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THIOPHENOXY PEPTIDES : A NEW CLASS OF HIV REPLICATION INHIBITORS

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Abstract : A new series of modified peptides inhibitors of HIV is reported. These peptides utilize the isosteric substitution of a methylene group by a sulfur atom in a phenylalanine residue. Two thiophenoxy peptides **9** and **11** inhibit *in vitro* HIV-1 replication in MT_4 cells with IC_{50} values of 5 and 10 μM respectively.

HIV produces a small dimeric aspartyl protease which specifically cleaves the polyprotein precursors encoding the structural proteins and enzymes of the virus.[1] This proteolytic activity is required for the production of mature infectious virions. Therefore, the aspartyl protease represents an attractive target for a therapeutic intervention. Medicinal chemists undertook to design and synthesize inhibitors of this critical aspartyl protease enzyme. This was based on the concept of transition state analogues.[2]

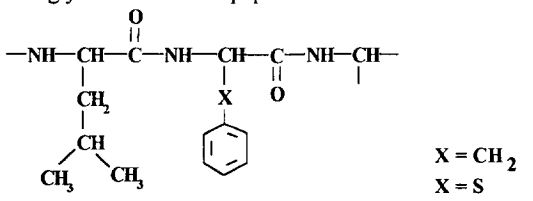
This concept consists of preparing the shortest peptide substrate in which the normally cleaved amide bond is replaced by a non-hydrolyzable surrogate that mimics the tetrahedral transition state motif. To date, a great number of various transition state cassettes have been introduced into the HIV-1 protease peptide, these being aminoethylene isosteres,[3,4] statine analogues,[5,6] phosphinic acid isosteres,[7] difluoroketones,[8] dihydroxyethylene and hydroxyethylamine isosteres.[9,10] HIV-1 protease inhibitors have also been designed based on the tertiary structure of the enzyme. These compounds can be classified as symmetric inhibitors,[11] and inhibitors of dimerization.[12,13] In an attempt to increase the overall scope of anti-HIV peptide approach, we have investigated a new concept of HIV inhibitors based on the following observations :

- if specific HIV protease substrates are not readily apparent from simple sequence analysis around the scissile bonds, then the N and C terminal sequence of structural proteins and enzymes found in mature infectious virions would show a remarkably diverse array of specific cleavage sites.[14,15,16]

- many peptides that model known sites of proteolytic processing within the HIV-1 polyproteins, have been shown to be accurately cleaved by purified synthetic or recombinant HIV-1 protease.

- several peptides were designed from the deduction of amino and carboxyl terminal sequencing of mature HIV-1 proteins. Among them, the synthetic peptide Ile-Arg-Lys-Ile-Leu-Phe-Leu-Asp-Gly-Ile **1** was found to be cleaved between the Leu and Phe residues. This corresponded to the normal pol 727 / 728 cleavage site.[15,17,18] Assuming that replacement in peptide **1** of the phenylalanine methylene group by an heteroatom like sulfur (Figure 1) should not change the cleavage site between Leu and Phe, we were able to report the synthesis and the surprising HIV-1 inhibitory properties of new thiophenoxy analogue isosteres of peptide **1** (Table 1).

Figure 1. Structure of α-glycine substituted peptide.



Leu-(S)-Phe

Table 1. Anti HIV-1 Activity of Synthetic [Leu-(S)-Phe] Containing Peptides.

N°	Synthetic-Leu(S)-Phe-peptides	IC^a_{50} μM	TI^b (ID^c_{50} / IC_{50})
1	Ile-Arg-Lys-Ile-Leu-Phe-Leu-Asp-Gly-Ile-OH	inactive	-
2	BocNH-Leu-(S)-Phe-OH	inactive	-
7	BocNH-Leu-(S)-Phe-OCH_2-CH=CH_2	inactive	-
8	Fmoc-Ile-Arg-Lys-Ile-Leu-(S)-Phe-Leu-Asp-Gly-NH_2	inactive/toxic	-
9	Ile-Arg-Lys-Ile-Leu-(S)-Phe-Leu-Asp-Gly-NH_2	5 ± 2	50
10	Fmoc-Ile-Arg-Lys-Ile-Leu-(S)-Phe-Leu-Asp-Gly-Ile-OH	100 ± 50	10
11	Ile-Arg-Lys-Ile-Leu-(S)-Phe-Leu-Asp-Gly-Ile-OH	10 ± 5	10

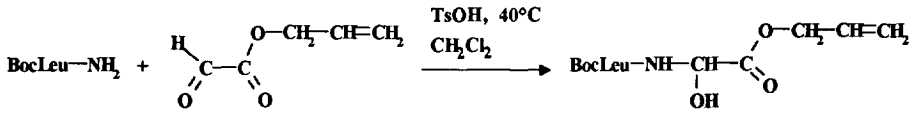
a) IC_{50} : concentration required to inhibit 50% syncitia formation. b) TI : therapeutic index.
c) ID_{50} : concentration required to cause death of 50% of uninfected MT_4 cells.

The basic template of the new peptide containing α-substituted glycine is represented in Figure 1. Usually an α-substituted glycine in which the α-carbon is linked to an N, O or S atom is unstable. Various N-acylated α-substituted glycines of this type have been described in the literature.[19,20] Acylation of the amino group provides stabilization of the molecule by delocalization of the electrons on nitrogen into the peptide bond. Instead of using simple N-acylation to provide chemical stability to such α-substituted glycines, we used a Leu-Phe

peptide bond in which the phenylalanine has been modified in order to mimic peptide **1** (Figure 1).

Peptides listed in Table 1 have been synthesized using the solid phase method. This synthesis required the use of the key synthon **2** prepared according to the following procedure (Schemes 1 and 2). Boc-Leucine amide **3** was condensed with allyl glyoxalate hydrate **4** to give the corresponding α-hydroxy derivative of Boc-Leu-Gly allyl ester **5** (Scheme 1) as a mixture of diastereoisomers (1:1).

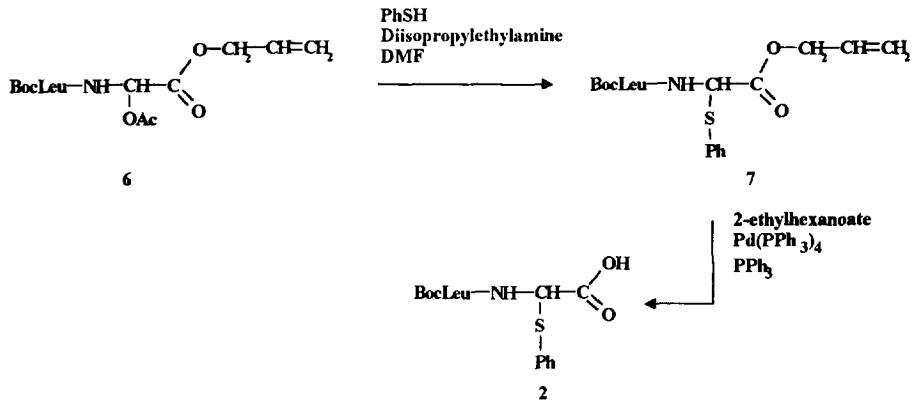
Scheme 1. Synthesis of the α-hydroxy derivative **5**.



3 **4** **5** 44%

Compound **5** was acetylated (Ac_2O, pyridine) to give **6**, the acetate group from which was displaced with thiophenol to give **7** (Scheme 2). Removal of the allyl ester group using the bis palladium triphenylphosphine complex $Pd(PPh_3)_4$ in the presence of PPh_3[21] led to the desired synthon **2** as a mixture of diastereoisomers (1:1). It should be noted that the use of other glyoxylic esters (t-butyl, benzyl, ethyl) were unsuitable for condensation onto the Boc-Leucine amide, mainly because the final saponification conditions led to the hydrolysis of the peptide bond.

Scheme 2. Synthesis of the key synthon **2**.



6 **7** **2**

Moreover, direct condensation between glyoxylic acid and Boc-Leucine amide failed under experimental test conditions. Because the solid phase synthesis of the peptides listed on Table 1 required grams of the synthon **2**, this preliminary study was performed using compound **2** as a mixture of diastereoisomers. Taking into account the unexpected anti-HIV results, separation by reverse phase HPLC[22] of mixture **2**, and enantiomeric synthesis of the corresponding peptides are in progress.

HIV-1 protease tests: The model peptides listed on Table 1 were incubated with partially purified HIV-1 protease using a standard procedure.[23] The cleavage products were analyzed by reverse phase HPLC. The results were not as expected: only the peptide model **1** was cleaved. Surprisingly, the sulfur containing peptides **2**, **7**, **8**, **9**, **10**, **11** were resistant to any proteolytic cleavage under the test conditions. Moreover, when the sulfur containing peptides were added to an assay using peptide **1** as model substrate, they were not inhibitors at concentrations of equal molarity to the substrates (about 2mM).

Antiviral activity: The compounds listed in Table 1 were tested for their ability to inhibit HIV-1 infection in cell culture. The fusogenic effect of HIV-1 in the MT_4 cell line[24] was determined as described by Rey and coworkers.[25,26] As shown in Table 1, some new thiophenoxypeptides were acting as inhibitors of HIV replication. The most active compounds were **9** and **11**. These results showed that protection of the N-terminus by an Fmoc group in compounds **8** or **10** caused loss of inhibition of the viral replication, compared to the free N-terminal homologs **9** and **11**. Billich and coworkers[23] showed that synthetic peptides containing 7 to 18 amino-acids, could be used as both model substrates and inhibitors for investigation of the protease. We found that the minimal length for the new α-S-phenyl substituted glycine peptides was 9 or 10 amino acids. In this finding both dipeptides **2** and **7** containing the S-phenyl moiety were inactive as inhibitors of viral replication. Concerning the C terminal residue, these preliminary results seem to indicate that the free carboxyl group compound **11** or the carboxamide compound **9** could be suitable for HIV replication inhibition.

This study has shown that moderately potent inhibitors of HIV-1 replication, incorporating a phenylalanine isostere (Figure 1) could be identified. To our knowledge it is the first time that synthetic peptides, which are not substrates or inhibitors of HIV-1 protease, are active on HIV-1 infection in MT_4 cell culture. This new class of synthetic peptides, based on the isosteric replacement of a methylene group by a sulfur atom in a phenylalanine residue positioned at the cleavage site of a HIV-protease synthetic peptide substrate, could be of interest. Indeed, the question of mechanism of action for this new class of compounds appears to be crucial. Experiments that could identify the target in the HIV replication cycle triggered by this new class of compounds will be continued. Before trying to determine the mechanism by which these compounds interfere with the viral replication cycle inside the cell, the results reported here suggest a problem of membrane permeability for these newly synthesised

peptides: indeed compounds like **9** and **11** with a free N-terminus showed the greatest anti-HIV effect, while more lipophilic peptides like **8** and **10** were found inactive in infected culture. In summary, a new series of HIV cell culture inhibitors which utilize the isosteric replacement of a methylene group by a sulfur atom in a phenylalanine residue, has been developed. The mechanism of action of the new synthetic compounds is unknown. After optimization of these initial leads, they could represent a new approach in the search for new anti HIV-drugs.

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