European Journal of Medicinal Chemistry 60 (2013) 144–154

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Linear and cyclic glycopeptide as HIV protease inhibitors

Sachin A. Pawar [a], Amit M. Jabgunde [b], Glenn E.M. Maguire [a], Hendrik G. Kruger [a], Yasien Sayed [c], Mahmoud E.S. Soliman [a,d], Dilip D. Dhavale [b,**], Thavendran Govender [a,*]

[a] Catalysis and Peptide Research Unit, School of Health Sciences, University of KwaZulu-Natal, South Africa
[b] Garware Research Centre, Department of Chemistry, University of Pune, Pune, India
[c] School of Molecular and Cell Biology, University of the Witwatersrand, Wits 2050, South Africa
[d] Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt

ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form
8 November 2012
Accepted 12 November 2012
Available online 29 November 2012

Keywords:
HIV-1 protease
Linear and cyclic glycopeptides
Solid and solution peptide synthesis
Molecular docking

ABSTRACT

Novel linear and cyclic glycotetrapeptides were designed, synthesized and tested for inhibition of the wild type C-SA HIV-1 protease enzyme. The incorporation of β-amino acid sugar to the linear and cyclic peptides resulted in a series of fifteen novel compounds. Linear glycopeptide **4a** and cyclic glycopeptide **6a** displayed significant activities against the HIV protease enzyme. The experimental results were compared with a computational approach using molecular docking. The sugar hydroxyl group at the C_3 position in linear (**4a**) as well as cyclic glycopeptide (**6a**), shows hydrogen bonding interaction with the enzymatic Asp25/Asp25′ residues in docking studies.

1. Introduction

Human immunodeficiency virus (HIV) is a pathogenic retrovirus and the causative agent of acquired immunodeficiency syndrome (AIDS) [1,2]. Inhibitors targeting reverse transcriptase, integrase, protease, co-receptor antagonists and the fusion of the virus to the host cells were developed and are available as commercial drugs for HIV treatment [3]. The assembly of new HIV virions is the ultimate step of the viral cycle which begins at the plasma membrane of the host cell [3]. HIV proteases cleave the viral polyprotein into individual functional HIV proteins that are required as the building blocks for the maturation of the virus [3].

Peptides containing sugar β-amino acids have significant impact in the medicinal chemistry research due to their wide applications. They have been used as chemotherapeutic [4], antiviral [2,5,6], antifungal [7], antitubercular [7], antibacterial [7], antiproliferative and apoptotic agents [8,9]. A sugar β-amino acid analogue (**A**) of natural product azumamide E (**B**) demonstrated better in vitro HDAC inhibitory activity than its synthetic natural compound (Fig. 1) [4]. The sugar β-amino acid analogue of somatostain (see Fig. 1, **C**) displayed apoptotic and antiproliferative activity [8,9].

The type VI β-turn [10] conformational motif of peptide derived inhibitors is thought to be especially important for improved HIV activity [10–14]. This is deduced from the selectivity shown by HIV protease in cleavage of proline–phenylalanine:tyrosine amide bonds in the matrix–capsid domain of the *gag-pol* polyproteins [10]. It is known that proline is an active β-turn turn inducer [15–18] and the proline–phenylalanine:tyrosine moiety exhibits type VI β-turn characteristics [10–14]. Recent literature reveals the key role that sugar β-amino acids play in forming turn structures in cyclic tetrapeptides [19]. The presence of known transition state analogues in cyclic peptides may make them interesting as potential protease inhibitors [13]. The use of hydroxy propyl amine (see Fig. 2) containing glycopeptide as HIV protease inhibitors was recently described by our group [2]. In this paper we compare the use of linear and cyclic glycopeptides incorporating a hydroxy

Abbreviations: AIDS, acquired immunodeficiency syndrome; CTC, 2-chlorotrityl chloride; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropyl ethylamine; DMF, *N*,*N*-dimethylformamide; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; EtOH, ethanol; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HDAC, histone deacetylases; HOBt, *N*-hydroxybenzotriazole; HBTU, N-[(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HIV, human immunodeficiency virus; MeOH, methanol; NMM, *N*-methylmorpholine; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; RP-HPLC, reverse phase high performance liquid chromatography; Ser, serine; SAA, sugar amino acid; TFA, trifluoroacetic acid; TIS, triisopropylsilane.

* Corresponding author. Tel.: +27 (0)31 260 8212; fax: +27 (0)31 260 7792.
** Corresponding author. Tel.: +91 (0)20 25691727; fax: +91 (0)20 25691728.
E-mail addresses: ddd@chem.unipune.ac.in (D.D. Dhavale), govenderthav@ukzn.ac.za (T. Govender).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.11.018

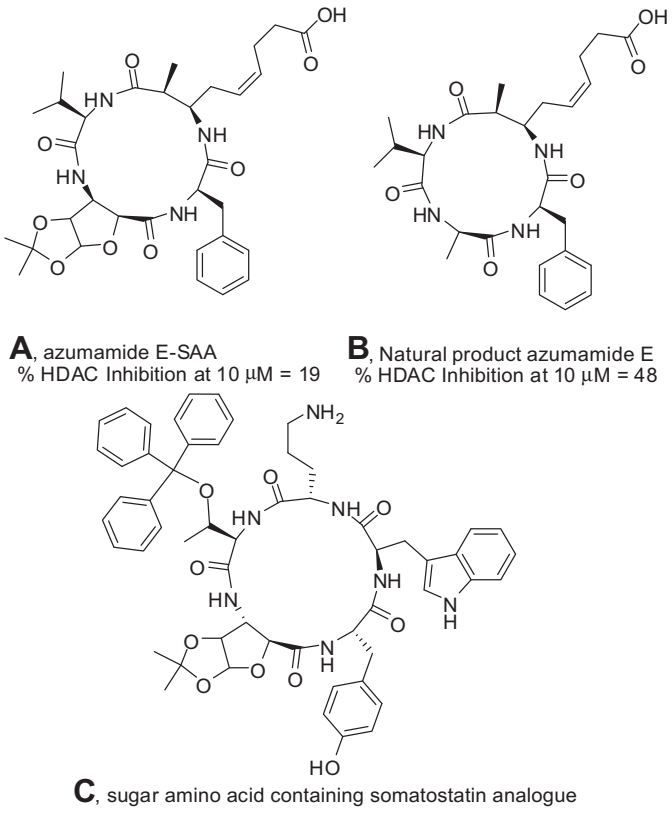

Fig. 1. Azumamide E-SAA (A) and its synthetic natural product (**B**) [4] and sugar β-amino acid containing somatostain analogue (**C**) [8,9].

propyl amine as a transition state analogue for HIV PR inhibition. We have recently observed the H-bonding interaction between hydroxyl group on sugar moiety of carbapeptide and ASP25/ASP25′ units of the HIV protease with docking studies [2]. The same observation was made in this study. The sugar moiety appears to induce a turn in the linear peptides so that the 3D structure corresponds quite well with the corresponding cyclic peptides. It appears that the hydroxyl propyl amine part of the glycol part acts as a transition state which interacts with the active site of the HIV protease (ASP25/ASP25′ units).

The solution synthesis of linear and cyclic glycopeptides containing sugar β-amino acid has been broadly reported and in all cases with low overall yields [4,5,19,20]. The solid phase synthesis of glycopeptides containing sugar β-amino acids was reported with overall yields of 20–40% [8,21–23]. In general, linear glycopeptides can be prepared more efficiently by using a solid phase approach and then subject to cyclization in solution [21,24]. The synthesized compounds (see Fig. 3, **2a**–**2c**, **3a**–**3c**, **4a**–**4c**, **5a**–**5c** and **6a**–**6c**)

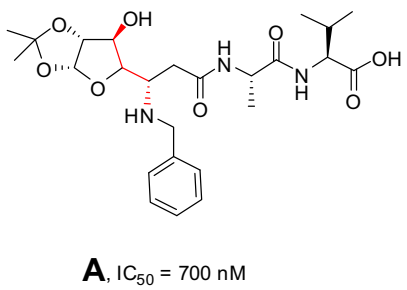

Fig. 2. Glycopeptide HIV protease inhibitor (**A**) involving hydroxy propyl amine [2].

were evaluated for HIV-PR activity and the experimental results were compared with a computational approach involving molecular docking.

2. Materials and methods

2.1. Over-expression, extraction and purification of the C-SA protease

Plasmid encoding HIV-1 subtype C protease (containing the mutation Q7K designed to reduce the hypersensitive autolytic site) is expressed as inclusion bodies in *Escherichia coli* BL21 S4 (DE3) pLysS cells. Briefly, *E. coli* cells harboring the plasmid DNA were grown at 37 °C in LB medium supplemented with 100 μg/mL of ampicillin and 35 μg/mL of chloramphenicol. The overnight culture was diluted 100-fold into fresh 2 × YT medium supplemented with ampicillin (100 μg/mL) and chloramphenicol (35 μg/mL) and grown at 37 °C. When the optical density (OD_{600}) of the culture reached 0.4–0.5, over-expression of the HIV-1 C-SA protease was induced by adding IPTG. IPTG was added to final concentrations of 0.4 mM. Over-expression of the protease was allowed to continue for 4 h. The cells were pelleted after growth and resuspended in ice-cold extraction buffer [10 mM Tris, 1 mM EDTA, and 1 mM PMSF (added only fresh before use), pH 8] and disrupted using an ultrasonicator. Following the addition of $MgCl_2$ and DNase I to final concentrations of 10 mM and 10 μg/mL, respectively, the culture medium was stirred on ice until the viscosity of the mixture decreased. The cells were then ruptured by sonication and centrifuged at 15 000 × *g* for 30 min at 4 °C. The pellet was resuspended in ice-cold extraction buffer containing 1% (v/v) of Triton X-100. Cell debris and protease-containing inclusion bodies were pelleted by centrifugation at 15 000 × *g* for 30 min at 4 °C. The pellet was then resuspended in a freshly prepared solubilization buffer containing 10 mM Tris, 2 mM DTT, 8 M urea, pH 8.0, at room temperature, and centrifuged at 15 000 × *g* for 30 min at 20 °C. The protease, in the supernatant, was purified by passing it through an anion exchange (DEAE) column previously equilibrated with solubilization buffer. Upon elution from the column, the protease was acidified by adding formic acid to a final concentration of 25 mM. Precipitation of significant amount of contaminating proteins occurred upon acidification. Following an overnight incubation, the precipitated contaminants were removed by centrifugation at 15 000 × *g* for 30 min at 4 °C. HIV-1 protease was refolded by dialysis into a 10 mM formic acid solution at 4 °C. Subsequently, the protease was dialyzed into storage buffer containing 10 mM sodium acetate, 1 mM NaCl and 1 mM DTT, pH 5.0. The folded protease was concentrated to a final volume of ~5 mL and stored at −20 °C.

2.2. In vitro HIV-1 protease activity

The catalytic activity of the HIV-1 protease was monitored following the hydrolysis of the chromogenic peptide substrate Lys-Ala-Arg-Val-Nle-*p*-nitro-Phe-Glu-Ala-Nle-NH_2. This substrate mimics the conserved KARVL/AEAM cleavage site between the capsid protein and nucleocapsid (CA-p2) in the Gag polyprotein precursor [25,26]. For this study the chromogenic substrate was synthesized using the Discovery CEM Liberty microwave peptide synthesizer on rink amide resin. The substrate was cleaved from the resin and simultaneously deprotected. It was then precipitated using cold ether and purified *via* reverse phase semi-preparative HPLC and characterized using mass spectrometry.

To determine the concentration of the inhibitors that resulted in 50% inhibition (IC_{50}) of HIV-1 protease inhibition activity, the protein (0.1 μM) and chromogenic substrate (50 μM) were added into a 150 μL microcuvette containing increasing concentrations of