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

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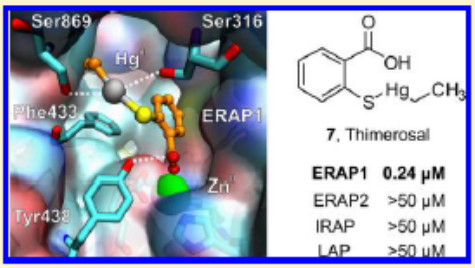
Supporting Information

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.



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.

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