Human acidic mammalian chitinase as a novel target for anti-asthma drug design using in silico screening

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ABSTRACT

Human acidic mammalian chitinase (hAMCase) was recently shown to be involved in the development of asthma, suggesting a possible application for hAMCase inhibitors as novel therapeutic agents for asthma. We therefore initiated drug discovery research into hAMCase using a combination of in silico methodologies and a hAMCase assay system. We first selected 23 candidate hAMCase inhibitors from a database of four million compounds using a multistep screening system combining Tripos Topomer Search technology, a docking calculation and two-dimensional molecular similarity analysis. We then measured hAMCase inhibitory activity of the selected compounds and identified seven compounds with IC_{50} values $\leqslant 100$ μM. A model describing the binding modes of these hit compounds to hAMCase was constructed, and we discuss the structure–activity relationships of the compounds we identified, suggested by the model and the actual inhibitory activities of the compounds.

1. Introduction

Chitinase is the enzyme that catalyzes the hydrolysis of chitin and is widely distributed in living organisms, including insects, nematodes, fungi, bacteria, plants and mammals, including humans.[1–3] Its substrate, chitin, is composed of linear polymers of β-1,4-linked N-acetyl-D-glucosamine (GluNAc) and is a major component of invertebrates. Chitin synthesis and metabolism are essential for the maintenance of invertebrate life;[4] as chitin forms, for example, the exoskeleton of insects and arthropods, the periostracum coating the shells of mollusks, the vesicular wall of protozoa, and the cell wall of fungi. Elevated chitinase activity has been demonstrated in the molting fluid and integument of insects during molting[5] and in the epidermis of nematodes during hatching.[6] Furthermore, fungal chitinase has been reported to modify chitin during morphogenesis.[7]

As mammals, including humans, lack chitin, it was believed that mammalian chitinases act as a protective mechanism against pathogenic organisms which are composed of chitin. In 2004, however, large amounts of acidic chitinase were observed in the lungs of mice used in an asthma model and in the lungs of asthmatic patients. Furthermore, a chitinase inhibitor was found to alleviate inflammation in the mouse model of asthma.[8] Since these findings suggested the involvement of human acidic mammalian chitinase (hAMCase) in the development of asthma, the possible application

of hAMCase inhibitors as novel therapeutic agents for asthma has been considered.

Until now, three natural products with potent inhibitory activity for chitinase, allosamidin,[9] argadin[10] and argifin,[11] have been widely used in chitinase research. However, allosamidin has a complex carbohydrate structure and its synthesis and derivatization is difficult. Although argadin and argifin are peptide compounds and their synthesis is relatively easy, their structures are not conducive to Absorption, Distribution, Metabolism, and Excretion (ADME) studies. Therefore, these natural products have been considered poor lead compounds for drug discovery research.

We have therefore been carrying out exploratory research into novel hAMCase inhibitors, using Oprea's concept of leadlikeness scores,[12] to identify lead compounds for drug discovery research, based on the results of an in silico drug discovery technique and a hAMCase assay system. In addition, we have analyzed binding modes between hit compounds and hAMCase using molecular docking calculations, and we discuss here the inhibitory activity of hit compounds in light of their structural interactions with hAMCase.

2. Methods

2.1. Preparation of protein structure for molecular docking calculation

At the beginning of this study, the Protein Data Bank (PDB) contained two crystal structures for hAMCase, PDB ID: 3FY1, which

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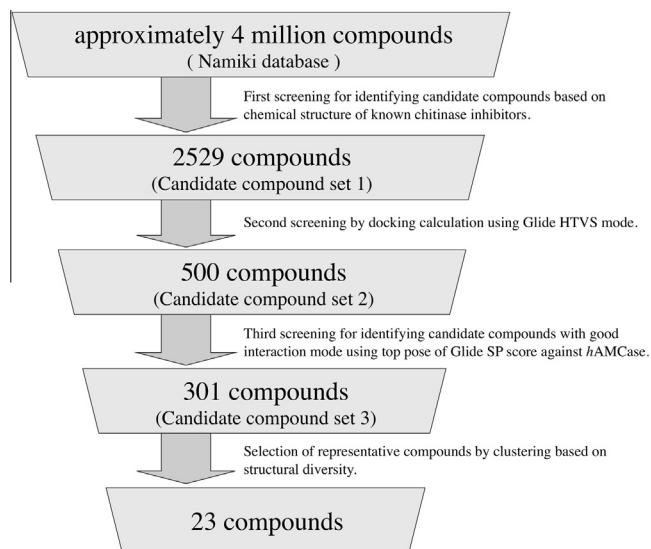E-mail address: hironos@pharm.kitasato-u.ac.jp (S. Hirono).

Figure 1. In silico screening procedure.

Figure 3. Correlation analysis for hAMCase inhibitory activity values (ln IC$_{50}$) and scores from Glide using HTVS mode for the four known low molecular-weight inhibitors (arrowed).

included two different coordinates (A chain and B chain) as a complex with methylallosamidin (holo structure), and PDB ID: 3FXY, which included four different coordinates (A, B, C, and D chains) with no ligand (apo structure).[13] We used all six of these coordinates in a cluster analysis of the conformation of the hAMCase active site, by calculating pairwise root-mean-square-deviations (RMSDs) for the amino acid residues around the active site using the conformer cluster module of MacroModel 9.9. From the results obtained, we determined the representative structure of hAMCase that we then used for molecular docking calculations.

2.2. In silico screening

Figure 1 shows the multistep in silico screening procedure used in this study. First, compounds with a similar functional group to the pharmacophore of argifin were retrieved from the Namiki chemical compound database, containing approximately four million compounds, provided by Namiki Shoji Co. Ltd (Tokyo, Japan). This was carried out using the Topomer Search module in SYB-YL8.1.[14] The Topomer Search is a 3D ligand-based virtual screening tool, which use one already-known inhibitor as a query molecule to search 'hit molecules' that exhibit similar three-dimensional

shapes. The similarity between a query molecule and a molecule from chemical database is estimated by comparing phamacophoric features and the shape similarity of the associated Topomers. Next, compounds fulfilling Oprea's criteria (number of hydrogen-bonding donors <5, number of hydrogen-bonding acceptors <8, molecular weight <450, log$P$ <4.5, number of cyclic groups <4, number of rotatable bonds <10) were selected as the primary candidate compound set.

After generating the conformation of the primary candidate compound set using LigPrep2.2 from the Schrödinger Suite 2008, we performed a high throughput docking calculation against the representative structure of hAMCase, using the HTVS mode of Glide 5.0 docking software. The top 500 compounds ranked by their Glide scores were selected as the secondary candidate compound set.

Finally, we selected those compounds to be purchased using a docking calculation with the SP mode of Glide 5.0, which is more precise than the HTVS mode, and by a molecular similarity analysis based on a two-dimensional (2D) fingerprint. In this approach, we first generated a conformer set for each compound in the
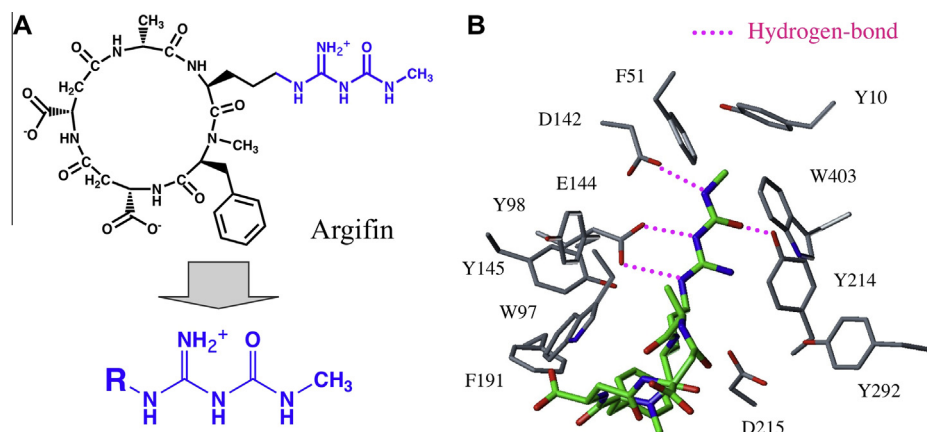

Figure 2. (A) Query structure based on argifin used in the identification of candidate compounds. (B) The crystal structure of argifin in 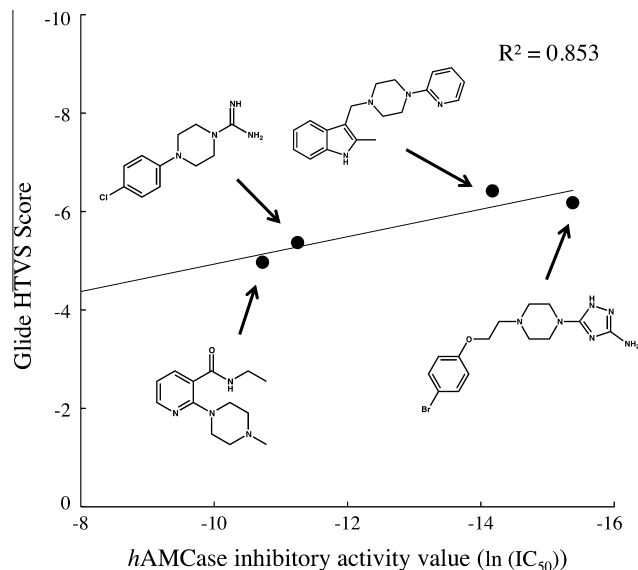complex with SmChiB (PDB ID: 1h0i).