ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Screening Identifies Thimerosal as a Selective Inhibitor of Endoplasmic Reticulum Aminopeptidase 1

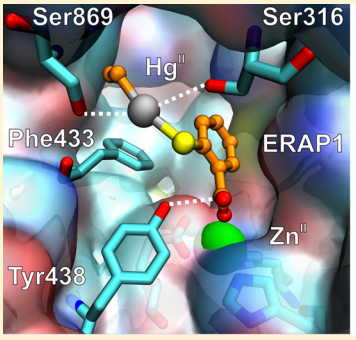
Athanasios Stamogiannos,[†,‡] Athanasios Papakyriakou,[†,‡] Francois-Xavier Mauvais,[§] Peter van Endert,[§] and Efstratios Stratikos*[,†]

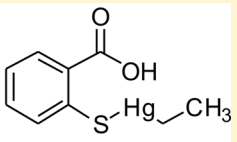
[†]National Center for Scientific Research Demokritos, Agia Paraskevi GR-15310, Athens, Greece

[§]Institut National de la Santé et de la Recherche Médicale, Unité1151; Université Paris Descartes, Sorbonne Paris Cité; Centre National de la Recherche Scientifique, Unité 8253, 75015 Paris, France

Supporting Information

ABSTRACT: We employed virtual screening followed by *in vitro* evaluation to discover novel inhibitors of ER aminopeptidase 1, an important enzyme for the human adaptive immune response that has emerged as an attractive target for cancer immunotherapy and the control of autoimmunity. Screening hits included three structurally related compounds carrying the (*E*)-*N*′-((1*H*-indol-3-yl)methylene)-1*H*-pyrazole-5-carbohydrazide scaffold and (2-carboxylatophenyl)sulfanyl-ethylmercury as novel ERAP1 inhibitors. The latter, also known as thimerosal, a common component in vaccines, was found to inhibit ERAP1 in the submicromolar range and to present strong selectivity versus the homologous aminopeptidases ERAP2 and IRAP. Cell-based analysis indicated that thimerosal can effectively reduce ERAP1-dependent cross-presentation by dendritic cells in a dose-dependent manner.





7, Thimerosal

ERAP1	**0.24 μM**
ERAP2	>50 μM
IRAP	>50 μM
LAP	>50 μM

KEYWORDS: *ERAP1, ERAP2, IRAP, aminopeptidase, inhibitor, immune system, antigenic peptide, docking*

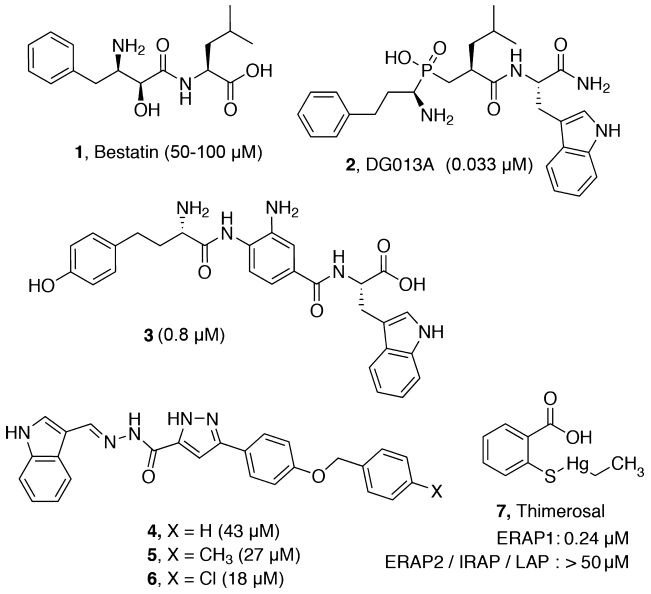
Endoplasmic reticulum (ER) aminopeptidases generate antigenic peptides for loading onto Major Histocompatibility Class I molecules (MHCI), which then interact with receptors on cytotoxic T-lymphocytes to initiate adaptive immune responses against infected or cancerous cells.[1,2] ER aminopeptidase 1 (ERAP1) is particularly effective in this function, and many *in vitro* and *in vivo* studies have established its role in regulating adaptive immune responses. For these reasons, ERAP1 is an attractive target for both cancer immunotherapy and the control of autoimmune reactions.[3,4] Indeed, ERAP1 down-regulation by available inhibitors has been reported to enhance cytotoxic responses versus cancer and suppress cellular autoimmune responses in Ankylosing Spondylitis.[4−6] Despite its biological importance, however, no clinical application of ERAP1 inhibitors have been reported, in part due to the lack of pharmacologically appropriate potent and selective inhibitors. Bestatin (ubenimex), a typical aminopeptidase inhibitor, has been evaluated in clinical settings but is a poor inhibitor of ERAP1.[7] Recent rational design efforts have yielded promising leads including a phosphinic pseudopeptide nanomolar inhibitor (DG013A, Chart 1) that displayed however low selectivity toward homologous enzymes, and 3,4-diaminobenzoic acid derivatives (such as **3**, Chart 1) that displayed a better selectivity profile albeit with modest potency.[8,9] In an effort to discover novel, nonpeptidic scaffolds that inhibit ERAP1 as leads for preclinical development we applied a combination of structure-based, ligand-based, and knowledge-based virtual screening approaches, taking advantage of key structural characteristics revealed in the recent crystal structures of ERAP1 and ERAP2 and their complexes with **1** and **2**, respectively.[8,10,11]

Toward this goal, we compiled a library of more than 265,000 compounds from selected collections of chemical vendors that are focused on drug-likeness and structural diversity (Table S1). The library was enriched with the National Cancer Institute's diversity set II (1364 compounds) and the DrugBank database comprising 6590 FDA-approved and experimental small-molecule drugs[12] We also performed a 3D pharmacophore search against the purchasable subset of the ZINC database (more than 20 million compounds)[13] using the online interface of ZINCPharmer.[14] The pharmacophore features of the query were extracted from the X-ray crystal structures of ERAP1 complex with bestatin and ERAP2 complex with DG013A,[8,10,11] which were further refined to a consensus pharmacophore (see the Computational Methods section, Table S2, and Figure S1 in the Supporting Information for more details). The filtered query results (3959 compounds) supplemented our small-molecule library for docking to ERAP1.

Received: February 26, 2016
Accepted: May 31, 2016

DOI: 10.1021/acsmedchemlett.6b00084
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Chart 1. Chemical Structures of Known ERAP1 Inhibitors and Inhibitors Identified in This Study[a]



[a]Values in parentheses indicate the IC_{50} for ERAP1.

Molecular docking was performed with AutoDock Vina,[15] using ERAP1 in the closed state as the target structure (PDB ID: 2YD0).[10] Approximately 2500 top-ranked complexes were examined on the basis of three knowledge-based criteria: (i) their zinc-binding potential, (ii) accommodation of an aromatic ring system inside the S1 pocket, and (iii) additional hydrogen bonding and hydrophobic interactions at the S1′−S2′ subsites. Accordingly, 24 compounds that satisfied all three criteria (Supporting Information, Table S3) were obtained and screened against ERAP1 using a previously established fluorigenic assay.[16]

The *in vitro* evaluation of the selected compounds revealed **4** as a low micromolar inhibitor of ERAP1 (Chart 1). The top-ranked predicted pose of **4** displayed coordination to the active site Zn(II) via the carbonyl group and a stacking interaction between the indole ring of **4** and Phe433 (Figure 1). The accommodation of the indole ring inside the S1 pocket of ERAP1 can be stabilized further via a hydrogen bond with the backbone carbonyl group of Glu183. The pyrazole carbohydrazide moiety of **4** is predicted to interact with the active site Tyr438 and the backbone of Ala318, whereas the benzyloxybenzene moiety is mainly engaged in aromatic−hydrophobic interactions.

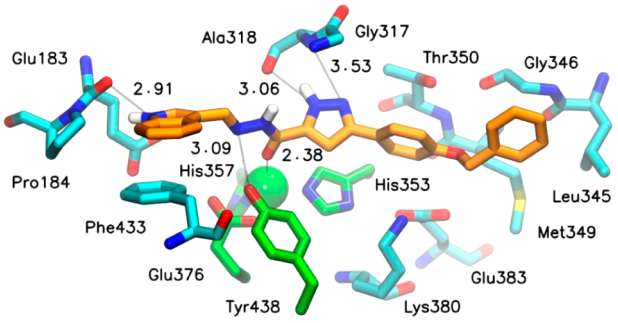


Figure 1. Predicted conformation of **4** bound to the active site of ERAP1. The inhibitor is shown with orange C, the interacting residues with cyan C, and the zinc-bound residues including the catalytic Tyr438 with green C; all N are blue, O are red, S are yellow, and Zn(II) is shown as a green sphere. Intermolecular hydrogen bonding distances between heavy atoms are given in Å.

To examine the potential of **4** for further optimization as ERAP1 inhibitor we performed a similarity search at 70% cutoff of the (*E*)-*N*′-((1*H*-indol-3-yl)methylene)-3-phenyl-1*H*-pyrazole-5-carbohydrazide scaffold against the purchasable ZINC database. The query results (1320 compounds) were docked against ERAP1, and visual inspection of the predicted bound conformations assisted the selection of a subset of 20 compounds for experimental evaluation (Table S4). The second round of *in vitro* screening revealed two inhibitors of ERAP1, **5** and **6** (Chart 1), with an approximately 2-fold increase in potency (representative titrations are shown in Figure 2). The activity gain of **5** and **6** can be attributed to a potential CH···O hydrogen or halogen bond with the hydroxyl group of Ser342, respectively (Figure S2). Although the identified inhibitors **4**−**6** are quite large and lipophilic (MW ≈ 450, *x* log *P* > 5) to be considered for further lead optimization, our data indicate that (*E*)-*N*′-((1*H*-indol-3-yl)methylene)-1*H*-pyrazole-5-carbohydrazide is a promising scaffold for the design of nonpeptidic inhibitors for ER aminopeptidases.

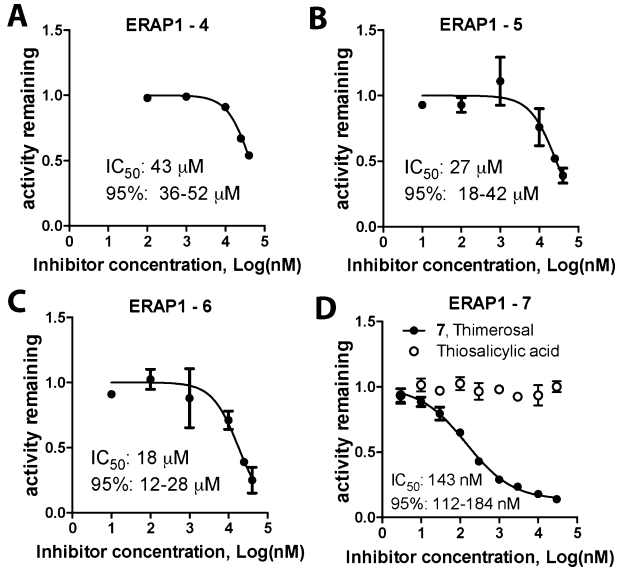


Figure 2. Representative titrations showing the ability of selected compounds to inhibit the ability of ERAP1 to hydrolyze the model fluorigenic substrate L-leucine-7-amino-4-methylcoumarin. Calculated IC_{50} values and 95% confidence interval are indicated.

Motivated by successful structure-based design strategies that were based on the use of a zinc-binding group,[17] we selected an additional subset of nine small-molecule drugs from the DrugBank database (Table S5). Their selection was assisted by visual inspection of the docked conformations but was based on their zinc-chelating potential, regardless of their estimated binding affinity for ERAP1 (see the Compound Selection section in the Supporting Information).

Of the small-molecule drugs that were evaluated (Table S5) only one was found to inhibit ERAP1. Compound **7** (Chart 1) displayed submicromolar inhibition of ERAP1 with an IC_{50} of 0.24 ± 0.11 μM (average of three separate experiments, representative titration shown in Figure 2D). Compound **7** was

DOI: 10.1021/acsmedchemlett.6b00084
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

initially selected for its potential bidentate coordination to the thiophilic Zn(II) through the thiosalicylic acid moiety. However, *in vitro* evaluation of thiosalicylic acid revealed no measurable inhibition of ERAP1 up to 30 μM (Figure 2D), suggesting that **7** inhibits ERAP1 through a different mechanism.

To examine the selectivity of the discovered ERAP1 inhibitors, we evaluated the two most potent compounds (**6** and **7**) against ERAP2 and IRAP, two enzymes that are homologous to ERAP1 and are also important for the generation of antigenic epitopes and the function of the adaptive immune system. Compound **6** inhibited ERAP2 and IRAP with similar potency (34 and 28 μM, respectively), but **7** was essentially inactive toward the two enzymes (Figure S3). The remarkable selectivity of **7** for ERAP1 with respect to the highly homologous ERAP2 and IRAP (51% and 46% sequence identity, respectively) suggests that inhibition is mediated by structural features that are unique to ERAP1. This idea is further supported by the inactivity of **7** against another zinc aminopeptidase of the M17 peptidase family, leucyl-aminopeptidase (LAP, Figure S4).

The inhibition profile of thimerosal (Figure 2D) displayed some atypical parameters and specifically a less than complete inhibition (10−20% residual enzymatic activity) and a hill constant of 0.73, which could indicate either negative cooperativity or multiple binding sites. To rule out any potential artifacts due to the nature of the fluorigenic enzymatic assay we also tested the ability of thimerosal to inhibit ERAP1 in a more physiologically relevant HPLC-based peptide trimming assay.[18] The rate of trimming of the N-terminus of the natural antigenic epitope precursor YTAFTIPSI was followed by reversed-phase HPLC in the presence or absence of **7** at 1 μM concentration (Figure 3). Thimerosal was able to

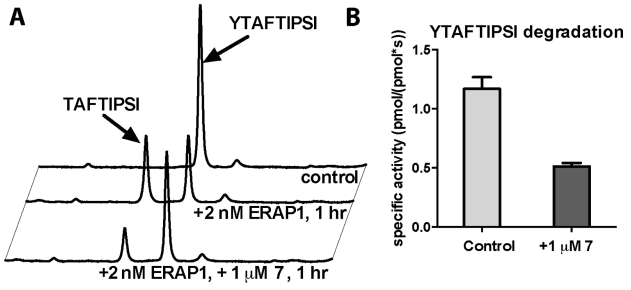


Figure 3. (A) Characteristic chromatograms showing the trimming of peptide YTAFTIPSI by ERAP1 in the presence or absence of **7**. (B) Calculated specific activity based on the surface of the peptide peaks in the chromatograms (average of three separate experiments).

reduce the rate of trimming by 2.3-fold, which is overall consistent with the previous *in vitro* measurements of inhibitor activity versus ERAP1. Still, the residual ERAP1 activity seen in this assay suggests that thimerosal may be less potent in inhibiting the trimming of large peptides by ERAP1.

To further investigate the mechanism by which **7** inhibits ERAP1 we employed Michaelis−Menten analysis using the chromogenic substrate L-pNA (Leucine-*para*-Nitroanilide).[8] The V_{max} of the reaction was unaffected by the presence of the inhibitor, whereas K_M was increased according to the expected inhibition, indicating that thimerosal acts as a competitive inhibitor of ERAP1 (Figure 4A,B).

Since thimerosal has been reported to be able to release ethylmercury that can bind covalently to cysteine residues, we

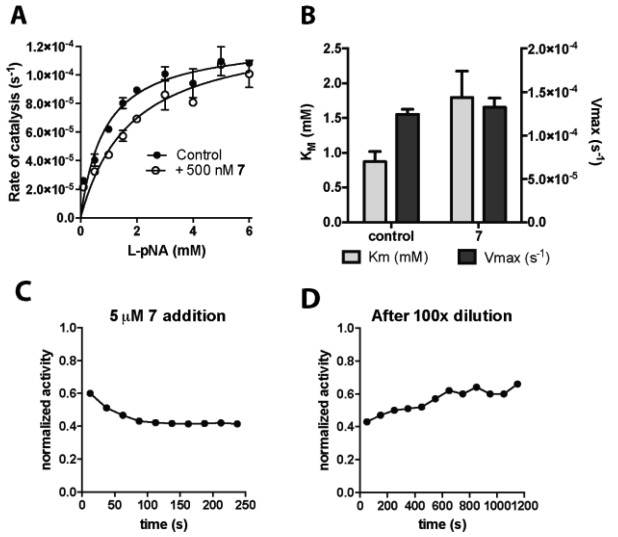


Figure 4. Kinetic analysis of inhibition of ERAP1 by **7** (thimerosal). (A) Michaelis−Menten analysis in the absence or presence of 500 nM **7**. (B) Calculated enzymatic parameters by fitting the experimental data of panel A to the classical Michaelis−Menten equation. (C) Normalized ERAP1 activity following the addition of **7** (15 s of dead mixing time). (D) Recovery of ERAP1 activity after 100-fold dilution of the sample used in (C).

investigated the reversibility of ERAP1 inhibition by **7**.[19] Rapid mixing of 5 μM thimerosal with ERAP1 in the presence of L-AMC resulted in loss of the majority of the enzymatic activity, although a slow-rate component was also evident. Dilution of this mixture by 100-fold resulted in a time-dependent recovery of enzymatic activity indicating that the interaction of ERAP1 with thimerosal is characterized by relatively slow k_{on} and k_{off} rates and is mostly reversible (Figure 4C,D). Overall, the selectivity of thimerosal for ERAP1 over other highly homologous enzymes, the competitive type of inhibition, in addition to evidence for reversibility, indicate that thimerosal inhibits ERAP1 through noncovalent interactions with non-conserved residues of the active site.

To provide a structural rationale for the ability of thimerosal to inhibit ERAP1 we evaluated its docked conformations at the active sites of ERAP1, ERAP2, and IRAP.[10,20,21] Several top-ranked poses among the docked conformations of **7** displayed monodentate binding of the carboxylate group (Figure 5A), in accordance with crystallographic structures of Zn(II) model

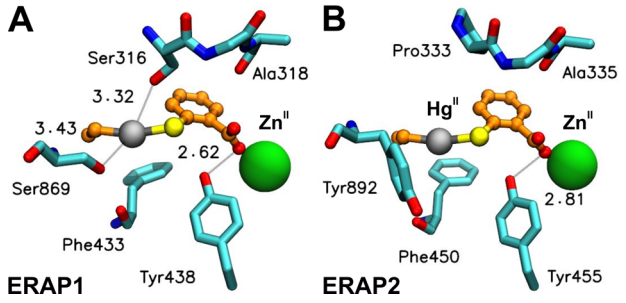


Figure 5. Putative binding mode of thimerosal at the catalytic zinc of ERAP1 (A) and ERAP2 (B). Hg(II) is shown as gray sphere, and all other atom colors are the same as those in Figure 1. Interactions between the ethylmercury moiety and the two nonconserved serine residues 316 and 869 are unique for ERAP1 and may be underlying the observed selectivity.

DOI: 10.1021/acsmedchemlett.6b00084
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

complexes with salicylate ligands, including thiosalicylic acid.[22] The complex of ERAP1 with **7** suggested two potential contacts between the mercury atom and the hydroxyl groups of Ser316 and Ser869 (Figure 5A). This type of interaction has been also observed in the X-ray structure of methylmercury complex with L-serine, where Hg(II) exhibits short intermolecular contacts with the carboxylate and the hydroxyl groups of the coordinated amino acid.[23] In contrast, such a configuration does not exhibit any contact with the corresponding residues Pro333 and Tyr892 of ERAP2 (Figure 5B). These two residues are also not conserved in IRAP or LAP, an observation that could explain the high selectivity of thimerosal for ERAP1.

ERAP1 has been described as a key enzyme for the generation of antigenic peptides in both direct and cross-presentation.[1,24] Cross-presentation by dendritic cells can proceed by at least two separate biochemical pathways, only one of which is dependent on ERAP1 enzymatic activity.[25]

To test whether thimerosal can affect ERAP1-dependent antigen presentation in live cells we incubated ERAP$^{+/+}$ and ERAP$^{-/-}$ BMDCs (bone marrow derived dendritic cells) with a fixed dose of ovalbumin (OVA) and increasing dosages of the inhibitor and then exposed them to transgenic OT-I CD8+ T cells as described in the methods section. Antigen cross-presentation to CD8+ T cells was quantified by measuring the IL-2 secretion by OT-I T cells, reflecting their activation.[26,27] Increasing concentrations of thimerosal reduced OVA presentation by ERAP$^{+/+}$ but not ERAP$^{-/-}$ BMDCs, consistent with an ERAP1-specific effect (Figure 6A). The calculated ED_{50} for this effect was found to be 930 nM, consistent with the *in vitro* affinity of thimerosal for ERAP1. No significant toxicity was observed with the concentrations of thimerosal tested in the cross-presentation assays (Figure S5). The inactive homologue thiosalicylic acid had no dose-dependent effect on OVA cross-presentation for either ERAP$^{+/+}$ or ERAP$^{-/-}$ BMDCs (Figure 6B). We conclude that thimerosal can affect ERAP1-dependent antigen presentation in cells.

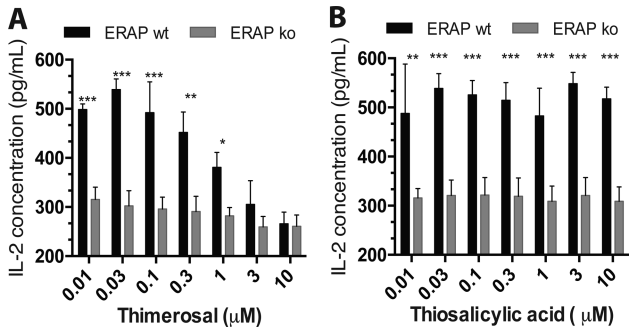


Figure 6. *In vitro* inhibitory effect of **7** (thimerosal) and inactive analogue thiosalicylic acid on cross-presentation by BMDCs. The IL-2 secretion of transgenic OT-I CD8+ T cells incubated with OVA loaded-ERAP$^{+/+}$ and ERAP$^{-/-}$ BMDCs in the presence of **7** (thimerosal) (A) or thiosalicylic acid (B) was assessed by ELISA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, as calculated by a two-way ANOVA test, with correction for multiple comparisons by the Bonferroni method. Histograms represent the mean of triplicates obtained in four independent experiments.

Thimerosal is commonly used as a preservative in vaccines because it is a potent antiseptic and antifungal agent. Although several concerns have been raised over the years regarding its safety due to its potential to release ethylmercury, a toxic substance, thimerosal is generally considered safe at the amounts used in vaccines (25−50 μg per dosage).[28,29] Still, other biological effects have been described including effects on mitochondrial respiration and the function of dendritic cells.[30,31] Our experiments indicate that thimerosal is a potent ERAP1 inhibitor and can affect cross-presentation by dendritic cells, a cell type important for vaccination responses. Although the low dosages of thimerosal in vaccines probably rule out any systemic effects, local, short-term effects on dendritic cells at the site of vaccination cannot be readily ruled out and should be further investigated.

In summary, we evaluated a collection of compounds as putative inhibitors of ERAP1, an enzyme important for adaptive immune responses and target for immunotherapy approaches.[3,4] We discovered two novel scaffolds as possible inhibitors of ERAP1, including thimerosal, a known pharmaceutical agent. Thimerosal was found to be a selective inhibitor of ERAP1 and to be able to affect antigen cross presentation by DCs. Although toxicity concerns may discourage the pharmacological use of thimerosal as an ERAP1 inhibitor, its strong selectivity profile and the effective utilization of unique structural elements in the active site of ERAP1 may constitute a valuable starting point for the development of potent and selective ERAP1 inhibitors that are currently lacking from the literature.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.6b00084.

Detailed description of computational and biochemical methods; collection of libraries screened; description of the 3D pharmacophore query; compounds used in this study and *in vitro* screening results; illustration of the predicted ERAP1−**6** complex; selectivity versus ERAP2 and IRAP; the effect of **7** on the activity of LAP; the toxicity of **7** on BMDCs; and supporting references (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: stratos@rrp.demokritos.gr or stratikos@gmail.com.

Author Contributions

‡A.S. and A.P. contributed equally to this work. A.S. performed all enzymatic experiments and analyzed data. A.P. designed and performed the virtual library screen, compound selection, and structural analysis. F.X.M. and P.v.E. designed and implemented the cross-presentation assay. E.S. supervised the project, analyzed data, and interpreted results. The manuscript was written through contributions of all authors, and all authors have given approval to the final version of the manuscript.

Funding

This research was funded by the European Union (EU Social Fund) and Greek National funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework: Research Funding Program of the General Secretariat for Research and Technology (Grant ERC-14 to E.S.). Funding was also provided by grants DEQ20130326539 from the Fondation pour la Recherche Médicale and ANR-14-CE11-0014 from the Agence Nationale de Recherche to P.v.E.

DOI: 10.1021/acsmedchemlett.6b00084
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

ER, endoplasmic reticulum; MHC, major histocompatibility molecules; ERAP1&2, endoplasmic reticulum aminopeptidase 1 and 2; IRAP, insulin regulated aminopeptidase; LAP, leucyl aminopeptidase; BMDCs, bone marrow derived dendritic cells; OVA, ovalbumin; IL-2, interleukin-2

■ REFERENCES

(1) Weimershaus, M.; Evnouchidou, I.; Saveanu, L.; van Endert, P. Peptidases trimming MHC class I ligands. *Curr. Opin. Immunol.* **2013**, *25*, 90−96.

(2) Evnouchidou, I.; Papakyriakou, A.; Stratikos, E. A New Role for Zn(II) Aminopeptidases: Antigenic Peptide Generation and Destruction. *Curr. Pharm. Des.* **2009**, *15*, 3656−3670.

(3) Stratikos, E. Regulating adaptive immune responses using small molecule modulators of aminopeptidases that process antigenic peptides. *Curr. Opin. Chem. Biol.* **2014**, *23C*, 1−7.

(4) Stratikos, E. Modulating antigen processing for cancer immunotherapy. *Oncoimmunology* **2014**, *3*, e27568.

(5) Cifaldi, L.; Romania, P.; Falco, M.; Lorenzi, S.; Meazza, R.; Petrini, S.; Andreani, M.; Pende, D.; Locatelli, F.; Fruci, D. ERAP1 regulates natural killer cell function by controlling the engagement of inhibitory receptors. *Cancer Res.* **2015**, *75*, 824−834.

(6) Chen, L.; Ridley, A.; Hammitzsch, A.; Al-Mossawi, M. H.; Bunting, H.; Georgiadis, D.; Chan, A.; Kollnberger, S.; Bowness, P. Silencing or inhibition of endoplasmic reticulum aminopeptidase 1 (ERAP1) suppresses free heavy chain expression and Th17 responses in ankylosing spondylitis. *Ann. Rheum. Dis.* **2016**, *75*, 916.

(7) Hattori, A.; Matsumoto, H.; Mizutani, S.; Tsujimoto, M. Molecular cloning of adipocyte-derived leucine aminopeptidase highly related to placental leucine aminopeptidase/oxytocinase. *J. Biochem.* **1999**, *125*, 931−938.

(8) Zervoudi, E.; Saridakis, E.; Birtley, J. R.; Seregin, S. S.; Reeves, E.; Kokkala, P.; Aldhamen, Y. A.; Amalfitano, A.; Mavridis, I. M.; James, E.; Georgiadis, D.; Stratikos, E. Rationally designed inhibitor targeting antigen-trimming aminopeptidases enhances antigen presentation and cytotoxic T-cell responses. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 19890−19895.

(9) Papakyriakou, A.; Zervoudi, E.; Tsoukalidou, S.; Mauvais, F. X.; Sfyroera, G.; Mastellos, D. C.; van Endert, P.; Theodorakis, E. A.; Vourloumis, D.; Stratikos, E. 3,4-Diaminobenzoic Acid Derivatives as Inhibitors of the Oxytocinase Subfamily of M1 Aminopeptidases with Immune-Regulating Properties. *J. Med. Chem.* **2015**, *58*, 1524.

(10) Kochan, G.; Krojer, T.; Harvey, D.; Fischer, R.; Chen, L.; Vollmar, M.; von Delft, F.; Kavanagh, K. L.; Brown, M. A.; Bowness, P.; Wordsworth, P.; Kessler, B. M.; Oppermann, U. Crystal structures of the endoplasmic reticulum aminopeptidase-1 (ERAP1) reveal the molecular basis for N-terminal peptide trimming. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 7745−7750.

(11) Nguyen, T. T.; Chang, S. C.; Evnouchidou, I.; York, I. A.; Zikos, C.; Rock, K. L.; Goldberg, A. L.; Stratikos, E.; Stern, L. J. Structural basis for antigenic peptide precursor processing by the endoplasmic reticulum aminopeptidase ERAP1. *Nat. Struct. Mol. Biol.* **2011**, *18*, 604−613.

(12) Knox, C.; Law, V.; Jewison, T.; Liu, P.; Ly, S.; Frolkis, A.; Pon, A.; Banco, K.; Mak, C.; Neveu, V.; Djoumbou, Y.; Eisner, R.; Guo, A. C.; Wishart, D. S. DrugBank 3.0: a comprehensive resource for 'omics' research on drugs. *Nucleic Acids Res.* **2011**, *39*, D1035−1041.

(13) Irwin, J. J.; Sterling, T.; Mysinger, M. M.; Bolstad, E. S.; Coleman, R. G. ZINC: a free tool to discover chemistry for biology. *J. Chem. Inf. Model.* **2012**, *52*, 1757−1768.

(14) Koes, D. R.; Camacho, C. J. ZINCPharmer: pharmacophore search of the ZINC database. *Nucleic Acids Res.* **2012**, *40*, W409−414.

(15) Trott, O.; Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455−461.

(16) Zervoudi, E.; Papakyriakou, A.; Georgiadou, D.; Evnouchidou, I.; Gajda, A.; Poreba, M.; Salvesen, G. S.; Drag, M.; Hattori, A.; Swevers, L.; Vourloumis, D.; Stratikos, E. Probing the S1 specificity pocket of the aminopeptidases that generate antigenic peptides. *Biochem. J.* **2011**, *435*, 411−420.

(17) Jacobsen, J. A.; Major Jourden, J. L.; Miller, M. T.; Cohen, S. M. To bind zinc or not to bind zinc: an examination of innovative approaches to improved metalloproteinase inhibition. *Biochim. Biophys. Acta, Mol. Cell Res.* **2010**, *1803*, 72−94.

(18) Georgiadou, D.; Hearn, A.; Evnouchidou, I.; Chroni, A.; Leondiadis, L.; York, I. A.; Rock, K. L.; Stratikos, E. Placental leucine aminopeptidase efficiently generates mature antigenic peptides in vitro but in patterns distinct from endoplasmic reticulum aminopeptidase 1. *J. Immunol.* **2010**, *185*, 1584−1592.

(19) Trumpler, S.; Lohmann, W.; Meermann, B.; Buscher, W.; Sperling, M.; Karst, U. Interaction of thimerosal with proteins-ethylmercury adduct formation of human serum albumin and beta-lactoglobulin A. *Metallomics* **2009**, *1*, 87−91.

(20) Birtley, J. R.; Saridakis, E.; Stratikos, E.; Mavridis, I. M. The Crystal Structure of Human Endoplasmic Reticulum Aminopeptidase 2 Reveals the Atomic Basis for Distinct Roles in Antigen Processing. *Biochemistry* **2012**, *51*, 286−295.

(21) Mpakali, A.; Saridakis, E.; Harlos, K.; Zhao, Y.; Papakyriakou, A.; Kokkala, P.; Georgiadis, D.; Stratikos, E. Crystal Structure of Insulin-Regulated Aminopeptidase with Bound Substrate Analogue Provides Insight on Antigenic Epitope Precursor Recognition and Processing. *J. Immunol.* **2015**, *195*, 2842−2851.

(22) Jacobsen, F. E.; Cohen, S. M. Using model complexes to augment and advance metalloproteinase inhibitor design. *Inorg. Chem.* **2004**, *43*, 3038−3047.

(23) Corbeil, M. C.; Beauchamp, A. L. Conformational-Changes in Serine on Methylmercury Complexation. *Can. J. Chem.* **1988**, *66*, 1379−1385.

(24) Firat, E.; Saveanu, L.; Aichele, P.; Staeheli, P.; Huai, J.; Gaedicke, S.; Nil, A.; Besin, G.; Kanzler, B.; van Endert, P.; Niedermann, G. The role of endoplasmic reticulum-associated aminopeptidase 1 in immunity to infection and in cross-presentation. *J. Immunol.* **2007**, *178*, 2241−2248.

(25) Saveanu, L.; Carroll, O.; Weimershaus, M.; Guermonprez, P.; Firat, E.; Lindo, V.; Greer, F.; Davoust, J.; Kratzer, R.; Keller, S. R.; Niedermann, G.; van Endert, P. IRAP Identifies an Endosomal Compartment Required for MHC Class I Cross-Presentation. *Science* **2009**, *325*, 213−217.

(26) Weimershaus, M.; van Endert, P. Preparation of dendritic cells by in vitro cultures. *Methods Mol. Biol.* **2013**, *960*, 351−357.

(27) Merzougui, N.; Kratzer, R.; Saveanu, L.; van Endert, P. A proteasome-dependent, TAP-independent pathway for cross-presentation of phagocytosed antigen. *EMBO Rep.* **2011**, *12*, 1257−1264.

(28) Baker, J. P. Mercury, vaccines, and autism: one controversy, three histories. *Am. J. Public Health* **2008**, *98*, 244−253.

(29) Dorea, J. G.; Farina, M.; Rocha, J. B. Toxicity of ethylmercury (and Thimerosal): a comparison with methylmercury. *J. Appl. Toxicol.* **2013**, *33*, 700−711.

(30) Goth, S. R.; Chu, R. A.; Gregg, J. P.; Cherednichenko, G.; Pessah, I. N. Uncoupling of ATP-mediated calcium signaling and dysregulated interleukin-6 secretion in dendritic cells by nanomolar thimerosal. *Environ. Health Perspect.* **2006**, *114*, 1083−1091.

(31) Sharpe, M. A.; Livingston, A. D.; Baskin, D. S. Thimerosal-Derived Ethylmercury Is a Mitochondrial Toxin in Human Astrocytes: Possible Role of Fenton Chemistry in the Oxidation and Breakage of mtDNA. *J. Toxicol.* **2012**, *2012*, 373678.

DOI: 10.1021/acsmedchemlett.6b00084
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX