Bioorganic & Medicinal Chemistry Letters 23 (2013) 4832–4836

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel selective inhibitors of aminopeptidases that generate antigenic peptides

Athanasios Papakyriakou [a,b], Efthalia Zervoudi [c], Emmanuel A. Theodorakis [a], Loredana Saveanu [d], Efstratios Stratikos [c], Dionisios Vourloumis [b,*]

[a] Department of Chemistry & Biochemistry, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0358, USA
[b] Chemical Biology Laboratories, National Center for Scientific Research 'Demokritos', Agia Paraskevi Attikis, GR 15310, Greece
[c] Protein Chemistry Laboratories, National Center for Scientific Research 'Demokritos', Agia Paraskevi Attikis, GR 15310, Greece
[d] Institut National de la Santé et de la Recherché Médicale, Unité 1013 and Université Paris Descartes, Sorbonne Paris Cité, Faculté de médecine, 75015 Paris, France

ARTICLE INFO

Article history:
Received 3 June 2013
Revised 12 July 2013
Accepted 15 July 2013
Available online 23 July 2013

Keywords:
Endoplasmic reticulum aminopeptidase
ERAP1
ERAP2
IRAP
Selective inhibitor
Rational design
Molecular modeling

ABSTRACT

Endoplasmic reticulum aminopeptidases, ERAP1 and ERAP2, as well as Insulin regulated aminopeptidase (IRAP) play key roles in antigen processing, and have recently emerged as biologically important targets for manipulation of antigen presentation. Taking advantage of the available structural and substrate-selectivity data for these enzymes, we have rationally designed a new series of inhibitors that display low micromolar activity. The selectivity profile for these three highly homologous aminopeptidases provides a promising avenue for modulating intracellular antigen processing.

Human aminopeptidases of the oxytocinase subfamily of M1 aminopeptidases have recently been shown to play important roles in the function of the human adaptive immune response.[1–4] Endoplasmic reticulum aminopeptidases 1 and 2 (ERAP1/2), as well as Insulin regulated aminopeptidase (IRAP) can generate key antigenic peptides that help the human body fight pathogens and cancer but at the same time they can also destroy several antigenic peptides by over-trimming.[5–7] Both functions can contribute to human disease by either promoting immune evasion or contributing to autoimmune responses.[8] Genetic polymorphisms in ERAP1 and ERAP2 have been associated with the individual's predisposition to numerous human diseases (reviewed in Ref. 8) and it has been suggested that this link is due to changes in their specificity and activity.[9–11]

Despite the important biological roles of ERAP1/2 on human health, very little is known on how to pharmacologically manipulate their function. Down-regulation of ERAP1 protein expression in experimental models has been shown to elicit novel cytotoxic responses in mice,[7] to induce Natural Killer cell responses against malignant cells leading to tumor rejection,[12] and to elicit non-classical Major Histocompatibility Class Ib cytotoxic T-lymphocyte responses in vivo.[13] Some of these effects could be reproduced using the non-specific metalloprotease inhibitor Leucinethiol.[7–13] These findings, along with the recent elucidation of the crystallographic structures of ERAP1,[14,15] ERAP2,[16] and the accumulation of a large amount of specificity data for these peptidases,[17] have spurred interest towards the development of potent and selective inhibitors for these enzymes that could potentially control the generation of specific subsets of antigenic epitopes. Achievement of selectivity may be even more important than high potency in this case, since complete incapacitation of antigenic peptide generation may not be desired therapeutically as opposed to subtle modulation of a particular epitope's generation. This concept has been supported physiologically by demonstrating that relatively small changes in the enzymatic activity of ERAP1 due to a single nucleotide polymorphism can be either protective or predisposing to autoimmunity.[8,10,18]

Since ERAP1, ERAP2 and IRAP are highly homologous, having sequencing identity at ~50%, the design of inhibitors that demonstrate any degree of selectivity between these enzymes is highly challenging. Towards our goal to achieve selectivity we utilized the acquired scientific experience from the matrix metalloproteinases (MMPs) family of zinc endopeptidases, which has been intensively pursued during the last three decades.[18–20] Specifically, it has been demonstrated that employing strong zinc chelators,[21–23]

* Corresponding author. Tel.: +30 210 6503624; fax: +30 210 6511766.
E-mail address: vourloumis@chem.demokritos.gr (D. Vourloumis).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.07.024

such as the hydroxamic acid zinc-binding group, usually raises undesirable off-target activity against other metalloproteins, as exemplified by the first generation of MMP inhibitors.[24,25] Additionally, it has been proposed that since the metal site is the most conserved feature across all MMPs, inhibition based on non-zinc chelating inhibitors would minimize or even eliminate the interaction with the catalytic zinc, leading to improved selectivity.[26–28] The latter was proven to be very successful for targeting enzymes with deep pockets, such as the S1′ subsite of MMP-13.[29]

It has become clear that a careful consideration of inhibitor backbones for targeting the substrate pockets of individual metalloenzymes, can lead to the development of highly selective inhibitors, irrespective of the zinc-binding group used. On these grounds, we based our design strategy on a relatively weak zinc chelator, aminobenzamide, borrowed from class I histone deacetylase inhibitors.[30] The amino-functionality at position 1 (numbering as of Fig. 1A) could either coordinate to the active-site zinc ion, or probably interact with a catalytically important glutamic acid residue (Glu-354/371/465 for ERAP1/ERAP2/IRAP), which is engaged in most zinc metalloproteases to polarize the water molecule for nucleophilic attack on the carbonyl group of the scissile bond.[31] The 2-benzamide functionality incorporates the coordinating carbonyl group and the α-amino acid-based moiety, as required for the enzymatic function in all natural substrates. Thus, the strong electrostatic interaction with the two conserved glutamic acid residues that harbor the α-amino terminus of natural substrates is preserved (Fig. 1). The P1 side-chain could be selected according to the target member and is expected to significantly contribute in achieving selectivity for one aminopeptidase over another, based on their individual spatial and electrostatic requirements. Expansion towards the primed subsites could be achieved through functionalization of the carboxylic acid at position 4, properly directing the inhibitor side-chain(s) Pn′ to target the corresponding substrate pockets.

The selectivity profile of the S1 subsite of ERAP1, ERAP2 and IRAP was recently investigated using a library of 82 fluorogenic substrates.[17] These studies suggested that ERAP1 displays a general preference for substrates comprising long, aromatic or hydrophobic P1 side-chains. In contrast, the S1 subsite of ERAP2 exhibits higher selectivity for positively charged groups (e.g., L-Arg), whereas IRAP combines the specificity of ERAP1 and ERAP2.[17] For these reasons we selected L-homo-phenylalanine (hPhe) as one of the preferred occupants for the S1 subsite. This decision was further supported by our molecular modeling calculations, which indicated that the long side-chain of hPhe would be ideally accommodated in the S1 pocket of ERAP1, providing π-stacking interactions with a conserved aromatic residue (Phe-433/Phe-450/Phe-544 for ERAP1/ERAP2/IRAP). Higher flexibility was allowed for the Pn′ position by employing a compilation of polar, non-polar and aromatic natural amino acids to attain the desired selectivity.

The targeted analogues were synthesized according to the procedure presented in Scheme 1. Thus, Boc-protected L-homo-phenylalanine **2**, selected as the amino acid of choice for optimizing the S1 lipophilic interactions as presented before, was coupled with di-aniline **1** under standard coupling conditions (HBTU, DIPEA) furnishing the corresponding amide in 82% yield. The high regioselectivity of the coupling transformation is

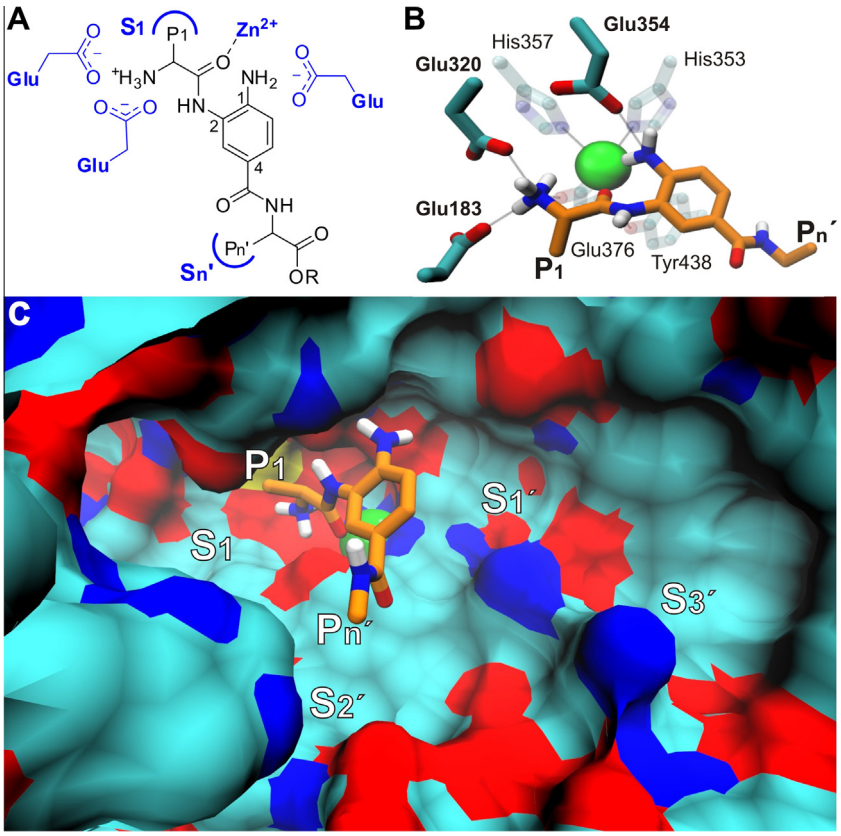

Figure 1. Inhibitor design strategy. (A) Schematic representation of the designed inhibitors (black) showing the interacting residues of the enzyme in blue. P1 and Pn′ represent amino acid side-chains that are accommodated at the S1 and Sn′ subsites of the aminopeptidases, respectively. (B) Molecular model of the designed scaffold with carbon atoms shown in orange. The active-site residues of ERAP1 are shown with cyan-stick carbons and Zn^{2+} with a green sphere; all oxygen and nitrogen atoms are red and blue, respectively. The α-amino terminal docking residues are Glu-183 and Glu-320. The zinc-coordinated residues His-353, His-357 and Glu-376, and the catalytically important Tyr-438 are shown transparent. (C) Surface representation of the active site of ERAP1 indicating the S1 and the putative S1′–S3′ subsites.