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Use of Proline Bioisosteres in Potential HIV Protease Inhibitors: Phenylalanine-2-thiophenoxy-3-pyrrolidinone: Synthesis and Anti-HIV Evaluation

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Abstract—The synthesis of new phenylalanine-2-thiophenoxy-3-pyrrolidinones is described. Anti-HIV recombinant protease assays and HIV infected cell culture assays (observation of *syncytia*) demonstrated the potent anti-HIV activity of this new class of pseudopeptides. © 2000 Published by Elsevier Science Ltd.

Introduction

We have recently reported the synthesis of new 3-pyrrolidinone type inhibitors of HIV-1 replication.[1,2] The design of these inhibitors was based on the observation that HIV protease displays an unusual preference for the Tyr-Pro or Phe-Pro primary cleavage site.[3,4] This observation led us to replace the 1-carboxy-pyrrole ring of the proline residue by a 3-pyrrolidinone moiety. In this feature these new proline mimicking moieties were coupled to various amino acids, including aromatic residue (Phe, Tyr). We have evaluated the anti-HIV potencies of this new class of analogues, through *syncytia* formation inhibition assay as first screening.[5,6] Indeed, many peptidomimetic inhibitors possessing effective activity on purified protease inhibition assays turned out to be sometimes inactive on HIV infected cells, because of lack of cell membrane permeation.

We describe herein the synthesis of new Phe-Pro dipeptide analogues in which the proline residue was replaced by a 2-thiophenoxy-3-pyrrolidinone (Fig. 1).

Introduction of a thiophenoxy group in position 2 of the pyrrolidinone ring could be of interest since enzymatic cleavage of the amide bond leads to an unstable phenylthioaminal intermediate, which rapidly undergoes a chemical rearrangement allowing the release of thiophenol as shown in Scheme 1. This observation was already reported by Kingsbury et al.[7] in the case of Ala-(*S*)-thiophenoxyglycine. Our research approach was first to use these reactive modified dipeptides as new scaffolds for the design of new potential anti-HIV agents.

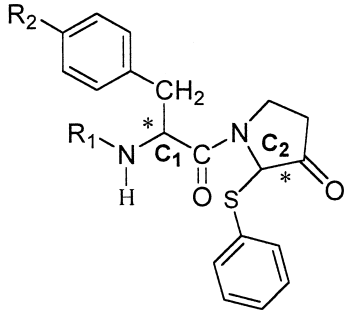

Figure 1.

Chemistry

Since 2-thiophenoxy-3-pyrrolidinone is an unstable species which cannot be isolated, the synthesis of the target compounds through the direct coupling between L-phenylalanine residue with 2-thiophenoxy-3-pyrrolidinone derivative cannot be considered. Therefore the synthesis of the new class of compounds (Fig. 1) required a specific synthetic route shown in Scheme 2.

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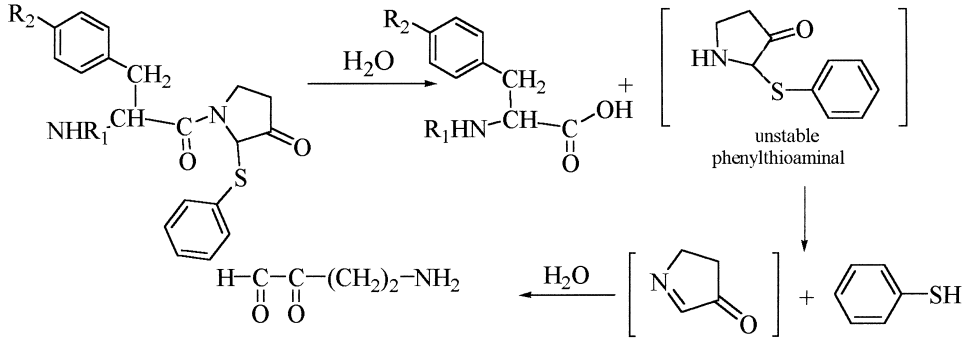
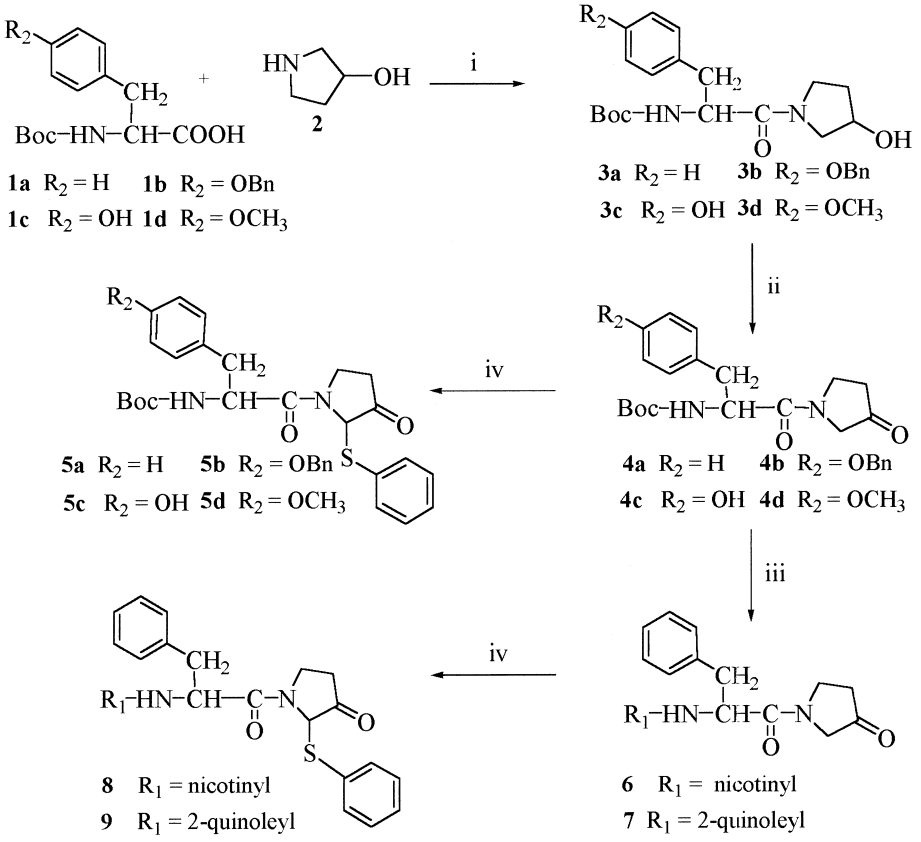
Compounds **3a–d** were obtained through standard coupling reactions between 3-pyrrolidinol **2** and the corresponding *N*-protected amino acid residues **1a–d** using BOP[8] as coupling reagent. Direct oxidation of derivatives **3a–d** under Swern[9] conditions led quantitatively to the corresponding 3-pyrrolidinone derivatives **4a–d**. In contrast, sulfenylation at the 2 position of the pyrrolidinone ring was rather difficult to perform and the obtained yields were quite low. The best results were obtained using the following conditions: 1 equiv of *sec*-butyllithium, 1.5 equiv of diphenyl thiosulfonate ($Ph_2S_2O_3$) and 1.5 equiv of 3-pyrrolidinone derivatives **4a–d** in toluene at −78 °C. In the same reaction conditions, analogues **5a–d** were isolated in 25, 17, 10 and 10% yield respectively. Similarly compounds **8** and **9** were obtained in respectively 10 and 22% yields from the *N*-deprotected precursors **6** and **7**. The low yields obtained in the desired mono 2-sulfenylated analogues



Scheme 1.



Scheme 2.

are due to the low regioselectivity of the enolate formation, which could lead to the formation of mono and polysulfenylated derivatives in positions 2 and 4 of the pyrrolidinone ring. Final compounds **5a–d** were obtained as diastereoisomeric mixtures. We observed no epimerisation of the C_1 stereogenic center, when *sec*-butyllithium is used in the following conditions (1 equiv *sec*-BuLi for 1,5 equiv of ketone), the α proton of the ketone is predominantly abstracted. Separation of these diastereoisomeric mixtures was achieved by semipreparative HPLC method using a reverse C18 waters spherisorb column and CH_3CN:H_2O (60:40) as eluent. Four pairs of diastereoisomers were obtained in which the asymmetric carbon C_1 coming from the (L)-phenylalanine residue is in the *S* configuration and asymmetric carbon C_2 bearing the thiophenoxy group at the 2 position of the pyrrolidinone ring is either *R* or *S*. It should be underlined that *R*, *S* assignment of the asymmetric carbon bearing the thiophenoxy group (compounds **5a$_1$**, **5a$_2$**, **5b$_1$**, **5b$_2$**, **5c$_1$**, **5c$_2$**, **5d$_1$**, **5d$_2$**) is not yet available (Table 1). Compounds **8** and **9** were tested as diastereoisomeric mixtures since HPLC separation was not satisfactory.

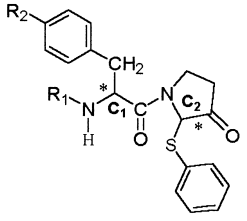
The structures of all these new analogues were determined by ^1H and ^{13}C NMR, mass spectrometry and elemental analyses. The synthesis of compound **5a$_3$** mixture of diastereoisomers (Table 1) which asymmetric carbon C_1 is in the D configuration and C_2 mixture of *R* and *S* configurations, was achieved starting from (D)-phenylalanine residue using the same procedure shown in Scheme 2.

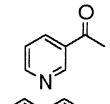
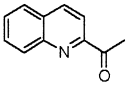
Antiviral Activity

The whole new synthesized analogues were evaluated for their anti-HIV activity on both screening assays: *syncytia* formation observation and recombinant protease assay. *Syncytia* formation: The fusogenic effect of HIV in MT_4 cells line[10] was determined as already described by Rey et al.[5,6] The appearance of *syncytia* was measured five days after infection, the inhibitory dose was expressed as the concentration of the tested compound which causes 50% inhibition of *syncytia* formation (EC_{50}). Results are presented in Table 1. Antiprotease assay: Evaluation of the synthesized compounds as HIV protease inhibitors was carried out according to a classical HIV recombinant protease assay.[11] Inhibitory activity of the compounds was expressed as IC_{50} values which correspond to the concentration required to inhibit 50% of the substrate hydrolysis by the recombinant HIV-1 protease. IC_{50} values are given in Table 1.

From the obtained results, it can be seen that some of the new analogues (**5a$_1$**, **5b$_1$** and **5c$_1$**), which are described in Table 1, elicited remarkable anti-HIV activities on both, infected MT_4 cells culture assay and recombinant HIV protease assay. It can be also observed that the configuration of the asymmetric

Table 1. Anti-HIV potencies of new phenylalanine-2-thiophenoxy-3-pyrrolidinone derivatives



Compound			Configuration		EC_{50}[a]	CC_{50}[b]	SI[c]	IC_{50}[d]
	R_1	R_2	C_1	C_2	μM	μM		μM
5a$_1$	Boc	H	L	*R*	1	>100	>100	0.008
5a$_2$	Boc	H	L	*S*	50	100	2	1
5a$_3$	Boc	H	D	(*R*,*S*)	NA[e]	>100	—	NA
5b$_1$	Boc	OBn	L	*R*	0.1	50	500	0.020
5b$_2$	Boc	OBn	L	*S*	NA	10	—	0.020
5c$_1$	Boc	OH	L	*R*	0.1	50	500	0.026
5c$_2$	Boc	OH	L	*S*	50	>100	>2	0.025
5d$_1$	Boc	OCH_3	L	*R*	NA	50	—	10
5d$_2$	Boc	OCH_3	L	*S*	NA	>100	—	10
8		H	L	(*R*,*S*)	1	50	50	0.1
9		H	L	(*R*,*S*)	NA	50	—	5

[a]EC_{50}: concentration in μM required to inhibit *syncytia* formation by 50% on MT_4 cells.
[b]CC_{50}: concentration in μM required to cause 50% death of uninfected MT_4 cells.
[c]SI: selective index = CC_{50}/EC_{50}.
[d]IC_{50}: enzyme inhibition values average of two separate runs.
[e]NA: nonactive at nontoxic concentrations.

carbons C_1 and C_2 appears to be crucial for anti-HIV activity particularly in the cell culture assay. Compound **$5a_3$** (Table 1) which asymmetric carbon C_1 is in D configuration and C_2 is a mixture of *R*, *S* configuration is denied of any anti-HIV activity in both assays. In contrast the most active compounds have the asymmetric carbon C_1 in the L configuration. Substitution on the aromatic ring of the phenylalanine residue also influenced the antiviral activity, the presence of a methoxy group (compound **5d**) abolished the antiviral activity while the presence of hydroxy or benzyloxy groups seems to improve the activity (compounds **$5c_1$** and **$5b_1$**) compared to that of compound **$5a_1$**. Concerning the recombinant HIV protease assay, the results are more difficult to explain since if as expected IC_{50} values found for diastereoisomers **$5a_1$** and **$5a_2$** (Table 1) are quite different, respectively 8 nm and 1 μM, in the case of the diastereisomers (**$5b_1$**, **$5b_2$**) or (**$5c_1$**, **$5c_2$**) (Table 1), the IC_{50} values for both isomers are similar, respectively 20 nm and 26 nm. More enzymatic experiments are required in order to clearly elucidate this point.

Conclusion

Nevertheless, this new series of 2-thiophenoxy-3-pyrrolidinone analogues appear to be a promising model for the design of potent HIV inhibitors, since these peptidomimetic compounds are not based on the transition state concept,[12,13] found in the clinically used antiprotease drugs. Moreover such peptidomimetic 'scaffolds' present two possible 'dressing' sites (R_1 and R_2) which could be used in combinational chemistry for the search of still more potent anti-HIV agents.

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