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ANTI-HIV ACTIVITY OF *N*-(2,3-DIHALOGENOPROPYL)- AND *N*-ALLYL-GLYCINE CONTAINING PENTAPEPTIDES.

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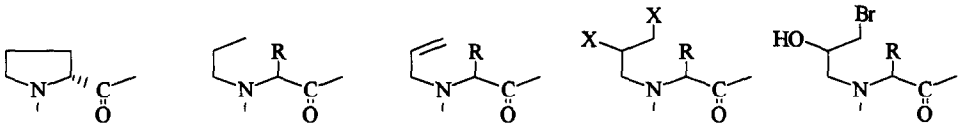
Abstract: A series of peptides containing *N*-(2,3-dihalopropyl)-glycine or alanine residues has been prepared as potential suicide substrates of the HIV *pol*-protease or as enzyme-activated prodrugs. Halogenation of unsaturated *N*-allyl peptide precursors in dichloromethane occurs with participation of a neighboring amide group and leads to halohydrins instead of the expected dihalides. Use of X_2/ LiX/ HOAc conditions gives the desired dihalogenated derivatives. These functionalized substrate analogs are not inhibitors of the enzyme. However, Boc-Ala-Phe-*N*-(2,3-dihalogenopropyl)-Gly-Ile-Val-OMe (halogen= Br and Cl) inhibit the cytopathic effect induced by HIV-1 in CEM cell cultures and the reverse transcriptase activity in cell culture supernatants. The corresponding unsaturated *N*-allyl precursor also displays an antiviral effect.

Human Immunodeficiency Virus produces a small homodimeric aspartyl proteinase (HIV *pol*-protease) which cleaves the polyprotein precursors encoding the structural proteins and enzymes of the virus. This proteolytic activity is absolutely required for the production of mature infectious virions and is therefore a target for synthetic inhibitors, in an approach to the therapy of AIDS [1,2]. An interesting feature of the HIV-1 proteinase is its rare ability to cleave substrates having a phenylalanine or a tyrosine at P_1 and a proline residue at P'_1 position (Schechter and Berger notation [3]). Indeed, few proteinases can efficiently cleave a peptide bond before an iminoacid [4]. Previously, we synthesized substrate analogs of the Ser-Gln-Asn-Tyr-Pro-Ile-Val sequence which spans the p17-p24 cleavage site of the Pr_{55}^{gag} polyprotein. These peptides contained a dehydropipecolic or an aminobenzoic acid derivative as substitutes of the prolyl P'_1 residue [5,6,7].



L-Pro **1** **2** **3**: X= Br **4**

a: R= H ; b: R= Me **3'**: X= Cl

Figure 1: structural analogy between L-Pro, *N*-propyl glycine **1a** (R= H) or alanine **1b** (R= CH_3), *N*-propenyl-Gly or Ala **2**, 2,3-dihalogenopropyl-Gly or Ala **3** and 3-bromo-2-hydroxypropyl Gly or Ala residues **4**.

We have now investigated *N*-substituted glycine or alanine derivatives as open mimics of proline (Fig 1). Such P'_1 analogs, when combined to appropriate $P_n..P_1$ and $P'_2..P'_n$ sequences, could lead to substrates or competitive inhibitors of the HIV protease. Moreover, the derivatives of **3** and **3'** contain a latent aziridinium

cation liable to be unmasked by the specific action of the HIV protease (Fig 2). They could behave as irreversible inhibitors of this enzyme by a suicide type mechanism (route *3*) or as enzyme activated inhibitors of another essential biomolecule of the virion by a pro-drug mechanism (route *4*) [8]. An analogous strategy has previously been used by R. A. Firestone and coll. for the transport and activation of Z-Gly-Phe nitrogen mustard conjugates into pinocytic cells [9].

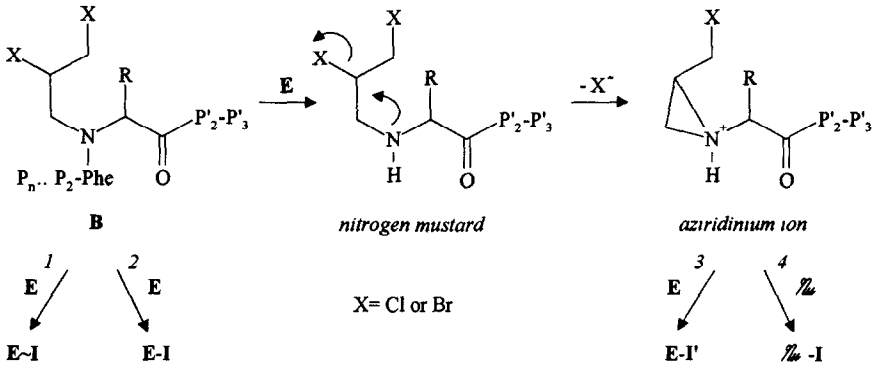


Figure 2: postulated interactions of compounds **B** with the *pol*-protease **E**. *1:* competitive inhibition, *2:* affinity labeling, *3:* suicide inhibition, *4:* prodrug (= essential biomolecule of the virion, different from **E**).

Synthesis

N-Boc-*N*-allyl-glycine and alanine synthons were obtained by selective N-alkylation of the corresponding *N*-Boc amino acid [10, 11]. Step-by-step elongation of the peptide chain using either TFA / CH_2Cl_2 or HCl / AcOEt for deprotection of the Boc group of the amino component and either the mixed anhydride or the DCC / HOBt method for activation of the carboxyl component, led successively to a series of unsaturated peptides **A** (Fig 3).

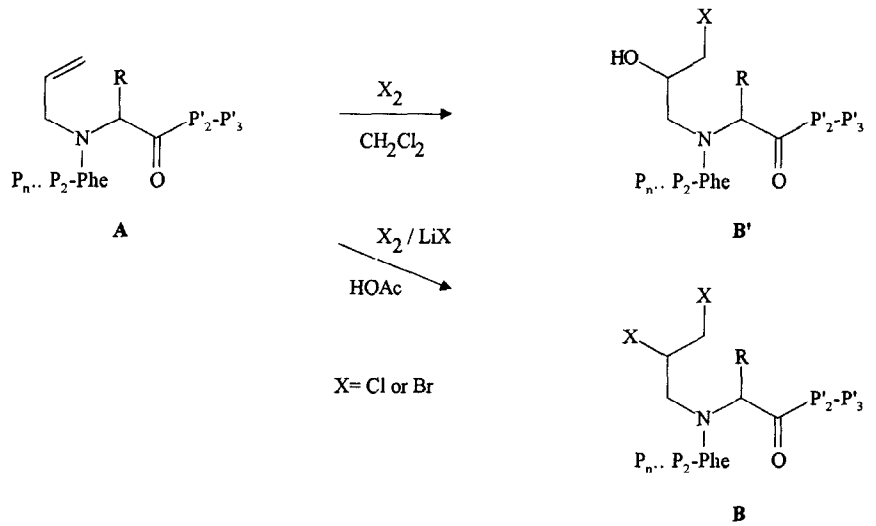


Figure 3: synthesis of the *vic*-dihalides **B** and halohydrins **B'**.

In CH_2Cl_2, bromine addition to the *N*-allyl-Gly or *N*-allyl-Ala residues of the peptides **A** gave a series of bromhydrin derivatives **B'** instead of the expected *vic*-dibromides **B**. This result is probably due to the participation of a neighboring amide group in the reaction, leading to an oxazolinium intermediate which is subsequently hydrolyzed [12, 13]. To avoid the participation of a benzamide function in a related reaction, S. Winstein *et al* have proposed the use of lithium bromide in acetic acid [12]. When applying analogous conditions (X_2, LiX, HOAc) to the electrophilic halogenation of the unsaturated peptides **A** the desired dibromides and dichlorides **B** (X= Br or Cl) were obtained (Fig 3). *Vic*-dihalides **B** and halohydrins **B'** have been characterized by elemental analysis and FAB mass spectrometry [14].

Tests for the inhibition of the HIV-1 proteinase

Series of compounds **A**, **B** and **B'** were assayed as inhibitors of the HIV-1 proteinase at 10^{-5} M, using three different substrates. The two-stage fluorescence assay (test A) used Suc-TLNFPIS-4-AMC in the first step, followed by aminopeptidase M release of the fluorescent AMC group [15]. The compounds were also tested in spectrophotometric assays using VSQNF(NO_2)PIV, 0.1 M NaCl (test B) or HKARVL-F(NO_2) EANLS-NH_2 (test C) [16]. Neither the starting N-allyl glycine or alanine containing peptides (series **A**) nor the compounds **B** and **B'** showed any significant inhibition (Table 1).

	A	B	C
A series (N-allyl)			
Boc-Phe-**2a**-Ile-Val-OMe	0	10	13
Boc-Ala-Phe-**2a**-Ile-Val-OMe	0	1	4
Boc-Ala-Ala-Phe-**2a**-Ile-Val-OMe	0	0	21
Boc-Phe-**2b**-Ile-Val-OMe	3	0	7
Boc-Ala-Phe-**2b**-Ile-Val-OMe	0	0	9
Boc-Ala-Ala-Phe-**2b**-Ile-Val-OMe	1	0	0
Boc-Ser-Ala-Ala-Phe-**2b**-Ile-Val-OMe	0	14	0
B series (dihalides)			
Boc-Phe-**3a**-Ile-Val-OMe	0	8	10
Boc-Phe-**3'a**-Ile-Val-OMe	0	0	2
Boc-Ala-Phe-**3a**-Ile-Val-OMe	0	3	0
Boc-Ala-Phe-**3'a**-Ile-Val-OMe	0	0	12

Table 1: Percentages of inhibition of the HIV-1 *pol*-protease with compounds **A**, **B** and **B'** at 10^{-5} M. [a] Assay A: Suc-TLNF-PIS-AMC, [b] Assay B: VSQNF(NO2)-PIV, [c] Assay C: HKARVLF(NO_2)-EANLS-NH_2.

Biological activity:

Some of the compounds belonging to the **A** and **B** series were evaluated for their protective activity against the cytopathic effect induced by the human HIV-1 (LAI strain) in CEM cell cultures [17] (Table 2). The Boc-Ala-Phe-*N*-(2,3-dihalogenopropyl)-Gly-Ile-Val-OMe, particularly the dibromide, showed a protective activity (EC-50= 111 µg/ml) without displaying a cytotoxic effect (IC-50 > 500 µg/ml). The *N*-allyl glycine or alanine containing precursors were more active (EC-50 = 8.15 and 80 µg/ml, respectively) than their halogenated derivatives.

	EC-50[a]	IC-50[b]	SI-50[c]	EC-50 (RT)[d]
A series (N-allyl):				
2a-Ile-Val-OMe	NA[e]	> 66.7		
Boc-**2a**-Ile-Val-OMe	NA	> 100		
Phe-**2a**-Ile-Val-OMe	NA	> 100		
Boc-Phe-**2a**-Ile-Val-OMe	NA	> 100		
*Boc-Ala-Phe-**2a**-Ile-Val-OMe*	8.15	35.7	4.03	10
Ala-Phe-**2a**-Ile-Val-OMe	NA	> 100		
Boc-Ala-Ala-Phe-**2a**-Ile-Val-OMe	NA	NA		
Boc-Phe-**2b**-Ile-Val-OMe	NA	> 10		
*Boc-Ala-Phe-**2b**-Ile-Val-OMe*	80	> 200	> 2.51	60
Boc-Ala-Ala-Phe-**2b**-Ile-Val-OMe	NA	2.9		
Boc-Ser-Ala-Ala-Phe-**2a**-Ile-Val-OMe	NA	> 100		
Boc-Ser-Ala-Ala-Phe-**2b**-Ile-Val-OMe	NA	> 20		
B series (dihalides):				
Boc-Phe-**3a**-Ile-Val-OMe	NA	42.51		
Boc-Phe-**3'a**-Ile-Val-OMe	NA	> 33.3		
*Boc-Ala-Phe-**3a**-Ile-Val-OMe*	111	> 500	> 4.50	200
*Boc-Ala-Phe-**3'a**-Ile-Val-OMe*	> 100	> 100		100

Table 2: Activities of the compounds against HIV-1 in CEM cells. Protective activity against the cytopathic effect induced by the human HIV-1 (LAI strain) in CEM cell was evaluated by MTT assay [17], and inhibition of reverse transcriptase activity in the supernatant of CEM cell cultures was evaluated by RT dosage [18]. [a] EC-50 (50 % antiviral effective dose): concentration in µg/ml that reduced by 50 % the HIV induced cytopathic effect; [b] IC-50: 50 % cytotoxic dose of compound (µg/ml) required to reduce the viability of uninfected cells by 50 %; [c] selectivity index: SI= ratio IC-50/ EC-50; [d] EC-50 (RT): dose of compound (µg/ml) that reduces reverse transcriptase activity in infected cell supernatant by 50 %; [e] NA: non active compound. All data represent the average value of at least two separate experiments

The antiviral activity was also evaluated by the inhibition of the reverse transcriptase activity in CEM cell culture supernatants [18, 19] (Table 2). Most of the compounds were inefficient inhibitors but Boc-Ala-Phe-*N*-(allyl)-Gly-Ile-Val-OMe (EC-50= 10 µg/ml) and its halogenated derivatives displayed antiviral effects (EC-50= 100 and 200 µg/ml for the *vic*-dichloride and the *vic*-dibromide, respectively).

The antiviral activity of the unsaturated peptides and of their *vic*-dihalide derivatives depends on the peptide chain length: shorter or longer analogs are not active. For the dibromides and dichlorides, an enzyme-activated antiviral mechanism (Figure 3, route *4:* protease-catalyzed hydrolysis, release of the aziridinium cation and reaction of this powerful electrophile with biomolecule Nu seemed possible. However, six or seven residues are usually required to achieve the minimal sequence for a peptide to become a substrate of the protease [1, 2]. Direct interaction of the molecules with an unknown biological target presumably leads to the observed antiviral activity. The antiviral effect of the unfunctionalized Boc-Ala-Phe-*N*-(allyl)-Gly-Ile-Val-OMe, which does not inhibit the protease either (Table 1), is higher than that of its halogenated derivatives (Table 2). The three related compounds could have a common objective, which is neither the *pol*-protease nor the reverse transcriptase.

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REFERENCES AND NOTES.

1. Meek TD (1992) *J. Enzyme Inhibition* 6, 65-98
2. Huff JR (1991) *J Med Chem* 34, 2305-2314
3. Schechter I, Berger A (1967) *Biochem Biophys Res Commun* 27, 157-162
4. Yaron A, Naider F (1993) *Crit Revs Biochem Mol Biol* 28, 31-81
5. Mazaleyrat JP, Rage I, Xie J, Savrda J, Wakselman M (1992) *Tetrahedron Lett* 33, 4453-4456
6. Xie J, Mazaleyrat JP, Savrda J, Wakselman M (1992) *Bull Soc Chim Fr* 129, 642-654
7. The synthesis of functionalized α-aryloxy- or α-arylamino-glycine and sarcosine and of 2-pyrrolecarboxylic acid containing peptides has also been undertaken. These molecules posess latent quinone methide, quinoniminium methide or azafulvenium electrophiles.
8. Silverman RB (1988) *Mechanism-based Enzyme Inactivation: Chemistry and Enzymology*" Vol I, CRC Press, Boca Raton, Florida, USA, p 6-7
9. Firestone RA, Pisano JM, Bailey PJ, Sturm A, Bonney RJ, Wightman P, Devlin, Lin CS, Keller D, Tway PC (1982) *J Med Chem* 25, 539-544
10. Cheung ST, Benoiton NL (1977) *Can J Chem* 55, 906-910
11. Mouna A, Nguyen C, Rage I, Xie J, Née G, Mazaleyrat JP, Wakselman M (1994) *Synth Commun.* 24, 2429-2436
12. Goodman L, Winstein S, Boschan R (1958) *J Am Chem Soc* 80, 4312-4317
13. Mc Manus SP, Caroll JT (1970) *J Org Chem* 35, 3768-3774
14. All compounds gave satisfactory analytical data. We just describe here the three active compounds: Boc-Ala-Phe-*N*-(allyl)-Gly-Ile-Val-OMe: m.p.= 86.3 °C, $[\alpha]_D^{25}$= -49° (c 0.1, MeOH). Anal., calc. for $C_{34}H_{53}N_5O_8$ (C,H,N): 61.88, 8.09, 10.61; found: 61.72, 8.03, 10.33. MS (FAB$^+$): m/z= 660 (MH$^+$). Boc-Ala-Phe-*N*-(2,3-dibromopropyl)-Gly-Ile-Val-OMe: m.p.= 134 °C, $[\alpha]_D^{25}$= -37.2° (c 1.2, MeOH). Anal., calc. for $C_{34}H_{53}Br_2N_5O_8$ (C,H,N): 49.82, 6.52, 8.54; found: 49.64, 6.63, 8.32. MS (FAB$^+$): m/z= 820 (MH$^+$). Boc-Ala-Phe-*N*-(2,3-dichloropropyl)-Gly-Ile-Val-OMe: m.p.= 158.5 °C, $[\alpha]_D^{25}$= -39.4° (c 0.52, MeOH). Anl., calc. for $C_{34}H_{53}Cl_2N_5O_8$ (C,H,N): 55.88, 7.30, 9.58; found: 55.59, 7.47, 9.38. MS (FAB$^+$): m/z= 730 (MH$^+$).
15. Hirel PH, Parker F, Boiziau J, Jung G, Outerovitch D, Dugue A, Peltiers C, Giulliacci C, Boulay R, Lelièvre Y, Cambou B, Mayaux JF, Cartwright T (1990) *Antiviral Chem Chemother* 1, 9-15
16. Pennington MW, Festin SM, Macchecchini ML, Dick F, Scaborough PE (1991) *Peptides 1990* Giralt E, Andreu D eds, ESCOM Sci Pub BV pp 787-789.
17. Pauwels R, Balzarini J, Baba M, Snoeck R, Schols D, Herdewijn P, Desmyter, J, De Clercq E (1988) *J Virol Meth* 20, 309-321.
18. Schwartz O, Hénin Y, Maréchal V, Montagnier L (1988) *Aids Res Hum Retroviruses 4, 441-448*
19. The compounds do not inhibit the catalytic activity of the reverse transcriptase *in vitro*.

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