



Identification of New Potential APE1 Inhibitors by Pharmacophore Modeling and Molecular Docking

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Apurinic/aprimidinic endonuclease 1 (APE1) is an enzyme responsible for the initial step in the base excision repair pathway and is known to be a potential drug target for treating cancers, because its expression is associated with resistance to DNA-damaging anticancer agents. Although several inhibitors already have been identified, the identification of novel kinds of potential inhibitors of APE1 could provide a seed for the development of improved anticancer drugs. For this purpose, we first classified known inhibitors of APE1. According to the classification, we constructed two distinct pharmacophore models. We screened more than 3 million lead-like compounds using the pharmacophores. Hits that fulfilled the features of the pharmacophore models were identified. In addition to the pharmacophore screen, we carried out molecular docking to prioritize hits. Based on these processes, we ultimately identified 1,338 potential inhibitors of APE1 with predicted binding affinities to the enzyme.

Keywords: APE1, molecular docking simulation, pharmacophore modeling, virtual screening

Introduction

DNA damage occurs naturally and due to the environment, altering the cell's abilities that are encoded by the DNA, and may lead to diseases, like cancer. Cells respond to DNA damage by DNA repair and cellular apoptosis [1, 2]. Apurinic/aprimidinic endonuclease (APE) is an enzyme that identifies damaged apurinic/aprimidinic sites in DNA, cuts the phosphodiester bond in the backbone of the sites, and has critical roles in the base excision pathway [3]. APE1 has recently been noted as a potential drug target for treating cancer, in that overexpression of the enzyme has been observed and shown to be associated with a poor response to cancer treatment, such as radiation and anticancer drugs, and a lower overall survival rate [4-7]. Antineoplastic agents that are to treat cancers are known to induce the expression of APE1, increasing the resistance of tumor cells to drug treatment. Thus, compounds that inhibit the activity of APE1 could be potential anticancer drugs with DNA-damaging antineoplastic agents used in the clinic [8].

For this reason, there have been several attempts to develop compounds targeting APE1. Currently, although there is no approved drug yet, three candidates—7-nitroindole-2-carboxylic acid (also known as CRT0044876) [9, 10], lucanthone (also known as Miracil D) [9], and methoxyamine (trademark TRC102)—are known to inhibit APE1 activity and are under examination in clinical trials. Lucanthone and CRT0044876 have rings similar to the deoxyribose sugar ring without a base and many hydrogen bond acceptors that can interact with hydrogen bond donors in the active site of APE1. These properties lead APE1 to stick in the site and prevent it from repairing DNA damage [11]. Methoxyamine is known to attack the open-ring form of AP sites to form an oxime linkage. In other words, methoxyamine blocks APE1 from accessing the lesion site rather than targeting the enzyme directly. This may lead to non-specific off-target effects [12, 13]. Although several inhibitors of APE1 have been discovered, most potent compounds have weaknesses [14]. Thus, it is necessary to find novel kinds of potential inhibitors targeting APE1. Here, we present our work, in which we applied pharmacophore modeling and

Received November 27, 2017; Revised December 1, 2017; Accepted December 1, 2017

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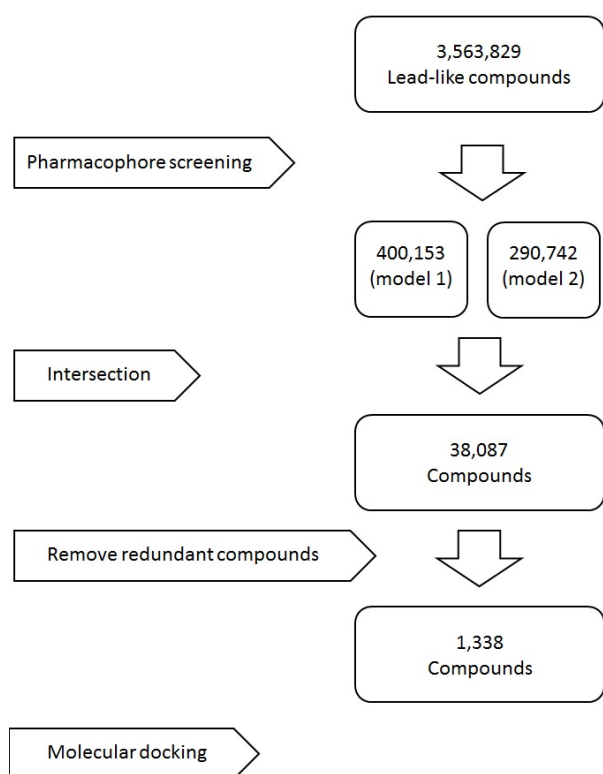


Fig. 1. Outline of overall procedures of the screen to find potential inhibitors of apurinic/aprimidinic endonuclease 1.

virtual screening. The overall procedures we carried out are illustrated in Fig. 1. We constructed pharmacophore models by capturing the common features of known inhibitors of APE1. The models were used to screen a vast number of lead-like compounds, and molecular docking was used to prioritize the hits of the screen.

Methods

Selection of ligands for pharmacophore modeling

From the ChEMBL [15] database, we retrieved 52 compounds known to be targets of APE1 and 51 compounds with an IC_{50} of less than $10 \mu M$. By eliminating redundancy, the number of compounds was reduced into 83. The list did not contain methoxyamine; so, methoxyamine was also added to the list.

We clustered these 84 compounds by Tanimoto distance, based on the PubChem fingerprint [16], and finally categorized them into two groups by excluding two outliers (CHEMBL1213633 and CHEMBL313493) (Fig. 2). A total of 49 molecules in group 1 (Fig. 3) and 33 molecules in group 2 (Fig. 4) were used to generate pharmacophore models 1 and 2, respectively.

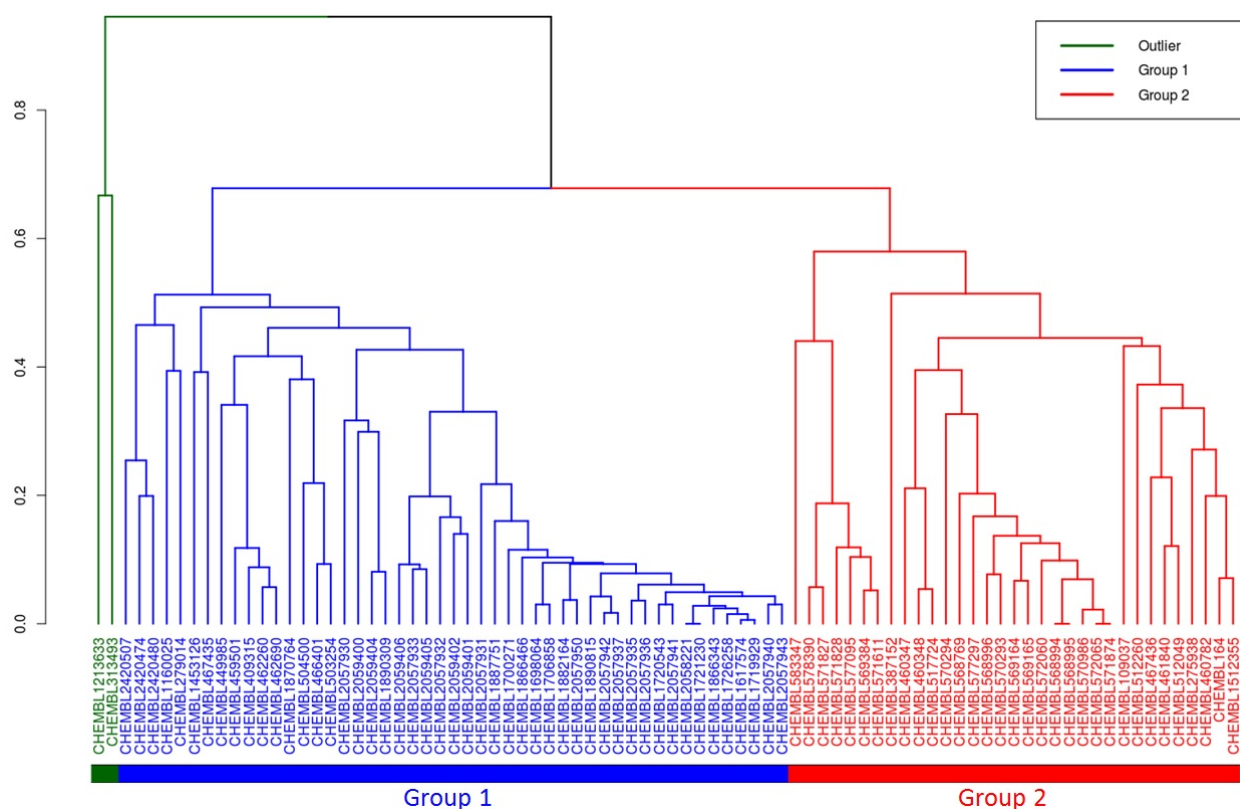


Fig. 2. Clustering of molecules for construction of pharmacophore models.

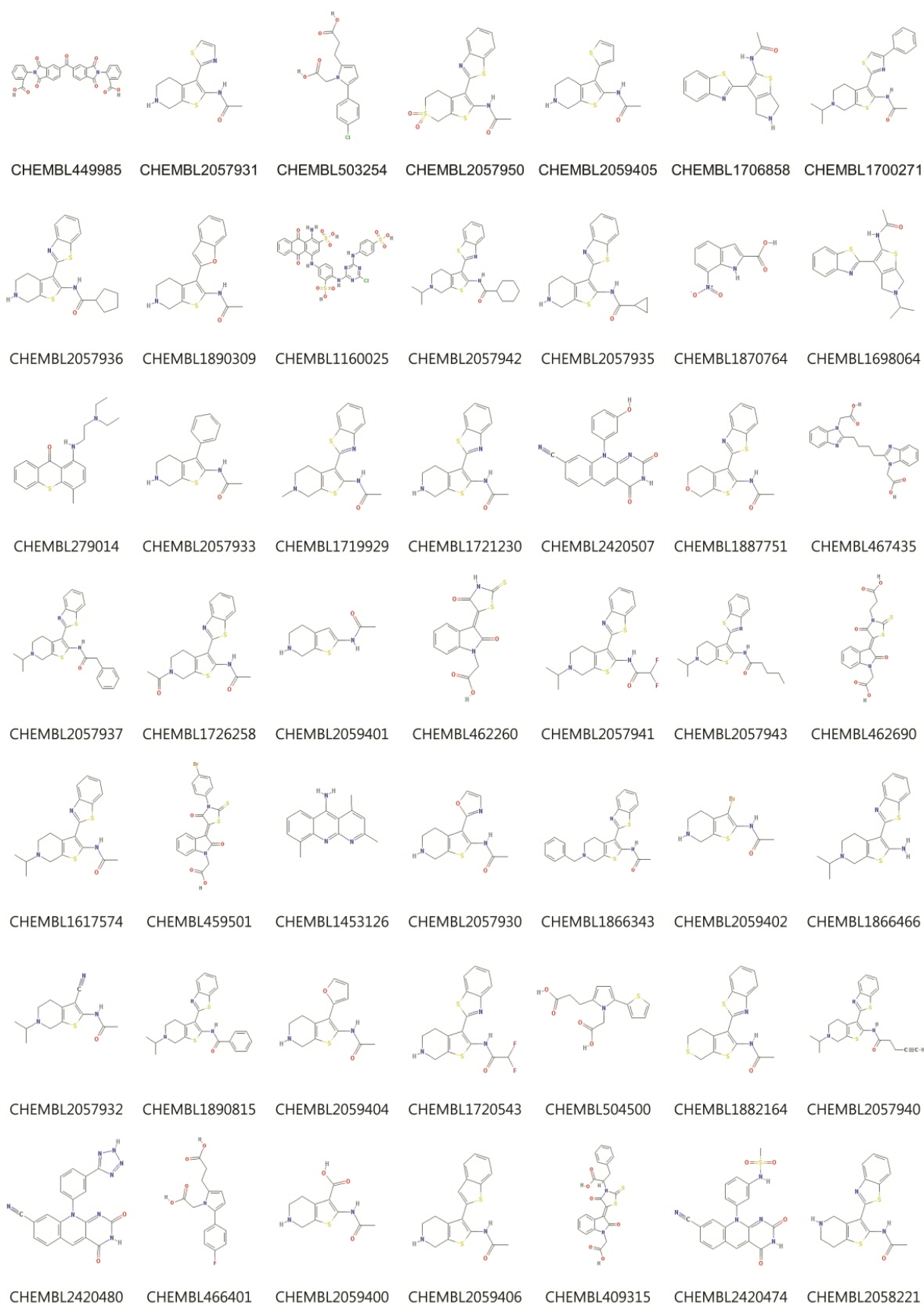


Fig. 3. Structures of the 49 compounds in group 1 used to generate pharmacophore model 1.

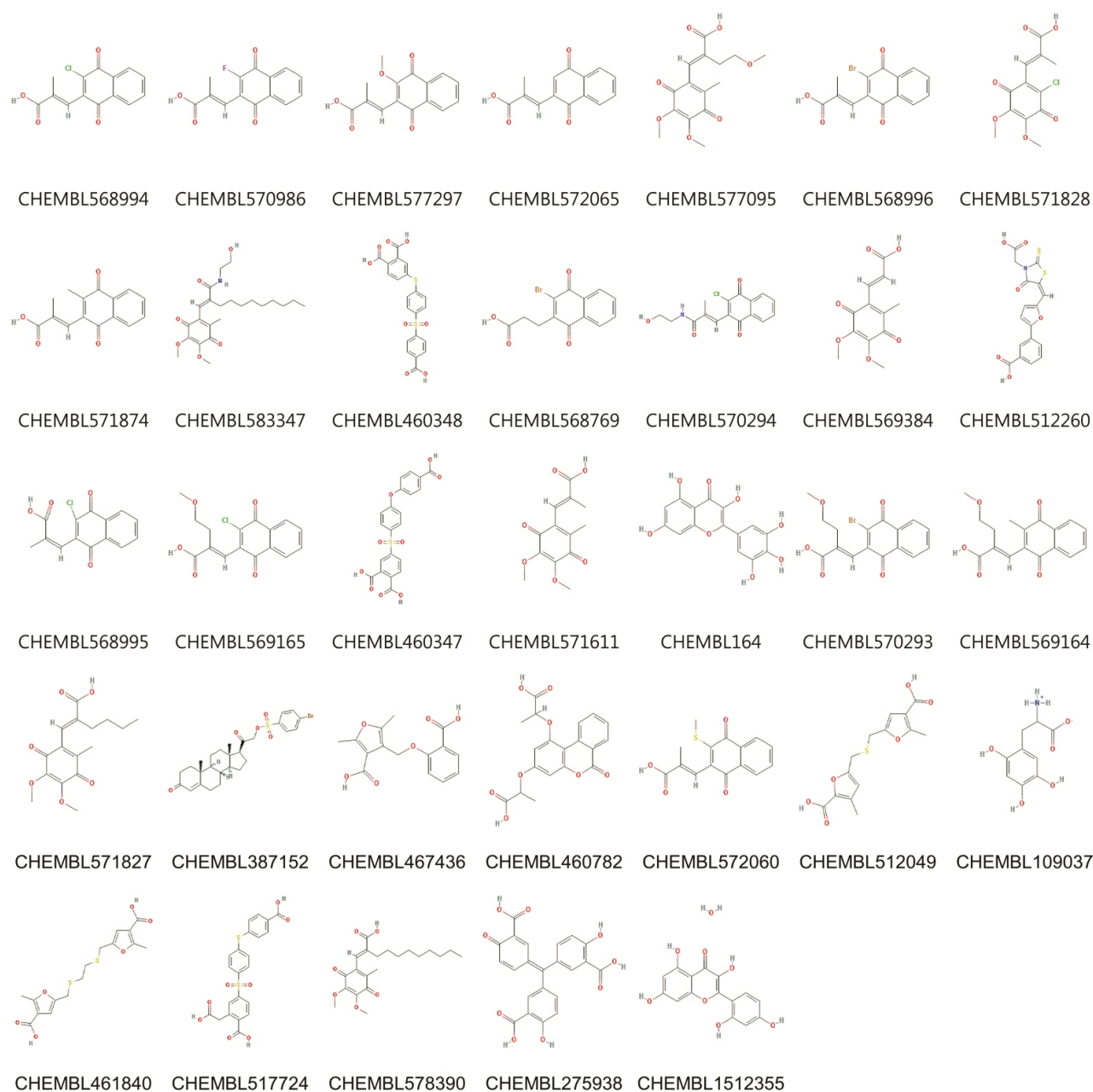


Fig. 4. Structures of the 33 compounds in group 2 used to generate pharmacophore model 2.

Generation of pharmacophore model

Ligandscout tools 4.1 [17] was used to generate the ligand-based pharmacophore models. Ligandscout is known to be able to increase the selectivity of a pharmacophore model with the excluded volume feature. To generate more flexible pharmacophore, the threshold of the portion of partially matching features was set to 20%.

Pharmacophore screen

For the initial set of the pharmacophore screen, we selected a lead-like subset [18], defined by the ZINC database (ZINC is not commercial) [19]. Similar to druglikeness [20], like Lipinski's rule of 5 [21], lead-like compounds are defined as being large enough to be validated in experiments but are smaller than most drugs, optimized too specifically, and more soluble than their drug-like compounds. ZINC provides a lead-like subset fulfilling leadlikeness as follows: (1) molecular weight between 250 and 350 Da,

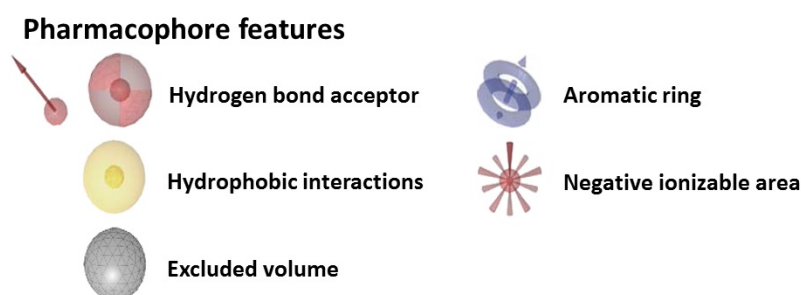
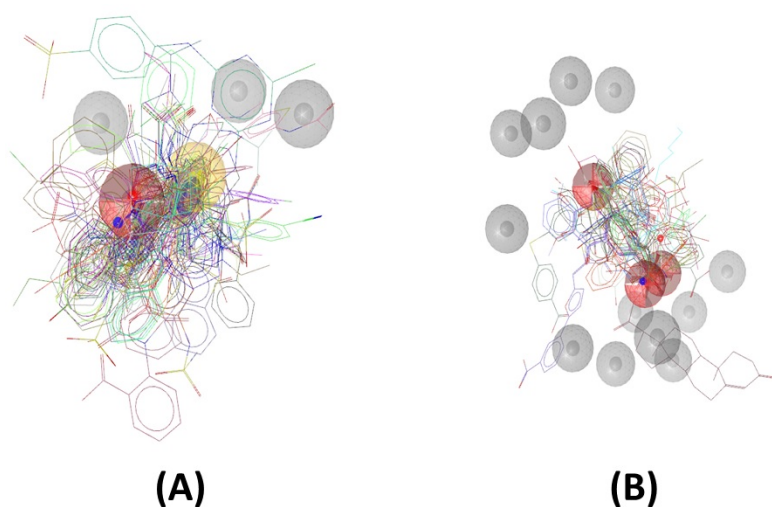


Fig. 5. Pharmacophore models used for screening. Models were generated by compounds in group 1 (A) and group 2 (B).

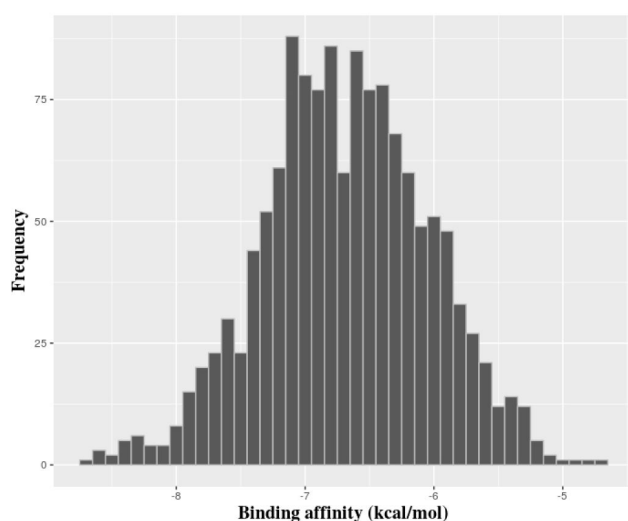


Fig. 6. Distribution of predicted binding affinities by molecular docking of potential inhibitors to apurinic/aprimidinic endonuclease 1.

(2) partition coefficient $\log p \leq 3.5$, and (3) no more than seven rotatable bonds. The structures of lead-like compounds in medium pH were downloaded and converted into a database for screening by *idbgen*, a component of Ligand-

scout. We carried out pharmacophore screens using *iscreen* in LigandScout for models 1 and 2 independently. Pharmacophore fit scores were also calculated by LigandScout based on the number of matching pharmacophore features and the root-mean-square deviation of the pharmacophore alignment.

Molecule docking simulation

To prioritize the hits of the pharmacophore screen, we docked the hits against previously determined structures of APE1 [22] (PDB ID: 1DEW) using AutoDock Vina [23]. A binding site of APE1 was assigned using the fpocket algorithm [24].

Results and Discussion

A total of 84 compounds from the ChEMBL database were first collected to generate a pharmacophore, but their structures and properties were too heterogeneous to get common features. Thus, we carried out clustering and categorized the compounds into two groups (Figs. 2–4). For each group of compounds, we generated the corresponding pharmacophore model. Pharmacophore model 1 was generated by 49 compounds from group 1. The model was

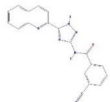


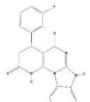
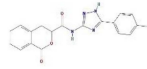
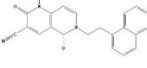
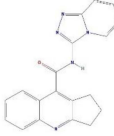

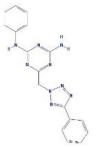
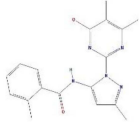
Structure	PubChem CID	Pharmacophore score 1	Pharmacophore score 2	Binding affinity (kcal/mol)
	53580594	41.00639	43.377747	-8.7
ZINC69565400				
	51603501, 28611523, 25248999	40.193176	44.288307	-8.6
ZINC20086366				
	4909512, 40928637	40.654366	43.33812	-8.6
ZINC09087097				
	5299120, 7305393	40.906654	44.409916	-8.6
ZINC04624380				
	17568456, 25987655	40.984146	42.76821	-8.5
ZINC14198982				
	56712240	39.836067	43.321037	-8.5
ZINC67676245				
	51113769	40.194237	43.284782	-8.4
ZINC46083122				
	843225, 3300507	41.150127	44.36736	-8.4
ZINC18161915				
	17448744	41.15087	43.60126	-8.4
ZINC12533962				
	51430633, 8516743	39.675964	43.53222	-8.4
ZINC07420850				

Fig. 7. Structures and scores of the screen of the top 10 hits.

composed of four features (one hydrophobic centroid, one aromatic ring, two hydrogen acceptors) and three exclusion volume spaces (Fig. 5A). Model 2 was generated by 33 compounds from group 2. The model was composed of four features (one negative ionizable and three hydrogen bond acceptors) and 12 exclusion volume spaces (Fig. 5B).

For 3,563,829 lead-like compounds retrieved from the ZINC database, we performed a pharmacophore screen based on pharmacophore models 1 and 2 independently. Among multiple subsets provided by ZINC, we chose the lead-like subset, not the drug-like set, because we aimed to provide a list of potential hits that could be optimized further by other groups, as well as our group.

As a result, 400,153 and 290,742 hits fulfilled the features of models 1 and 2, respectively. The intersection of the two lists of hits, which fulfilled all features of both models, consisted of 38,087 compounds. To remove structurally similar compounds, we clustered the 38,087 hits by hierarchical clustering, based on the Tanimoto distance in PubChem Fingerprint. According to the result of the clustering, we ruled out redundant compounds that had similar compounds (Tanimoto coefficient >0.8). Thus, 1,338 hits eventually remained as potential inhibitors of APE1.

We carried out molecular docking of the hits against APE1 to prioritize the hits using AutoDock Vina. Fig. 6 depicts the distribution of the predicted binding energies of the hits of the pharmacophore screen by docking. After molecular docking, we did not filter out compounds based on a particular threshold of the predicted value of the binding affinity but instead provide the top 10 hits in Fig. 7, their predicted binding poses in Supplementary Fig. 1, and all of the hits in Supplementary Table 1. This is because although Shityakov and Förster [25] reported that a compound having a binding affinity predicted by AutoDock Vina of lower than -6 kcal/mol could be considered an active hit, the values are only predictive and rely on a somewhat empirical energy

function. In other words, predicted binding affinities should be used restrictively to help those who want to validate hits to determine the priority of subjects of an assay. Fig. 8 shows the alignments of the best hits into each pharmacophore model; all of the hits map well to the pharmacophore models. Of note, the rank of the docking results does not mean pharmacophore fitness, and all of the inhibitor compounds we found here can be mapped to the models well. The figure of pharmacophore alignment was made to provide an example showing that our hits can be mapped properly.

In summary, we screened more than 3 million lead-like compounds by pharmacophore modeling, and 1,338 hits were suggested to be potential inhibitors of APE1. However, this work has a limitation, due to the lack of experimental validation. Nevertheless, the list of hits in this work could reduce the time and cost of researchers who want to develop novel anticancer drugs inhibiting the activity of APE1, since we prioritized candidates of the experiments and since all of them have lead-like properties, which means that the hits are appropriate for further optimization and development into drugs.

Currently, there are several approaches that apply hits from a pharmacophore screen for further development in to a novel drug. Fei *et al.* [26] first developed a pharmacophore model of a drug target, like our method; then, 3D-quantitative structure-activity relationship (QSAR) modeling was used for validation and further virtual screening. Wieder *et al.* [27] proposed a novel approach combining pharmacophore modeling and molecular dynamics (MD) simulations, and they showed that their methods were likely to result in more robust hits. Like these approaches, the results from pharmacophore modeling could be adopted in other *in silico* methods, such as molecular docking, QSAR modeling, and MD simulation. It is worth combining these methods and our results to get more robust results. If further integrative approaches and *in vitro* or *vivo* assays of hits validate our

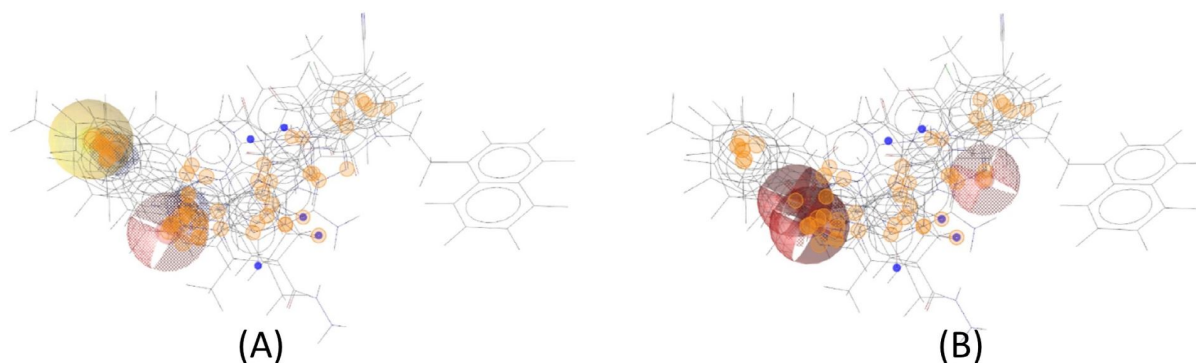


Fig. 8. Mapping of top 10 hits to pharmacophore models. (A) Model 1. (B) Model 2.

results, our method could be applied to other drug targets, in addition to APE1.

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Authors' contribution

Conceptualization: ML

Data curation: IWL

Formal analysis: IWL, ML

Funding acquisition: ML

Methodology: IWL

Writing – original draft: IWL, JY, GL, ML

Writing – review and editing: ML

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (NRF-2017R1C1B2008617 and NRF-2017-M3A9B6061511), KREONET (Korea Research Environment Open NETWORK) which is managed and operated by KISTI (Korea Institute of Science and Technology Information). In Won Lee was supported by a Sangji University scholarship for research assistants.

Supplementary materials

Supplementary data including one table and one figure can be found with this article online at <http://www.genominfo.org/src/sm/gni-15-147-s001.pdf>.

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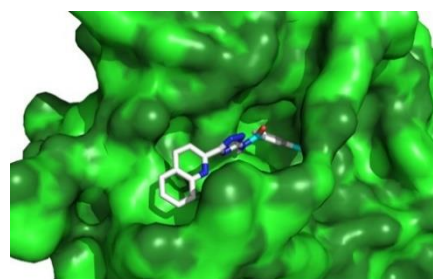
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SUPPLEMENTARY INFORMATION

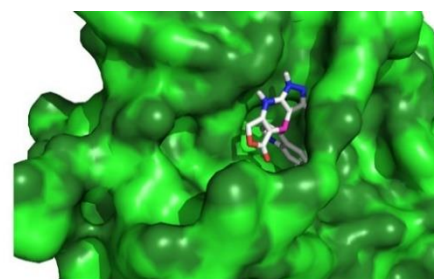
Identification of New Potential APE1 Inhibitors by Pharmacophore Modeling and Molecular Docking

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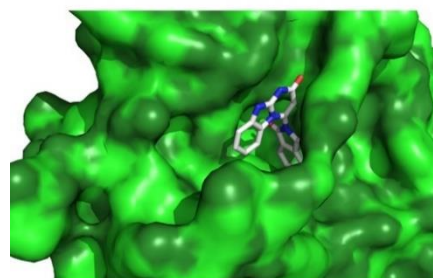
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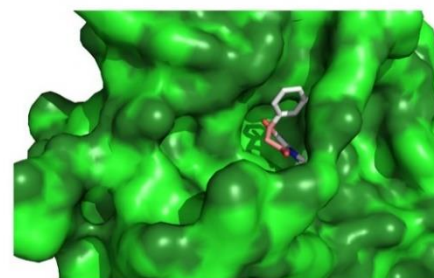
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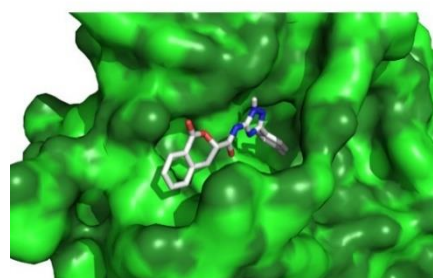
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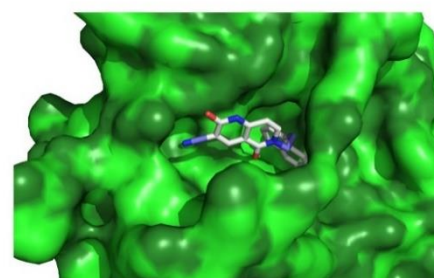
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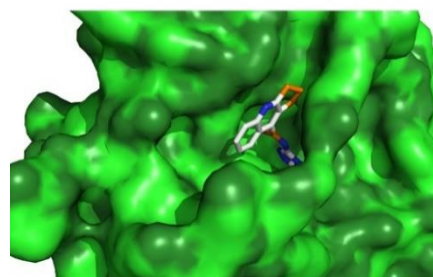
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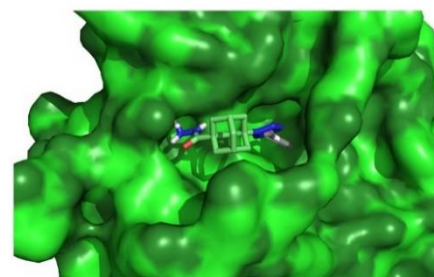
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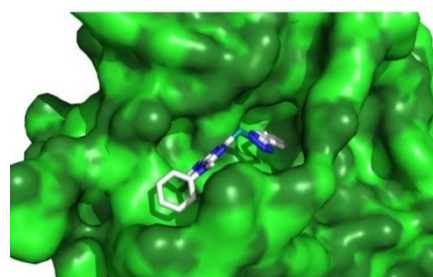
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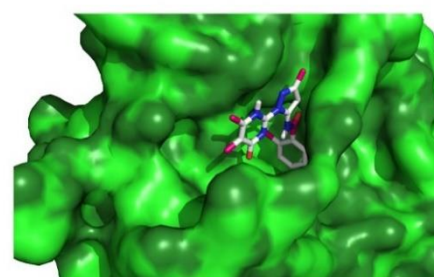
ZINC46083112



ZINC18161915



ZINC12533962



ZINC07420850

Supplementary Fig. 1. Predicted binding poses of the top 10 hits and AP endonuclease 1.

Supplementary Table 1. List of 1,338 potential inhibitors of AP endonuclease 1 by pharmacophore screening and molecular docking

ZINC ID	PubChem CID	Pharmacophore score 1	Pharmacophore score 2	Binding affinity
ZINC69565400	53580594	41.00639	43.377747	-8.7
ZINC20086366	51603501,28611523,25248999	40.193176	44.288307	-8.6
ZINC09087097	4909512,40928637	40.654366	43.33812	-8.6
ZINC04624380	5299120,7305393	40.906654	44.409916	-8.6
ZINC14198982	17568456,25987655	40.984146	42.76821	-8.5
ZINC67676245	56712240	39.836067	43.321037	-8.5
ZINC46083122	51113769	40.194237	43.284782	-8.4
ZINC18161915	843225,3300507	41.150127	44.36736	-8.4
ZINC12533962	17448744	41.15087	43.60126	-8.4
ZINC07420850	51430633,8516743	39.675964	43.53222	-8.4
ZINC07646218	8752998	41.03507	43.322594	-8.4
ZINC22263860	24698181	40.134243	45.798782	-8.3
ZINC28716248	35873053,35873054	40.797756	43.46943	-8.3
ZINC16956851	27444029	40.977768	43.403572	-8.3
ZINC00032557	582551	41.26294	45.560642	-8.3
ZINC09691166	17407752,9246897	40.98685	44.572247	-8.3
ZINC71803297	56825790	41.01689	42.84125	-8.3
ZINC03900862	2849543,51397836	40.186153	43.359375	-8.2
ZINC19373000	51590423,46973619	41.066746	44.160034	-8.2
ZINC07813720	8933276,16339923	40.81163	45.81323	-8.2
ZINC00081470	4195018,700996	40.310978	44.68419	-8.2
ZINC67677150	50966999	40.068657	43.8693	-8.1
ZINC48252179	47058741	41.00527	44.103455	-8.1
ZINC20086368	28611526,25248999	40.192806	44.28888	-8.1
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