Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 5, pp. 653-656, 1994

0960-894X/94 $6.00+0.00

0960-894X(94)E0024-9

SYNTHESIS OF C_2-SYMMETRIC INHIBITORS OF THE HIV-1 PROTEASE, WITH N,N'-SUBSTITUTED ETHYLENEDIAMIDE AND ETHYLENEDIAMINE LINKERS.

Jean-Paul Mazaleyrat*, Isabelle Rage, Jaroslav Šavrda, Malek Mouna and Michel Wakselman

CNRS-CERCOA, 2, rue Henri Dunant, F-94320 Thiais, France

Raymond Boulay and Yves Lelièvre

Rhône-Poulenc Rorer, 13, quai Jules Guesde, F-94403 Vitry-sur-Seine, France

Abstract: Coupling of Z-(L)-phenylalanine with either ethylenediamine or N,N'-dimethylethylenediamine, followed by N-deprotection and either direct coupling with Z-(L)-valine or amide reduction prior to coupling, gave C_2-symmetric compounds [Z-Val-Phe-N(R)CH_2-]$_2$ (R= H, CH_3) and [Z-Val-Phe(ΨCH_2-N)-N(R)CH_2-]$_2$ (R= H, CH_3, CH_2COOH, CH_2CH_2OH) which were moderately active inhibitors of the HIV-1 protease.

Inhibition of the HIV protease has emerged as one of the most interesting therapeutic targets for the treatment of AIDS. The key function of this protease of the aspartic class, post-translational processing of the Pr_{55}^{gag} and $Pr_{160}^{gag\text{-}pol}$ HIV polyprotein products, is essential for the viral life cycle: site-directed mutagenesis of either the HIV protease active site or the *gag* and *pol* cleavage sites, produces viral particles which are morphologically immature and non infectious. Starting from the Phe-Pro cleavage site frequently observed, a number of substrate-based inhibitors have been reported, in which the scissile bond has been replaced with non cleavable isosteres such as reduced amide, hydroxyethylene, hydroxyethylamine, acting as transition state analogues with tight binding to the Asp_{25}-$Asp_{25'}$ residues of the catalytic site.[1]

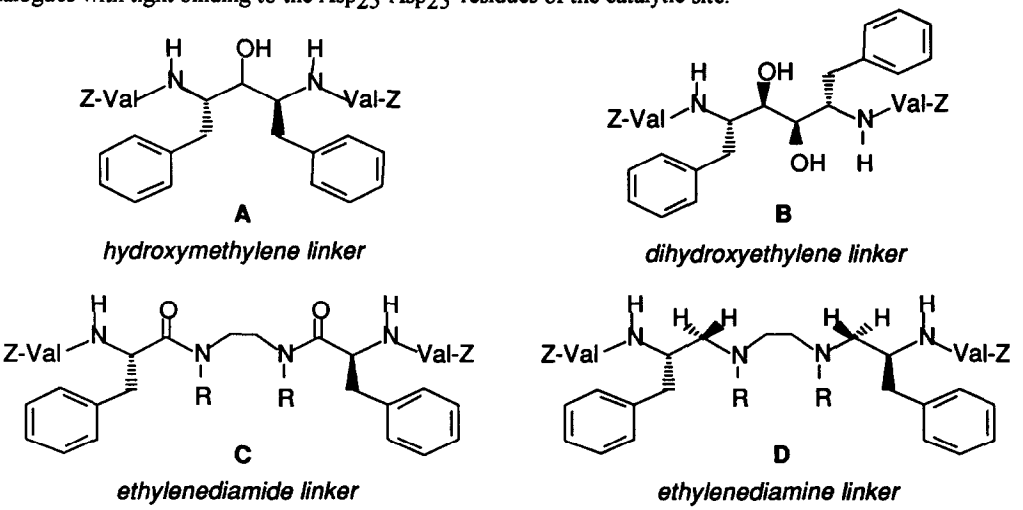

Figure 1

C_2-symmetric inhibitors of the HIV protease constitute a particularly interesting class of compounds, by exploiting a unique structural aspect of this enzyme, which in its active form is a C_2-symmetric homodimer with the symmetry axis bissecting the substrate binding groove.[1] Compounds of type **A** and **B** (Fig. 1), containing a hydroxymethylene and a dihydroxyethylene unit, respectively, as central linker of two symmetrical moietys that

incorporate binding elements of substrate, have been proven to be potent inhibitors of the HIV protease.[2,3] From those lead compounds, several others C_2-symmetric or pseudosymmetric inhibitors have been investigated.[3,4]

In the present paper, we report the synthesis of a series of parent compounds of type **C** and **D**, incorporating either 1,2-ethylenediamide or 1,2-ethylenediamine linkers, and their evaluation as inhibitors of the HIV protease. Such molecules present some interesting features: *(i)* possibility of interaction of the two NH groups of the linker with the carboxylic functions of the Asp_{25}-$Asp_{25'}$ residues of the catalytic site *(ii)* possibility of substitution of the hydrogens of the linker NH groups by R substituents for potential improvement of both binding and selectivity (resistance to cleavage by others proteases) *(iii)* possibility of size modulation of the ethylene bridge *(iv)* low peptidic character in the case of compounds of type **D** *(v)* straightforward synthesis. Derivatives of type **C**, such as $(Tyr\text{-}D\text{-}Phe\text{-}NHCH_2\text{-})_2$, had been previously investigated only as bivalent opioid peptide analogs,[5] until the recent report by Holmes et *al* [6] of a series of penicillin-derived molecules incorporating a central ethylenediamide linker, as novel potent inhibitors of the HIV protease. To our knowledge, no assays of C_2-symmetric compounds of type **D** as HIV protease inhibitors have been reported.

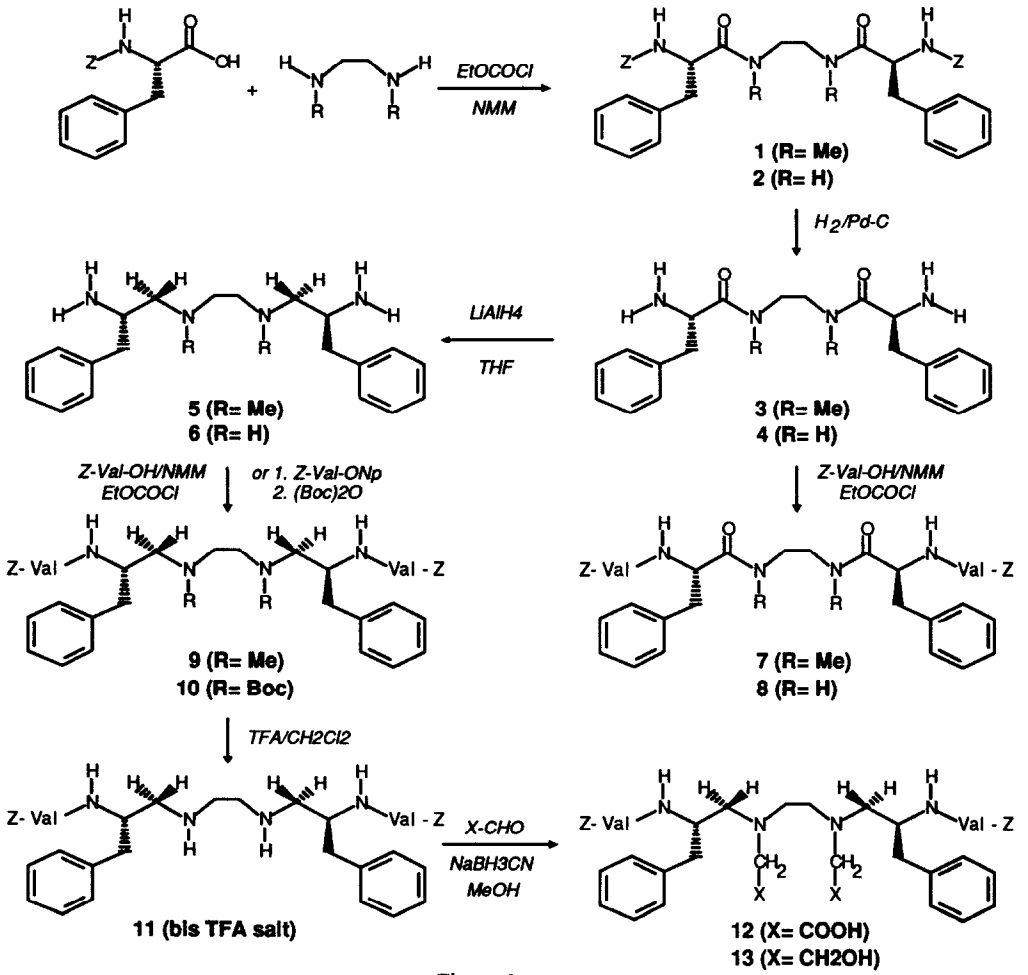

Figure 2

The derivatives **1-13** of both **C** and **D** types were obtained by using a single synthetic pathway (Fig. 2). Coupling of Z-(L)-phenylalanine (2 equiv. mol/mol) with either N,N'-dimethyl-1,2-ethylenediamine or 1,2-ethylenediamine by the mixed anhydride method, gave **1** [7] (72 %) and **2** [7] (91 % crude), respectively. N-deprotection of **1** and **2** by hydrogenolysis over Pd-C (Parr apparatus) in MeOH/H_2O 4:1 for **1** and in hot DMF for **2** (highly insoluble in water and most organic solvents), gave **3** [7] (78 %) and **4** [7] (99 % crude), respectively. Coupling of **3** and **4** with Z-(L)-valine by the mixed anhydride method gave **7** [7] (90 %) and **8** [7] (72 %), respectively. Alternatively, reduction of the amide functions of **3** and **4** was accomplished by using a large excess of lithium aluminum hydride in refluxing THF for 48 h, which led to the tetramines **5** [7] (85 % crude) and **6** [7] (77 % crude), respectively. A single diastereomer was obtained (by ^1H and ^{13}C NMR), showing the absence of epimerization at the Cα carbons of the two phenylalanine residues. Coupling of **5** with Z-(L)-valine by the mixed anhydride method gave **9** [7] (90 %), while coupling of **6** with Z-(L)-valine *p*-nitrophenyl ester (2 equiv. mol/mol) in dichloromethane at room temperature, allowed the selective acylation of the terminal primary amino groups.[8] In the latter case, the crude CH_2Cl_2 solution obtained after completion of the coupling reaction was treated with an excess of Boc-anhydride (3 equiv. mol/mol) at room temperature for 24 h, to give the fully protected derivative **10** [7] (51 %) which could be purified by chromatography.[9] It is noteworthy that the orthogonal Z/Boc N-protection of the compound **10** allows substitutions or chain elongations on both central and terminal nitrogens. Selective deprotection of the Boc protecting groups of the central nitrogens in TFA/CH_2Cl_2 1:1 at 0 °C (1 h) to room temperature (1 h) gave **11** [7] (76 %), obtained as a (bis)-trifluoroacetate. Reductive alkylation of **11** with either glyoxylic acid or hydroxyacetaldehyde and cyanoborohydride in methanol at room temperature gave **12** [7] (78 %) and **13** [7] (97 %), respectively.

The derivatives **7,8** (type **C**) and **9-13** (type **D**) were assayed at 10^{-5} M as inhibitors of the HIV-1 protease, against three different substrates: TLNFPIS-AMC (Test I),[11] SQN-(*p*NO_2)F-PIV (Test II) and HKARVL-(*p*NO_2)F-EAnLS_{NH2} (Test III).[12] The compounds **7** and **8** gave 16% and 25% inhibition in test III, respectively, but were inactive in tests I and II. Similarly, the derivatives **9**, **10** and **11** were moderately active in tests I and II (3-43% inhibition), but inactive in test III. Only the compounds **12** and **13** responded in all tests I, II and III, giving 36% (I), 53% (II), 33% (III) and 8% (I), 30% (II), 9% (III) inhibition, respectively. As far as comparison between **7-13** is allowed at such relatively weak activities, these results suggest that ethylenediamine linker of type -$CH_2N(R)CH_2CH_2N(R)CH_2$- with R= CH_2COOH or CH_2CH_2OH could be considered as well as the known ethylenediamide linker -$CONHCH_2CH_2NHCO$- [6] as central binding element in these series of inhibitors. However, the present molecules inhibit the HIV protease at concentrations 10^4 - 10^5 times higher than **A** (IC_{50}= 3 nM),[3a] **B** (IC_{50}= <1 nM) [3a] and the penicillin-derived dimers of type **C** (IC_{50}= 0.9-4.8 nM),[6a] which reflects a very substantial difference in binding. From cristallographic results, the occurence of a tight binding interaction between the central OH group(s) of **A** and **B** and the carboxylic functions of the catalytic Asp_{25}-$_{25'}$ residues has been demonstrated.[2a,3c] On the other hand, the ethylenediamide linker in C_2- symmetric penicillin-derived dimers of type **C** has been shown to have poor contacts at the catalytic aspartates, the high efficiency in binding rather being due to the excellent occupancy at the S_1/S'_1 and S_2/S'_2 subsites.[6c] Thus, the low efficiency in binding of compound **8**, structurally related to those last compounds, has to be due to poorer S_1/S'_1 and S_2/S'_2 contacts. By analogy, this is probably also true for all the derivatives **7-13**.

Acknowledgement. We thank A.N.R.S. for financial support of this work.

References and Notes

1. For recent reviews, see (a) Tomasselli, A. G.; Howe, W. J.; Sawyer, T.K.; Wlodawer, A.; Heinrikson, R. L., *Chem. Today*, **1991**, *9*, 6-27. (b) Meek, T. D., *J. Enzyme Inhibition*, **1992**, *6*, 65-98.
2. (a) Erickson, J.; Neidhart, D. J.; Van Drie, J.; Kempf, D. J.; Chun Wang, X.; Norbeck, D. W.; Plattner, J. J.; Rittenhouse, J. W.; Turon, M.; Wideburg, M.; Kohlbrenner, W. E.; Simmer, R.; Helfrich, R.; Paul, D A.; Knigge, R., *Science*, **1990**, *249*, 527-533. (b) Kempf, D. J.; Norbeck, D. W.; Codacovi, L. M.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Paul, D. A.; Knigge, M. F.; Vasavanonda, S.; Craig-Kennard, A.; Saldivar, A.; Rosenbrook, W.; Clement, J. J ; Plattner, J. J.; Erickson, J., *J. Med. Chem.*, **1990**, *33*, 2687-2689.
3. For the most recent papers, see (a) Kempf, D. J.; Codacovi, L. M.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Saldivar, A.; Vasavanonda, S.; Marsh, K. C.; Bryant, P.; Sham, H. L.; Green, B. E.; Betebenner, D. A.; Erickson, J.; Norbeck, D. W., *J. Med. Chem.*, **1993**, *36*, 320-330. (b) Kort, J. J.; Bilello, J. A.; Bauer, G.; Drusano, G. L., *Antimicrob. Agents Chemother.*, **1993**, *37*, 115-119. (c) Dreyer, G. B.; Boehm, J. C.; Chenera, B.; DesJarlais, R. L.; Hassel, A. M.; Meek, T. D.; Tomaszek, T. A.; Lewis, M., *Biochemistry*, **1993**, *32*, 937-947.
4. See for example (a) Bone, R.; Vacca, J. P.; Anderson, P. S.; Holloway, M. K., *J. Am. Chem. Soc.*, **1991**, *113*, 9382-9384. (b) Jadhav, P. K.; Woerner, F. J., *BioMed. Chem. Lett.*, **1992**, *2*, 353-356. (c) Yu, K. L.; Harte, W. E.; Spinazze, P.; Martin, J. C., Mansuri, M. M., *BioMed. Chem. Lett.*, **1993**, *3*, 535-538. (d) Abdel-Meguid, S. S.; Zhao, B.; Murthy, K. H. M.; Winborne, E.; Choi, J. K.; DesJarlais, R. L.; Minnich, M. D.; Culp, J. S.; Debouck, C.; Tomaszek Jr., T. A.; Meek, T. D.; Dreyer, G. B., *Biochemistry*, **1993**, *32*, 7972-7980. (e) Babine, R. E.; Zhang, N.; Jurgens, A. R.; Schow, S. R.; Desai, P. R.; James, J. C.; Semmelhack, M. F., *BioMed. Chem. Lett.*, **1992**, *2*, 541-546. (f) Babine, R. E.; Zhang, N.; Schow, S. R.; Jirousek, M. R.; Johnson, B. D.; Kerwar, S. S.; Desai, P. R.; Byrn, R. A.; Hastings, R. C.; Wick, M. M., *BioMed. Chem. Lett.*, **1993**, *3*, 1589-1594.
5. Lipkowski, A. W.; Konecka, A. M.; Sroczynska, I.; Przewlocki, R.; Stala, L.; Tam, S. W., *Life Sci.*, **1987**, *40*, 1457-1460.
6. (a) Holmes, D. S.; Clemens, I. R.; Cobley, K. N.; Humber, D. C.; Kitchin, J.; Orr, D. C.; Patel, B.; Paternoster, I. L.; Storer, R., *BioMed. Chem. Lett.*, **1993**, *3*, 503-508. (b) Humber, D. C.; Bamford, M. J.; Bethell, R. C.; Cammack, N.; Cobley, K.; Evans, D. N.; Gray, N. M.; Hann, M. M.; Orr, D. C.; Saunders, J.; Shenoy, B. E. V.; Storer, R.; Weingarten, G. G.; Wyatt, P. G., *J. Med. Chem.*, **1993**, *36*, 3120-3128. (c) Wonacott, A.; Cooke, R.; Hayes, F. R.; Hann, M. M.; Jhoti, H.; McMeekin, P.; Mistry, A.; Murray-Rust, P.; Singh, O. M. P.; Weir, M. P., *J. Med. Chem.*, **1993**, *36*, 3113-3119.
7. All compounds gave satisfactory analytical data (^{1}H NMR at 300 MHz, C,H,N analysis and/or FAB spectroscopy). The complete details of synthesis will be published elsewhere in a full account of this study. Unless noted, the given yields correspond to isolated pure compounds.
8. Byk, G.; Gilon, C., *J. Org. Chem.*, **1992**, *57*, 5687-5692.
9 Attempts to purifye the free diamine by t.l.c. on silicagel were unsuccessful, probably because of the occurence of silica catalyzed intramolecular transacylations.[10] Chromatographic separation repeatedly gave samples presenting again several spots. In a control experiment, analytically pure bis-trifluoroacetate **11** (one spot on t.l.c. and one set of signals in the ^{1}H NMR spectrum -DMSO d_6-) was shaken in 0.2 N aq. NaOH. The extracted (EtOAc) corresponding free diamine was clearly homogeneous by ^{1}H NMR (only one set of signals in DMSO-d_6) but presented two main close spots on t.l.c. (SiO_2; CH_2Cl_2/MeOH 9:1). The crude diamine was dissolved in TFA/CH_2Cl_2 1:1 to be converted back to the bis-trifluoroacetate **11**. After evaporation of the solvents, the obtained crude sample of **11** was strictly identical to the starting one by t.l.c. and ^{1}H NMR. This shows that N to N acyl shift, which has been shown to be general acid catalyzed in the case of glutamine,[10] could occur on silicagel for the free diamine, but is not presently observed in either acidic or basic solution.
10 Martin, B.; Parcell, A.; Hedrick, R. I., *J. Am. Chem. Soc.*, **1964**, *86*, 2406-2413.
11 Hirel, P-H.; Parker, F.; Boiziau, J.; Jung, G.; Outerovitch, D.; Dugue, A.; Peltiers, C.; Giulliacci, C.; Boulay, R.; Lelievre, Y.; Cambou, B.; Mayaux, J. F ; Cartwright, T., *Antiviral Chem. Chemother.*, **1990**, *1*, 9-15.
12 Pennington, M. W.; Festin, S. M.; Macchecchini, M. L.; Dick, F.; Scarborough, P. E., *Peptides 1990*, E. Giralt and D. Andreu (Eds), ESCOM Science Publishers B. V., 1991, pp. 787-789.

(*Received in Belgium* 24 *September* 1993; *accepted* 1 *December* 1993)