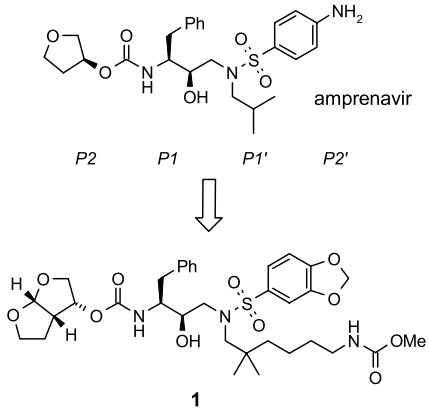


Figure 1.

modifying the P2, P1′, and P2′ side chains of the amprenavir scaffold.

A key element of our strategy was the possibility of improving enzyme potency through the introduction of additional enzyme–inhibitor binding interactions by tethering capped aminoalkyl moieties to the P1′ isobutyl side chain. More specifically, we were interested in building in hydrogen bonding interactions between distal P1′ acylamino groups and the guanidine side chain of Arg 8 in the protease enzyme. The SAR data showed a clear dependence of enzyme and antiviral activity on the nature of the amino capping group and the length

Keywords: HIV protease; Antiviral; Arylsulfonamide; Drug resistance; Antiretroviral; Hypersusceptibility.
* Corresponding author. Tel.: +1 919 483 2750; fax: +1 919 483 6053; e-mail: john.6.miller@gsk.com
† Present address: Eli Lilly and Company, Indianapolis, IN, USA.
‡ Present address: Regeneron Pharmaceuticals Inc., Tarrytown, NY, USA.
§ Present address: King Pharmaceuticals R&D, Cary, NC, USA.

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.05.129

of the tether. Both of these observations are consistent with a new enzyme–inhibitor interaction.

Herein we describe our efforts to further improve on the arylsulfonamide scaffold by shifting the capped alkylamino moieties from the P1′ isobutyl side chain to an oxygen atom located in the *para* position of the P1 phenyl group (Fig. 2). Based on the homodimeric C_2 symmetric structure of the HIV-protease enzyme, it might be possible to achieve binding interactions through P1 side chain extensions analogous to those that appear to be operative in the P1′ chain-extended series.

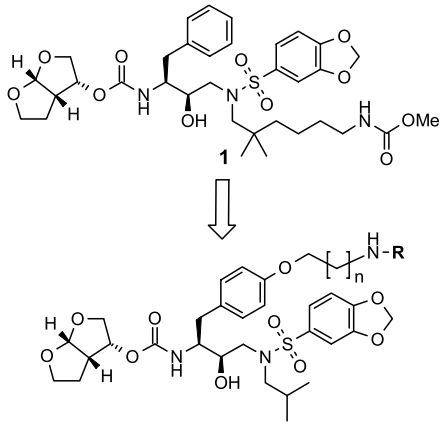
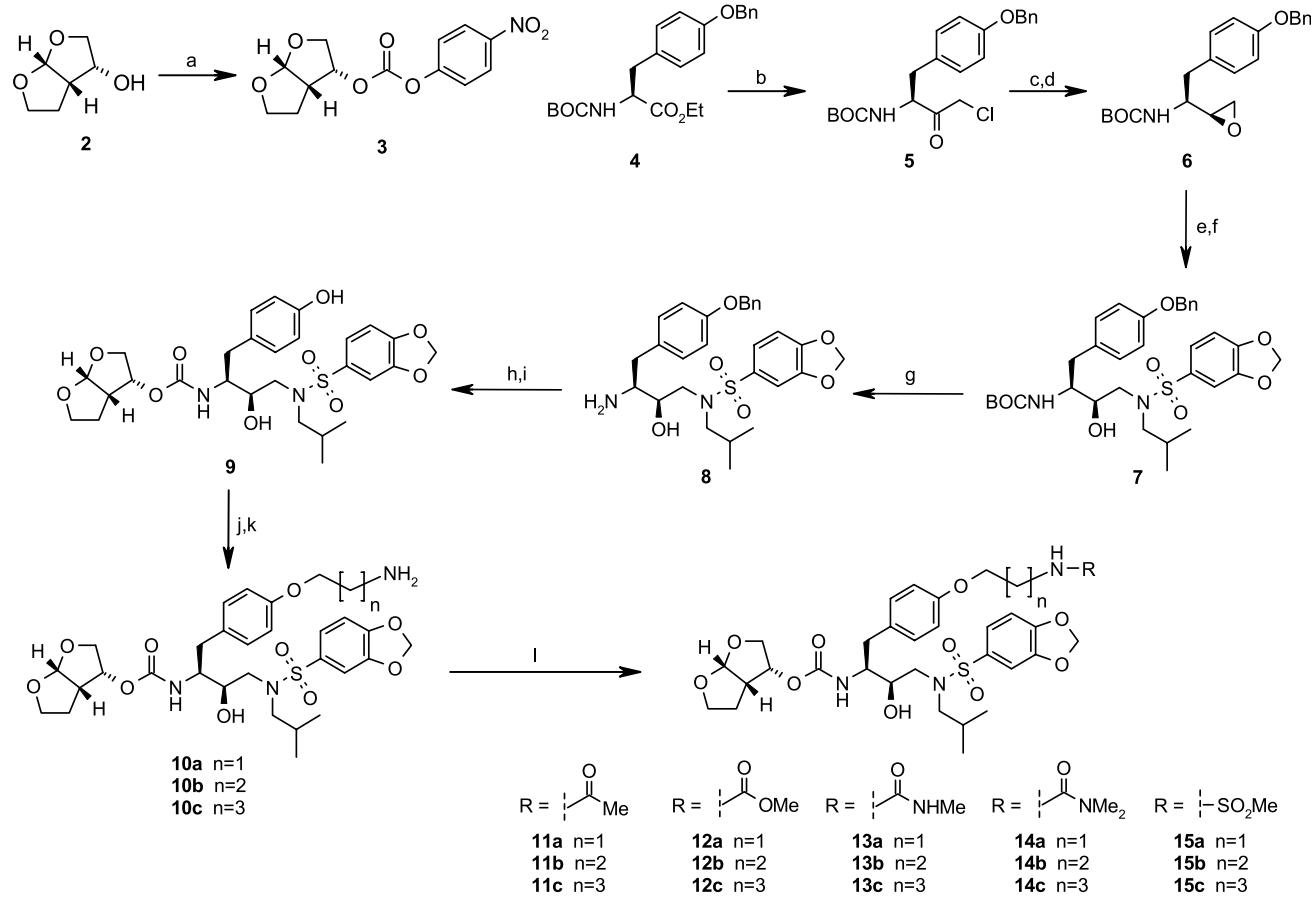


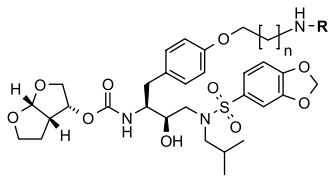
Figure 2.

To optimize any potential new P1 side chain–enzyme interactions, an efficient synthetic approach should allow for the straightforward variation of both tether chain length and the amino capping groups. The synthesis of compounds in the P1 chain-extended series is illustrated in Scheme 1. The route begins with tyrosine derivative **4**, which was converted to chloromethyl ketone **5** employing literature methods.[4] Diastereoselective sodium borohydride reduction of **5** afforded a chlorohydrin intermediate which was converted to epoxide **6** by treatment with ethanolic KOH. Ring opening of **6** with isobutylamine followed by reaction with 3,4-methylenedioxybenzenesulfonyl chloride[5] gave sulfonamide **7**. TFA-induced cleavage of the BOC group afforded primary amine **8**. The bicyclic bis-THF subunit was introduced at the P2 position by acylation with the PNP-carbonate derivative of optically pure alcohol **2**.[6]



Scheme 1. Reagents and conditions: (a) 4-Nitrophenylchloroformate, pyridine, CH_2Cl_2, 89%; (b) ICH_2Cl, LDA, THF, −78 °C, 70%; (c) $NaBH_4$, EtOH/THF; (d) KOH, EtOH, 83% from **5**; (e) isobutylamine, *i*-PrOH, reflux; (f) 3,4-methylenedioxybenzenesulfonyl chloride, DIEA, CH_2Cl_2, 86% from **6**; (g) TFA, CH_2Cl_2 then aqueous NaOH, 100%; (h) compound **3**, DIEA, MeCN, 96%; (i) H_2, 10% Pd(C), THF, 98%; (j) $HOCH_2(CH_2)_nNHBOC$, di-*t*-butylazodicarboxylate, Ph_3P, CH_2Cl_2, 70–95%; (k) TFA, CH_2Cl_2 then aqueous NaOH, 76–94%; (l) for **11a–c**: acetyl chloride, DIEA, CH_2Cl_2, 79–95%; for **12a–c**: methyl chloroformate, DIEA, CH_2Cl_2, 85–92%; for **13a–c**: methyl isocyanate, THF, 83–96%; for **14a–c**: dimethylcarbamyl chloride, DIEA, CH_2Cl_2, 85–98%; for **15a–c**: methanesulfonyl chloride, DIEA, CH_2Cl_2, 83–100%.

Table 1. Antiviral data for P1 chain-extended arylsulfonamides[a]



Entry	*n*	R	HXB2 IC_{50} (nM)	EP13 IC_{50} (nM)	D545701 IC_{50} (nM)
10a	1	H	180	150	130
10b	2	H	67	50	65
10c	3	H	15	14	28
11a	1		140	52	53
11b	2		57	18	20
11c	3		39	13	16
12a	1		3.0	2.9	7.1
12b	2		2.8	2.6	7.1
12c	3		2.6	2.6	6.6
13a	1		200	131	130
13b	2		119	56	43
13c	3		60	26	22
14a	1		37	9.1	11
14b	2		25	11	11
14c	3		65	22	32
15a	1		62	9.1	20
15b	2		41	20	21
15c	3		15	5.9	9.2
1	—	—	2.2	19	15
Atazanavir	—	—	25	93	70
Lopinavir	—	—	40	400	>1000
Indinavir	—	—	50	330	440
Amprenavir	—	—	130	440	>1000
Nelfinavir	—	—	320	450	>1000

[a] With the exception of compound **15a**, all IC_{50} values represent averages of at least three experimental results.

Catalytic hydrogenolysis of the *O*-benzyl group afforded phenol intermediate **9**. The P1 aminoalkyl linkers were installed by Mitsunobu reaction with BOC protected aminoalkanols followed by TFA deprotection of the BOC group. Finally, the amino capping groups were introduced via reactions of primary amine intermediates **10a–c** with various electrophiles.

Table 1 shows the in vitro antiviral data for 18 new compounds in the P1 chain-extended series. In addition, analogous data are shown for the earlier generation arylsulfonamide **1** and five currently marketed HIV protease inhibitors, also determined using our in-house antiviral assays. The antiviral IC_{50}s for wild-type HIV virus (HXB2)[7] and two multi-PI resistant viruses

(EP13 and D545701)[8] were determined in an MT4 cell line.[9]

The primary amine derivatives **10a–c** show a clear increase in wild-type and mutant antiviral activity upon extending the P1 chain from $n = 1$ to $n = 3$. This trend is consistent with a new side chain interaction which is strongest for $n = 3$. However, in the absence of enzyme inhibitory activities[10] or X-ray crystallographic data supporting a new interaction, the strength of this inference is limited. Clearly, factors beyond intrinsic enzyme activity, such as cell penetration and protein binding, influence the observed trends in cellular antiviral activities.

Acetyl derivatives **11a–c** are in general more potent than the analogous primary amines and they show a striking inverse resistance profile with approximately threefold increases in mutant activities versus wild-type. This inverse resistance behavior, often referred to as 'hypersusceptibility'[11], compares favorably to the threefold and >25-fold decrease in D545701 activities for atazanavir and lopinavir, respectively. Methyl carbamates **12a–c** comprise the most potent class of molecules in the series with single digit nanomolar IC_{50}s for all three virus strains tested. Interestingly, there appears to be no dependence of potency on the length of the P1 tether for the carbamate derivatives. The impressive potencies, along with the absence of a chain length SAR, suggest that their activities are probably more a function of physiochemical parameters than any specific P1 side chain binding interactions. Perhaps the methyl carbamate moiety imparts favorable polarity properties leading to an enhancement in cell penetration.

A comparison of monomethyl urea derivatives **13a–c** to dimethyl ureas **14a–c** is interesting. The monomethyl compounds show a consistent chain length SAR similar to that observed for primary amines **10a–c** and acetamides **11a–c**. In contrast, the dimethyl derivatives display a relatively flat chain length SAR and they are, as a class, more potent than the analogous monomethyl compounds. The methanesulfonyl derivatives **15a–c** show a chain length SAR against the wild-type virus that is consistent with compounds **10**, **11**, and **13**; however, the activity trends against the two mutant viruses are rather ill-defined.

Table 2 summarizes the in vivo pharmacokinetic analysis of methyl carbamate derivatives **12a** and **12b**. Both compounds possess negligible oral bioavailability in both rat and dog. However, when **12a** was co-administered with the potent cytochrome P450 inhibitor ritonavir, a dramatic increase from 0% to 33% oral bioavailability was achieved. Poor oral bioavailability is one of the general shortcomings of the HIV protease drug class. This can be effectively offset by 'boosting' systemic drug exposures via ritonavir co-administration and in fact this has become standard clinical practice (e.g., co-formulation of ritonavir with lopinavir in the commercial preparation of Kaletra).[12]

In summary, we have discovered a novel series of P1 chain-extended arylsulfonamide HIV-PIs that show very potent antiviral activity and in many cases inverse antiviral resistance profiles. As a class, the methyl carbamates **12a–c** are the most potent with mutant antiviral potencies of up to 35 times that of the currently marketed PI atazanavir. In addition, they show significant improvements in mutant antiviral activities versus our earlier generation arylsulfonamide series (i.e, compound **1**). Structural studies aimed toward elucidating any potential new enzyme–inhibitor interactions and the origin of the inverse resistance behavior are currently in progress and will be reported in due course.

Table 2. In vivo pharmacokinetic parameters for **12a** and **12b**

Entry	Species	Dose (mg/kg)	IV clearance (mL/h/kg)	$t_{1/2}$ (h)	% F
12b	Rat	1.0	43	0.2	~3
12b	Dog	0.5	30	1.1	~1
12a	Rat	1.0	55	1.6	~1
12a	Dog	0.5	68	nd	0
12a	Dog	0.5[a]	nd	0.6	33[b]

[a] Co-administered with 4 mg/kg ritonavir.
[b] Calculated by dividing the ritonavir-boosted oral AUC by the non-boosted IV AUC.

References and notes

1. (a) Palella, F. J.; Delaney, K. M.; Moorman, A. C.; Loveless, M. O.; Fuhrer, J.; Satten, G. A.; Aschman, D. J.; Holmberg, S. D. *N. Engl. J. Med.* **1998**, *338*, 853; (b) Hammer, S. M.; Squires, K. E.; Hughes, M. D.; Grimes, J. M.; Demeter, L. M.; Currier, J. S.; Eron, J. J.; Feinberg, J. E.; Balfour, H. H.; Deyton, L. R.; Chodakewitz, J. A.; Fischl, M. A.; Phair, J. P.; Pedneault, L.; Nguyen, B. Y.; Cook, J. C. *N. Engl. J. Med.* **1997**, *337*, 725.
2. (a) Boden, D.; Markowitz, M. *Antimicrob. Agents Chemother.* **1998**, *42*, 2775; (b) Tisdale, M.; Myers, R. E.; Maschera, B.; Parry, N. R.; Oliver, N. M.; Blair, E. D. *Antimicrob. Agents Chemother.* **1995**, *39*, 1704.
3. Miller, J. F.; Furfine, E. S.; Hanlon, M. H.; Hazen, R. J.; Ray, J. A.; Robinson, L.; Samano, V.; Spaltenstein, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 959.
4. Chen, P.; Cheng, P. T. W.; Spergel, S. H.; Zahler, R.; Wang, X.; Thottathil, J.; Barrish, J. C.; Polniaszek, R. P. *Tetrahedron Lett.* **1997**, *18*, 3175.
5. Tao, E.V.P.; Miller, W.D. U.S. Patent 5,387,681, 1995.
6. Ghosh, A. K.; Kincaid, J. F.; Walters, D. E.; Chen, Y.; Chaudhuri, N. C.; Thompson, W. J.; Culberson, C.; Fitzgerald, P. M. D.; Lee, H. Y.; McKee, S. P.; Munson, P. M.; Duong, T. T.; Darke, P. L.; Zugay, J. A.; Schleif, W. A.; Axel, M. G.; Lin, J.; Huff, J. R. *J. Med. Chem.* **1996**, *39*, 3278.
7. The wild-type virus HXB2 was derived from the pHXB2-D molecular clone For further details see: Fischer, A. G.; Collati, E.; Ratner, L.; Gallo, R. C.; Wong-Stall, F. *Nature (London)* **1985**, *316*, 262.
8. The EP13 and D545701 strains are engineered viruses containing mutations associated with clinically observed PI therapy failure in patients exposed to multiple protease inhibitors. They contain the following mutations relative to the consensus sequence of wild-type virus. EP13: protease—M46I, L63P, A71V, V82F/L, I84V; no reverse transcriptase mutations. D545701: protease—L10I, L19Q, K20R, E35D, M36I, S37N, M46I, I50V, I54V, I62V, L63P, A71V, V82A, L90M; reverse transcriptase—E28K,

K32E, V35I, T39S/T, E40D/V/Y/F, M41M/L, K43E, Y181Y/C.

9. Daluge, S. M.; Purifoy, D. J. M.; Savina, P. M.; St Clair, M. H.; Parry, N. R.; Dev, I. K.; Novak, P.; Ayers, K. M.; Reardon, J. E. *Antimicrob. Agents Chemother.* **1994**, *38*, 1590.
10. During the progression of our SAR program we reached a level of enzyme potency optimization at which the majority of compounds being synthesized had enzyme K_is below the lower limit (approximately 10 pM) measurable using standard steady-state kinetics techniques. As a result, enzyme inhibition data was no longer useful in driving our SAR program and we were forced to rely exclusively on cellular antiviral data.
11. (a) Leigh Brown, A. J.; Frost, S. D. W.; Good, B.; Daar, E. S.; Simon, V.; Markowitz, M.; Collier, A. C.; Connick, E.; Conway, B.; Margolick, J. B.; Routy, J. P.; Corbeil, J.; Hellmann, N. S.; Richman, D. D.; Little, S. J. *J. Virol.* **2004**, *78*, 2242; (b) Resch, W.; Ziermann, R.; Parkin, N.; Gamarnik, A.; Swanstrom, R. *J. Virol.* **2002**, *76*, 8659.
12. (a) Kumar, G. N.; Jayanti, V. K.; Johnson, M. K.; Uchic, J.; Thomas, S.; Lee, R. D.; Grabowski, B. A.; Sham, H. L.; Kempf, D. J.; Denissen, J. F.; Marsh, K. C.; Sun, E.; Roberts, S. A. *Pharm. Res.* **2004**, *21*, 1622; (b) Hurst, M.; Faulds, D. *Drugs* **2000**, *60*, 1371.