Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 4, pp. 445-450, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0960-894X/96 $15.00 + 0.00

S0960-894X(96)00035-2

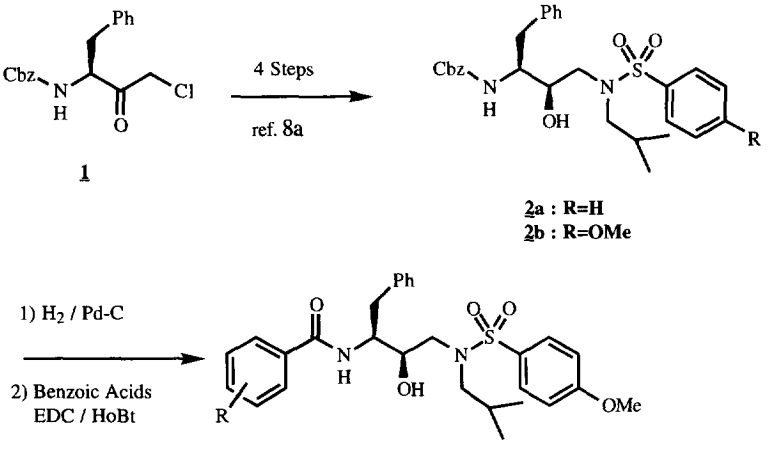
(Hydroxyethyl) Sulfonamide HIV-1 Protease Inhibitors : Identification Of The 2-Methylbenzoyl Moiety At P-2.

John N. Freskos,* Deborah E. Bertenshaw, Daniel P. Getman, Robert M. Heintz, Brent V. Mischke, Lisa W. Blystone, Martin L. Bryant, Christine Funckes-Shippy, Kathyrn A. Houseman, Nandini N. Kishore, Geralyn P. Kocan, and Pramod P. Mehta

Departments of Medicinal Chemistry and Infectious Diseases, Searle Discovery Research, 700 Chesterfield Parkway, St. Louis, Missouri 63198 and Searle Discovery Research, 4901 Searle Parkway, Skokie, Illinois 60077.

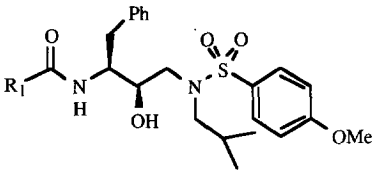
Abstract : We have discovered a potent low molecular weight series of HIV-1 Protease inhibitors incorporating the (R)-(hydroxyethyl) sulfonamide isostere.

The human immunodeficiency virus type-1 (HIV-1), the causative agent of AIDS, encodes for a unique aspartyl protease, which has been shown to play a critical role in the life cycle of the virus.[1] Recently, clinical trials with two different inhibitors of this protease have demonstrated a dramatic, albeit temporary, reduction in viral load and an increase in CD4 cell counts in AIDS patients.[2,3] Numerous examples of potent inhibitors of this protease have been reported and reviewed.[4-6] Previously the development of potent inhibitors of this enzyme incorporating the (R)-(hydroxyethyl)urea isostere,[7] and more recently, the (R)-(hydroxyethyl)sulfonamide isostere[8] were reported. Due to the high potency of the sulfonamide isostere, we have been able to develop inhibitors containing relatively simple P-2 groups. Our recent disclosure of the (R)-(hydroxyethyl)sulfonamide isostere identified **2a** as a potent inhibitor lead.[8a] A systematic SAR study demonstrated a clear preference for the R-stereochemistry at the key hydroxyl and identified the isobutyl group as optimum for potency. Addition of a p-methoxy group, **2b**, led to a furthur increase in potency. With the right side of these inhibitors optimized we then sought replacements for the Cbz group of **2b**. Described herein is a series of simple benzoyl containing compounds that maintain high enzyme potency and, in certain cases, exhibt potent antiviral activity coupled with good oral bioavailability.



Scheme 1.

The chemistry used to prepare these inhibitors is outlined in Scheme 1. The Cbz protected (hydroxyethyl) sulfonamide **2b** was prepared in 4 steps from the commercially availabile Cbz-L-phenylalanine chloromethyl ketone as previously described.[8a] Hydrogenation of the Cbz protecting group afforded the free amine which was coupled with either a benzoic acid using standard (EDC / HOBt) conditions or a benzoyl chloride in the presence of triethylamine. All of the starting acids or acid chlorides were purchased from commercial vendors. The IC_{50} values for inhibition of recombinant HIV-1 protease were determined using the spectrofluorometric assay developed by Toth and Marshall and are shown in Table 1.[9] The replacement of the Cbz group of **2b** by an unsubstituted benzoyl group led to a decrease in potency. The addition of an ortho methyl group (entry 4) led to a 3-fold increase in potency, whereas the meta (entry 5) and para (entry 6) substituted isomers were similar to phenyl. We hypothesized that the introduction of the ortho methyl group oriented the aryl ring perpendicular to the amide carbonyl group and placed the methyl substituent in the lipophilic pocket of the S-2 subsite. Increasing the size of the ortho group (entries 7-9) resulted in decreased potency, whereas an ortho-chloro (entry 10) showed comparable activity to **4**. As expected, introduction of a polar residue (entries 11 and 12) resulted in dramatically decreased potency. Although **2b** and **4** were potent inhibitors of HIV-1 protease, neither exhibited good antiviral activity in a cell culture assay. We attributed this poor correlation between their $IC_{50's}$ and $EC_{50's}$ to their high lipophilicity and sought to moderate this property by the addition of polar substituents on to the aromatic ring.



Compd. #	R_1	IC_{50}(nM)
2a	$PhCH_2O$	6
2b	$PhCH_2O$	3
3	Ph	14
4	o-$(CH_3)C_6H_5$	5
5	m-$(CH_3)C_6H_5$	27
6	p-$(CH_3)C_6H_5$	15
7	o-$(CH_2CH_3)C_6H_5$	10
8	o-$(CH(CH_3)_2)C_6H_5$	49
9	o-$(CF_3)C_6H_5$	37
10	o-$(Cl)C_6H_5$	6
11	o-$(OCH_3)C_6H_5$	190
12	o-$(OH)C_6H_5$	900

Table 1.

The benzoic acid analogs chosen to test this hypothesis are shown in Table 2.[10] The acids **A** and **C** were purchased from Aldrich. The 4-amino-2-methylbenzoic acid, **B**, was prepared from 2-iodo-5-nitrotolune via carbonylation followed by reduction of the nitro group.[11] The acids **D** and **F** were initially prepared from the corresponding amines by diazotization and decomposition of the diazo intermediates in hot water.[12,13] The 4-hydroxy-2-methylbenzoic acid, **E**, was prepared via a Diels Alder reaction between 2-trimethylsilyloxy-1,3-cyclohexadiene and methyl propiolate followed by hydrolysis.[14] Standard (EDC / HOBt) coupling of these acids yielded the desired inhibitors. The IC_{50} determinations of the 2-methyl-5-substituted isomers showed a slight potency decrease relative to the other isomers. These compounds were screened for antiviral activity against the HIV_{IIIB} strain of HIV-1 in a CEM cell culture assay and the results are shown in Table 3.[15]

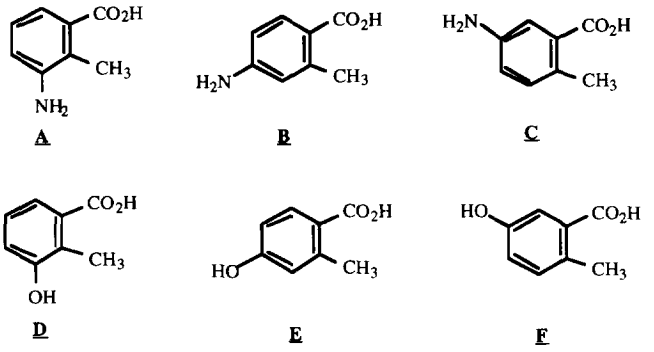
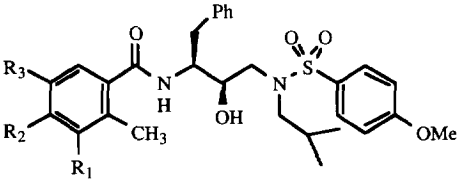


Table 2.

In the hydroxyl series, the 3- and 4-substituted hydroxy compounds (entries 13-14) showed no decrease in enzyme inhibition relative to **4** whereas the 5 substituted isomer (entry 15) showed a slight decrease in potency. A similar trend was observed in the amino series (entries 16-18). When evaluated in cell culture against HIV-1 the antiviral activity was significantly improved and a much better correlation between IC_{50} and EC_{50} values were observed. The most potent compound identified was **13** which exhibited an EC_{95} of 32 ng/mL (59 nM) against HIV-1.

The oral bioavailablity of these inhibitors was ascertained using a pharmacokinetic screen in rats.[16] Best results were seen with the 3-substituted-2-methyl isomers. The 3-hydroxy-2-methylbenzoyl sulfonamide, **13**, exhibited a $T_{1/2}$ of ~1 hour, C_{max} of 270 ng/mL and 12 % oral bioavailability. The 3-amino-2-methylbenzoyl sulfonamide, **16**, exhibited a $T_{1/2}$ of ~1 hour, C_{max} of 1800 ng/mL and 54 % oral bioavailability.

In summary, we have discovered a promising series of low molecular weight HIV-1 protease inhibitors that exhibit potent antiviral activity and substantial oral bioavailability.



Compd #	R_1	R_2	R_3	IC_{50}(nM)	EC_{50}(nM)	TD_{50}(nM)
4	H	H	H	5	200	> 10,000
13	OH	H	H	3	7	> 10,000
14	H	OH	H	5	9	> 10,000
15	H	H	OH	40	nd	
16	NH_2	H	H	6	18	> 10,000
17	H	NH_2	H	9	18	> 10,000
18	H	H	NH_2	10	82	> 10,000

Table 3.

Acknowledgements: The authors wish to thank Mr. Jim Allman for assistance in carrying out the reaction described in reference 12. Acquisition of mass spectra by the Monsanto Physical Science Center is greatly appreciated

References :

1. Kohl, N. E.; Emini, E. A.; Schlief, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. *Proc. Nat'l. Acad. Sci.* **1988**, *85*, 4686.
2. Wei, X.; Ghosh, S. K.; Taylor, M. E.; Johnson, V. A.; Emini, E. A.; Deutsch, P.; Lifson, J. D.; Bonhoeffer, S.; Nowak, M. A.; Hahn, B. H.; Saag, M. S.; Shaw, G. M. *Nature* **1995**, *373*, 117.
3. Ho, D. D.; Neumann, A. U.; Perelson, A. S.; Chen, W.; Leonard, J. M.; Markowitz, M. *Nature* **1995**, *373*, 123.
4. Darke, P. L.; Huff, J. R. *Advances in Pharmacology*; August, J. T.; Anders, M. W.; Murad, F., Eds.; Academic: San Diego, 1994; Vol. 25, pp 399-454.
5. Thaisrivongs, S.; *Annual Reports in Medicinal Chemistry;* Bristol, J. A., Ed.; Academic: New York, 1994; Vol. 29, pp 133-144.
6. Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305.
7. Getman, D. P.; DeCrescenzo, G. A.; Heintz, R. M.; Reed, K. L.; Talley, J. J.; Bryant, M. L.; Clare, M.; Houseman, K. A.; Marr, J. J.; Mueller, R. A.; Vazquez, M. L.; Shieh, H. S.; Stallings, W. C.; Stegman, R. A. *J. Med. Chem.* **1993**, 36, 288.
8. (a) Vazquez, M. L.; Bryant, M. L.; Clare, M.; DeCrescenzo, G. A.; Doherty, E. L.; Freskos, J. N.; Getman, D. P.; Houseman, K. A.; Julien, J. A.; Kocan, G. P.; Mueller, R. A.; Shieh, H. S.; Stallings, W. C.; Stegman, R. A.; Talley, J. J. *J.Med.Chem.* **1995**, *38*, 581. (b) Vazquez, M. L.; Mueller, R. A.; Talley, J. J.; Getman, D. P.; DeCrescenzo, G. A.; Freskos, J. N. **WO 94/04492** March 3, 1994. (c) Tung, R. D.; Murcko, M.A.; Bhisetti, G. R. **WO 94/05639** March 17, 1994. (d) Kim, E. E.; Baker, C. T.; Dwyer, M.D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navio, M. A. *J. Amer. Chem. Soc.*

1995, *117*, 1181.

9. Toth, M. V.; Marshall, G. R. *Int. J. Pept. Protein Res.* **1990**, *36*, 544.
10. Simultaneously with our efforts, the Agouron/Lilly groups were investigating 2-methyl benzoyl groups in the P-2 position of their (hydroxyethyl)amine series of inhibitors and had identified the 2-methyl-3-hydroxybenzoyl group in the clinical candidate AG-1343. For leading references to their work see : Kalish, V. J.; Tatlock, J. H.; Davies, J. F., Kaldor, S.W.; Dressman, B.A., Reich, S.; Pino, M.; Nyugen, D.; Appelt, K.; Musick, L.; Wu, B.; *Bioorg. Med. Chem. Lett.* **1995**, *5*, 727.
11. Burmagin, N. A.; Nitkin, K. V.; Beletskaya, I. P. *J. Organomettalic Chem.* **1988**, 563.
12. We also investigated the preparation of 2-methyl-3-hydroxybenzoic acid from 7-amino-1,5-naphthlene-disulfonic acid disodium salt in 50 % NaOH at 275^{0}C. Interestingly, using the reaction conditions of ref. 13a (initial pressure of 600 psi N_2) we found that after 1 hour at 275 ^{0}C that the pressure leveled off at 1400 psi and the reaction was done. Isolated yields were ~70% as in ref. 13a.
13. (a) Dean, R. E.; Midgley, A.; White, E. N.; McNeil, D. *J. Chem Soc.* **1961**, *116*, 2773. (b) Fieser, L. F.; Lothrop, W. C.*J. Amer. Chem. Soc.* **1936**, *58*, 749.
14. (a) Harland, P. A.; Hodge, P. *Synthesis* **1982**, 223. (b) Freskos, J. N.; Morrow, G. .W.; Swenton, J. S. *J. Org. Chem.* **1985**, *50*, 805.
15. Drug canidates were evaluated against the HIV_{IIIB} strain of HIV-1 in CEM cells at a multiplicity of infection of 0.1 for there ability to prevent HIV induced cell death. Compounds were evaluated in triplicate at varying doses and compared to (1) untreated, uninfected cell control samples, (2) drug treated uninfected cell toxicity controls, (3) untreated infected virus control samples. Drug was added on days 0, 2, and 5, the assay terminated on day 7, and cell death assesed using 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT). This assay detects drug induced supression of viral CPE, as well as drug cytotoxicity, by measuring the generation of MTT-formazan by surviving cells. AZT and DDI were included as positve controls, and typically provided EC_{50} values of 1- 5 ng/mL and 250-1.0000 ng/mL, respectively.
16. The oral bioavailabity of these inhibitors was ascertained using a pharmacokinetic screen in rats (4 rats/group). The inhibitors were dosed IV (5mpk) in a buffered Tween solution or orally (20mpk) as a carbomethoxy cellouse suspenstion. Blood was drawn at 5, 30 minutes, 1, 2, 4, and 6 hour intervals in heparinized tubes. Plasma samples from 4 Rats were pooled at each time point, proteins were precipatated with acetonitrile, and compound levels were determined by the previously described enzyme assay. The ratio of area under the curve (AUC) of the plasma concentration curve for oral dosing relative to IV dosing (corrected for dose) gave us the oral bioavailability.[17]
17. Ludy J. R.; BaMaung N.; Soderquist J.; Fung A. K. L.; Stein H.; Klienert H. D.; Marcotte P. A.; Egan D. A.; Bopp B.; Merits I.; Bolis G.; Greer J.; Perun T. P.; Plattner J. J. *J. Med. Chem.* **1988**, *31*, 2264.

(*Received in USA* 12 *December* 1995; *accepted* 17 *January* 1996)