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Identification of novel ASK1 inhibitors using virtual screening

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ABSTRACT

Apoptosis signal-regulating kinase 1 (ASK1, also called MAP3K5) is a mitogen-activated protein kinase kinase kinase (MAP3K) that plays important roles in stress-induced cell death and inflammation, and is expected as a new therapeutic target for cancer, cardiovascular diseases, and neurodegenerative diseases. We identified novel ASK1 inhibitors by virtual screening from the public chemical library collected by Chemical Biology Research Initiative (CBRI) at the University of Tokyo.

1. Introduction

Apoptosis signal-regulating kinase 1 (ASK1, also called MAP3K5) is a member of the mitogen-activated protein (MAP) kinase kinase kinase (MAP3K) family that activates the c-Jun N-terminal kinase (JNK) and p38 MAP kinase signaling cascades.[1] ASK1 has been reported to be involved in cell death and inflammation induced by various types of stressors including oxidative stress, endoplasmic reticulum (ER) stress, and cytotoxic cytokines.[2] Recently, ASK family proteins have been reported to play key roles in cancer, cardiac diseases and neurodegenerative diseases, and others.[3]

In the case of cancer, ASK1 has appeared to be required for the production of inflammatory cytokines such as TNF-α and IL-6 induced by treatment with TPA (12-*O*-tetradecanoylphorbol-13-acetate) in the skin tumorigenesis model, so ASK1 functions as a tumor promoter in the promotion stage by inducing inflammation.[4] Regarding cardiac diseases, cardiac hypertrophy induced by Angiotensin II (AngII), the central product of the rennin–angiotensin system was significantly suppressed in ASK1-deficient mice. These data suggest that ASK1 plays an important role in AngII signaling, leading to cardiac hypertrophy.[5] Concerning neurodegenerative diseases, it has been suggested that ASK1 may contribute to the pathology of Alzheimer's diseases, polyglutamine disease and familial amyotrophic lateral sclerosis (ALS) through the induction of neuronal cell death. Thus, ASK1 has the potential to become a therapeutic target for these neurodegenerative diseases.[2] Accordingly, ASK1 inhibitors are expected to be utilized as a new chemotherapeutic approach for the aforementioned diseases. However, there have been few reports about ASK1 inhibitors.

In the past several years, we have attempted to find various kinds of biologically active compounds from a commercially available compound database using virtual screening and have reported successful results.[6] Furthermore, this public chemical library has been collected and supplied by the Chemical Biology Research Initiative (CBRI)[7] at the University of Tokyo in Japan since 2007. We also used this chemical library and virtual screening approach (Fig. 1), and novel ASK1 inhibitors were found.

2. Results and discussion

2.1. Ligand-based virtual screening

ASK1 inhibitors containing a fused heterocyclic ring have been reported by Takeda Pharmaceutical Co., Ltd.[8] Since the inhibitory activities of these compounds are very high, we used them as queries for a similarity search.

Three compounds (IC_{50} = 14–19 nM) selected as queries are shown in Table 1, and we carried out a similarity search against the public chemical library of CBRI (CBRI Library) to find novel inhibitors possessing new scaffolds. We first calculated a fingerprint based on the descriptors of the BIT_MACCS and then carried out a similarity search using three compounds. Through these steps and visual inspection, we selected 248 compounds.

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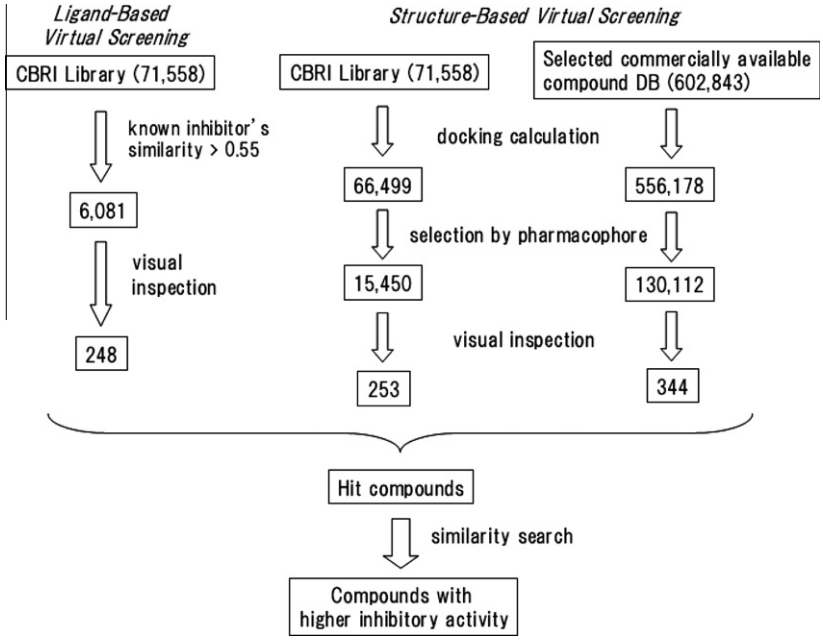
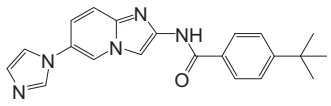
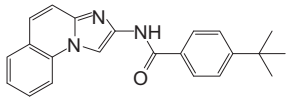
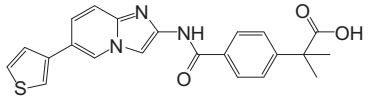


Figure 1. Virtual screening protocol; ligand-based virtual screening and structure-based virtual screening.

Table 1
Compounds as queries for similarity search

Compound	Structure	IC_{50} (nM)
1		14
2		15
3		19

These compounds selected by similarity search were tested in an ASK1 inhibition assay, and eight compounds showed appreciable inhibition at 50 μM. Four of these eight compounds exhibited greater than 30% inhibition at this concentration (Table 2).

2.2. Structure-based virtual screening

As reported previously,[6] we usually use a protein–ligand complex model built by molecular-dynamics simulation for more efficient structure-based virtual screening. In this case, we used X-ray crystal structure (PDB code: 2CLQ) and compound **2** to build the protein–ligand complex model (Fig. 2). In this binding mode, the main chain of Val757 made two hydrogen bonds with compound **2**. Using this complex model, we conducted structure-based virtual screening using DOCK4[9] against the CBRI Library and commercially available compound database.[10] At the time we carried out these screenings, the CBRI Library contained 71,558 compounds. To compensate for the numbers of compounds for structure-based virtual screening, we also screened against a commercially available compound database. After docking calculation, we selected compounds by pharmacophore which could possibly make hydrogen bonds with the hinge region of the ATP binding site.

Through structure-based virtual screening as shown in Figure 1, we selected 253 compounds from CBRI Library and 344 compounds from the commercially available compound database. Using these compounds, we performed an ASK1 inhibition assay. As a result, five compounds from the CBRI Library and seven compounds from the commercially available compound database exhibited greater than 30% inhibition at 50 μM, as shown in Table 2. These compounds' inhibitory activity was still very low, so we conducted a subsequent similarity search using these compounds as queries to obtain compounds with higher inhibitory activity.

2.3. Second screening (similarity search)

Using the above hit compounds as queries, we carried out similarity search with BIT_MACCS fingerprint. As a result, we were able to obtain five compounds with higher inhibitory activity (Table 3, % inhibition @ 10 μM > 50).

In these compounds, the purine scaffold is conspicuous. It is likely possible to replace the various building blocks which bind to the purine ring. Predicted binding modes are shown in Figure 3; the purine ring may make hydrogen bonds with the hinge region of the ASK1 kinase domain, and there is CH–π interaction in the purine ring and a side chain of Leu810. Such interactions would stabilize the protein–ligand binding mode.

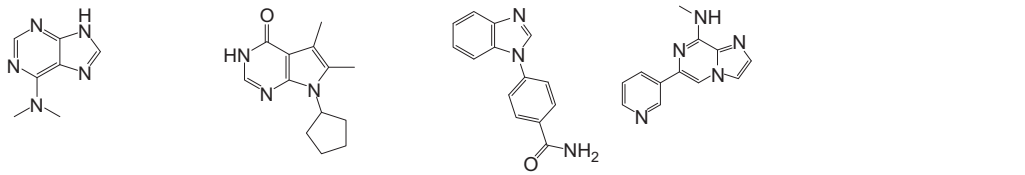
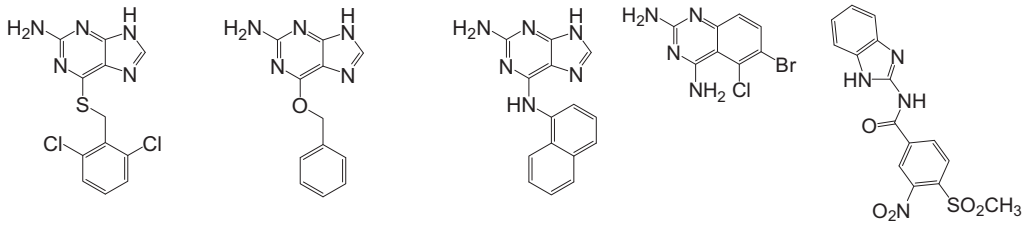
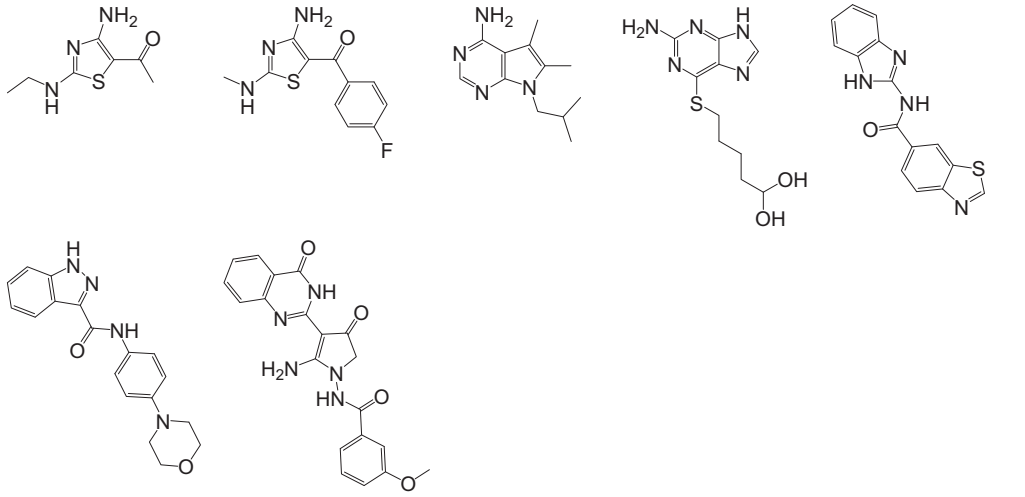
Furthermore, the IC_{50} values of compounds in Table 3 were determined (Table 4). In these compounds, compound **4** had the most potent inhibitory activity (IC_{50} = 13.3 μM).

Compounds other then purine derivatives also have novel scaffolds for ASK1 inhibitors. Consequently, we were successful in finding novel ASK1 inhibitors using information about known inhibitors and a virtual screening approach.

3. Conclusion

Using virtual screening, such as ligand-based virtual screening and structure-based virtual screening, we identified novel ASK1 inhibitors from the CBRI Library and a commercially available compound database. Importantly, these compounds have new scaffolds for ASK1 inhibitors. Since the inhibitory activities of these

Table 2
Hit compounds of ligand-based virtual screening and structure-based virtual screening (% inhibition @ 50 μM > 30)

Methods	Hit compounds
LBDD from CBRI Library	
SBDD from CBRI Library	
SBDD from commercially available compound database	

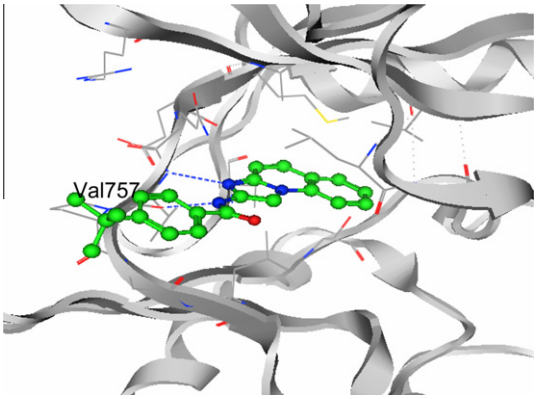


Figure 2. Protein–ligand complex model. In this model, compound **2** was used as a ligand.

compounds are still lower than we expected, we would like to further develop this search to seek more potent ASK1 inhibitors.

The results described in this paper indicate that our virtual screening is very efficient for finding a novel scaffold on a target protein's active compounds. Furthermore, at present, the CBRI Library contains approximately 200,000 compounds, such as those commercially available and owned in various Japanese universities, thus making it more and more useful for seeking compounds which suit individual purposes.

4. Experimental

4.1. Compounds

Tested compounds in this paper were purchased from several suppliers. Their characterizations and purity were confirmed using LC–MS and HPLC.

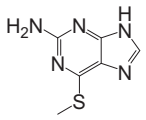
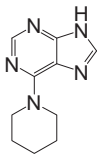
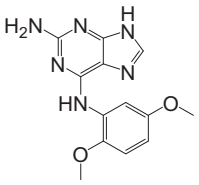
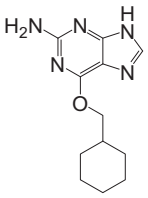
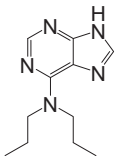
4.2. Similarity search

We calculated a fingerprint based on the descriptors of the BIT_MACCS: MACCS structural keys for all compounds in CBRI Library, and conducted a similarity search using MOE[11] with compounds **1**–**3** as queries. Then, we selected compounds with similarity more than 0.55 to compounds **1**–**3**, respectively, and chose compounds for in vitro assay by visual inspection.

4.3. Protein–ligand complex model

The protein–ligand complex model was built using molecular-dynamics (MD) simulation by MOE. In the MD calculations, the system was gradually heated to 300 K, and an additional 1 ns simulation at constant temperature and volume (NVT ensemble, NPA algorithm) was carried out. The equilibrated system was slowly cooled to 0 K and then energy-minimized.

Table 3
Compounds with high inhibitory activity in 2nd screening (% inhibition @ 10 μM > 50)

Compounds with high inhibitory activity				
4	**5**	**6**	**7**	**8**
				

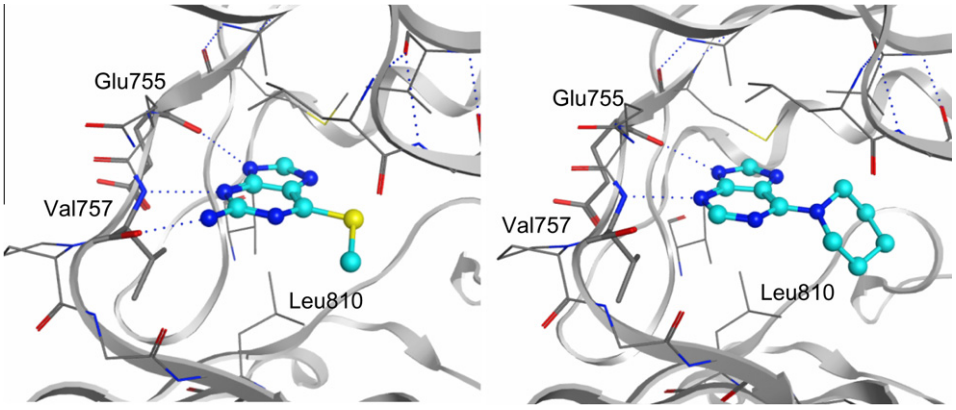


Figure 3. Predicted binding mode of two active compounds in ASK1 inhibitory assay. Purine ring may make two hydrogen bonds with hinge region, and there is CH–π interaction in the purine ring and a side chain of Leu810. These interactions are thought to stabilize the protein–ligand binding state.

Table 4
IC_{50} values of compounds in Table 3

Compound	IC_{50} (μM)
4	13.3
5	17.0
6	18.9
7	25.1
8	25.8

4.4. Docking calculation

We conducted docking calculation using DOCK4[9] for structure-based virtual screening. For more efficient screening, we used small-scale compound database (approximately a 1000-compound testset database) initially to determine the most appropriate protein–ligand complex model built by MD simulation. These 1000 compounds were selected randomly from the commercially available compound database. The small scale testset database has these 1000 compounds and several known ASK1 inhibitors. By docking calculation using this testset database, we compared the enrichment of known ASK1 inhibitors among representative protein–ligand complex models built by MD simulation. Thus, we decided to adopt a protein–ligand complex model (Fig. 2) for large-scale virtual screening using CBRI Library and selected commercially available compound database.

4.5. Kinase assay

Activity was measured by mobility shift assay. The reaction mixture containing 1.5 μM fluorescent peptide, FAM-GNTGTYTKK-NH2 (Toray Research Center, Tokyo, Japan), 200 μM ATP, 1.67 μg/ml ASK1 (Carna Biosciences, Kobe, Japan), 8 mM MOPS (pH 7.0), 10 mM $MgCl_2$, 1 mM DTT, 1% Protease Inhibitor Cocktail Set V (Merck Darmstat, Germany), 1% Phosphatase Inhibitor Cocktail Set III (Merck), 0.01% Brij-35, and a test compound (5% DMSO) was incubated at room temperature for 60 min and the reaction was stopped by adding 140 mM EDTA. The phosphorylated and unphosphorylated peptides were separated and detected by LabChip EZ Reader II (Caliper Life Science, Hopkinton, MA).

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