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

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Apurinic/apyrimidinic 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/apyrimidinic endonuclease (APE) is an enzyme that identifies damaged apurinic/apyrimidinic 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 nonspecific 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 out work, in which we applied pharmacophore modeling and
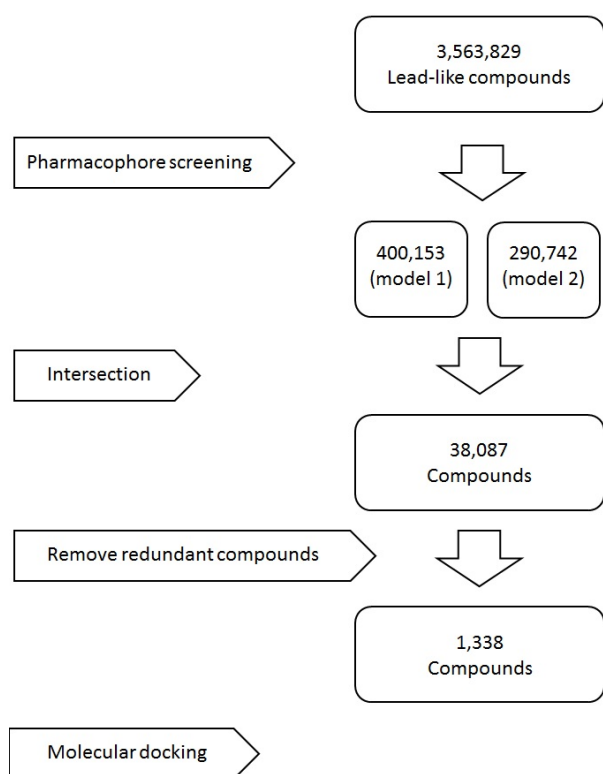
Fig. 1. Outline of overall procedures of the screen to find potential inhibitors of apurinic/apyrimidinic endonuclease 1.

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 modes 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 μM. 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.
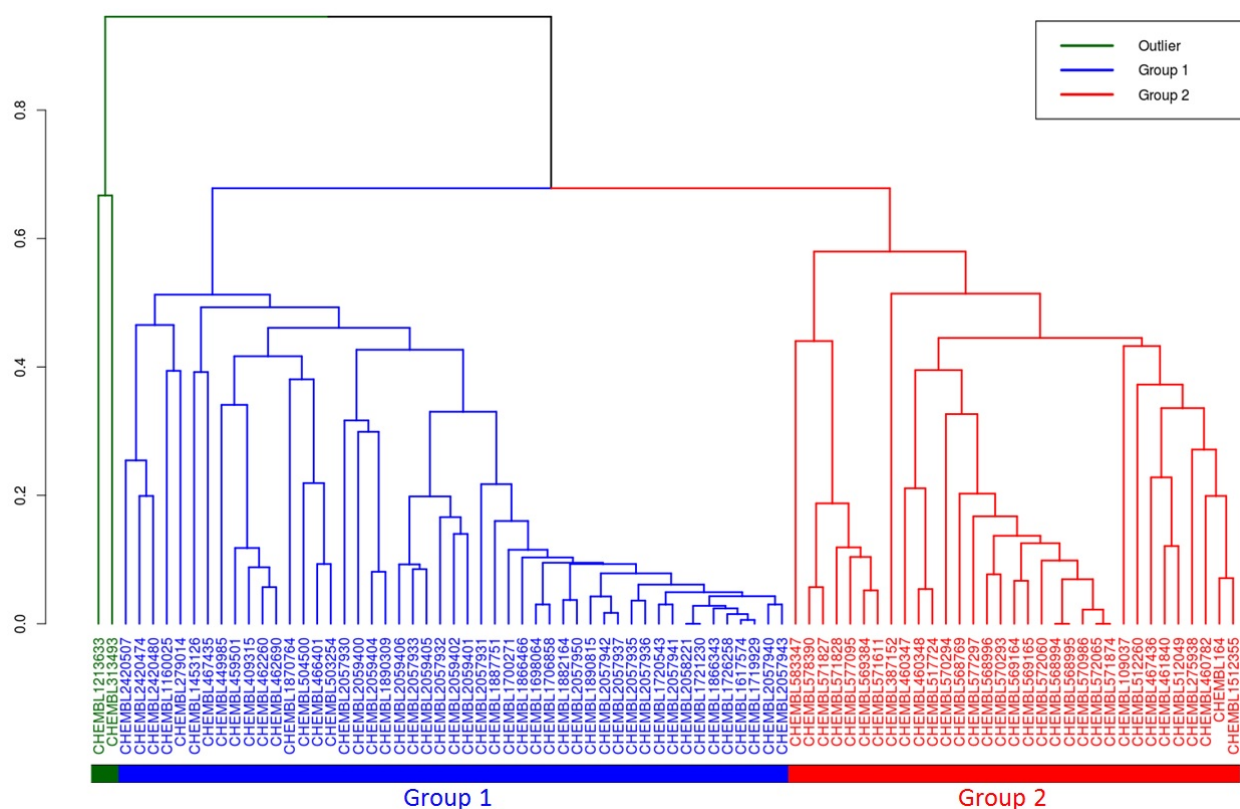

Fig. 2. Clustering of molecules for construction of pharmacophore models.
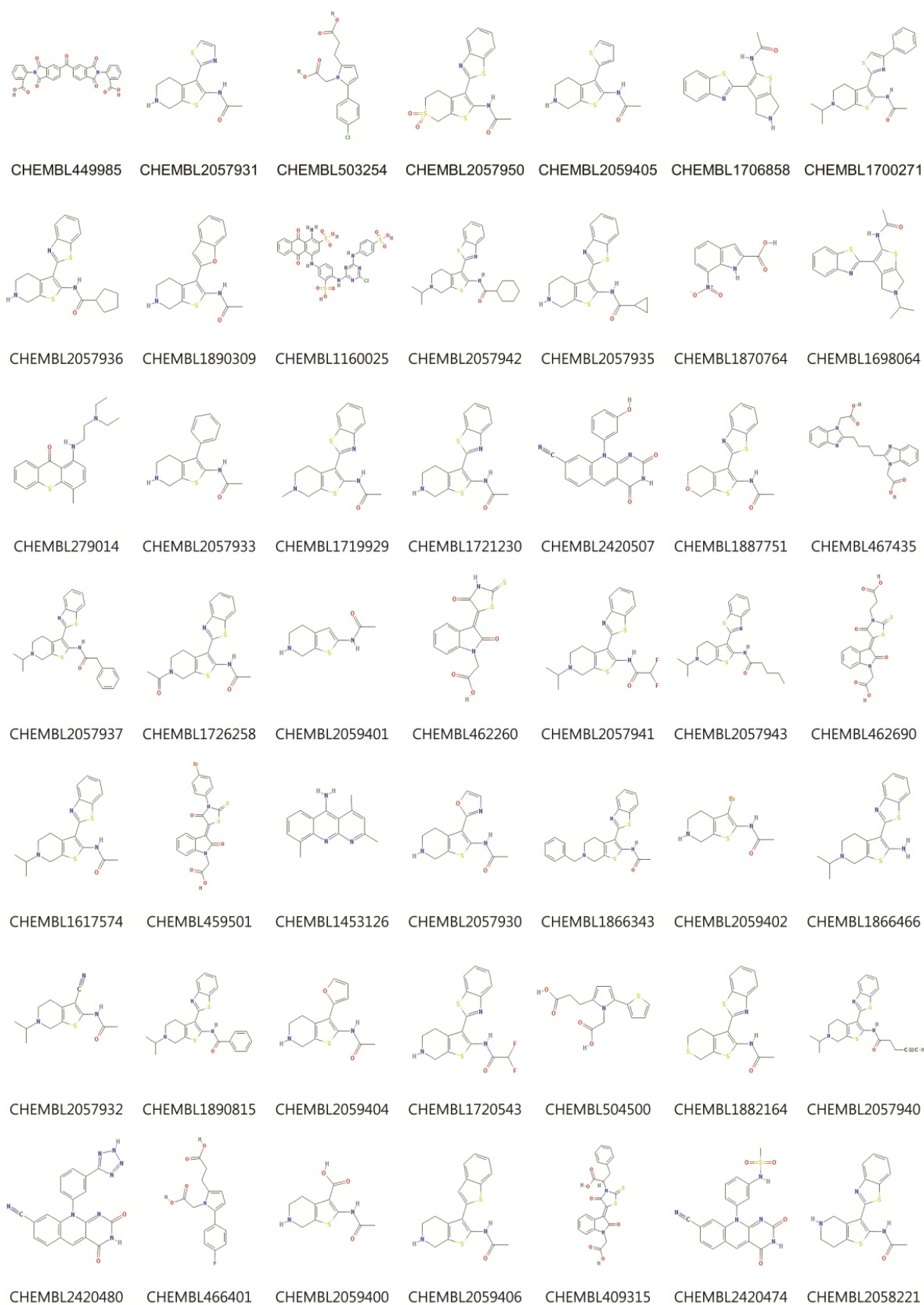
CHEMBL449985   CHEMBL2057931   CHEMBL503254   CHEMBL2057950   CHEMBL2059405   CHEMBL1706858   CHEMBL1700271

CHEMBL2057936   CHEMBL1890309   CHEMBL1160025   CHEMBL2057942   CHEMBL2057935   CHEMBL1870764   CHEMBL1698064

CHEMBL279014   CHEMBL2057933   CHEMBL1719929   CHEMBL1721230   CHEMBL2420507   CHEMBL1887751   CHEMBL467435

CHEMBL2057937   CHEMBL1726258   CHEMBL2059401   CHEMBL462260   CHEMBL2057941   CHEMBL2057943   CHEMBL462690

CHEMBL1617574   CHEMBL459501   CHEMBL1453126   CHEMBL2057930   CHEMBL1866343   CHEMBL2059402   CHEMBL1866466

CHEMBL2057932   CHEMBL1890815   CHEMBL2059404   CHEMBL1720543   CHEMBL504500   CHEMBL1882164   CHEMBL2057940

CHEMBL2420480   CHEMBL466401   CHEMBL2059400   CHEMBL2059406   CHEMBL409315   CHEMBL2420474   CHEMBL2058221

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

CHEMBL568994	CHEMBL570986	CHEMBL577297	CHEMBL572065	CHEMBL577095	CHEMBL568996	CHEMBL571828
CHEMBL571874	CHEMBL583347	CHEMBL460348	CHEMBL568769	CHEMBL570294	CHEMBL569384	CHEMBL512260
CHEMBL568995	CHEMBL569165	CHEMBL460347	CHEMBL571611	CHEMBL164	CHEMBL570293	CHEMBL569164
CHEMBL571827	CHEMBL387152	CHEMBL467436	CHEMBL460782	CHEMBL572060	CHEMBL512049	CHEMBL109037
CHEMBL461840	CHEMBL517724	CHEMBL578390	CHEMBL275938	CHEMBL1512355		

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,
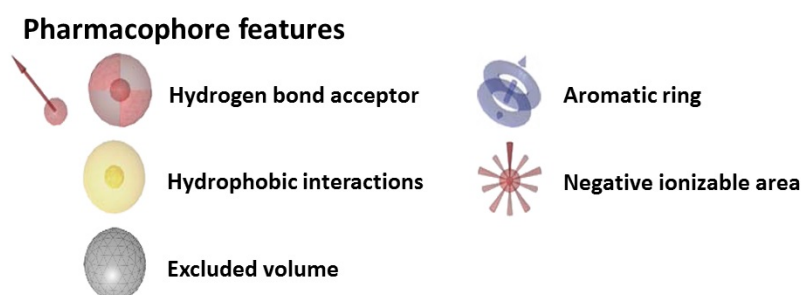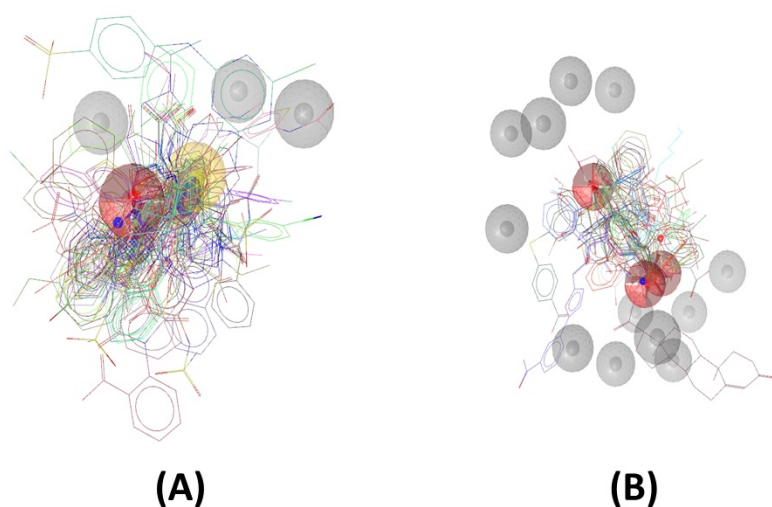
Pharmacophore features

Hydrogen bond acceptor

Hydrophobic interactions

Excluded volume

Aromatic ring

Negative ionizable area

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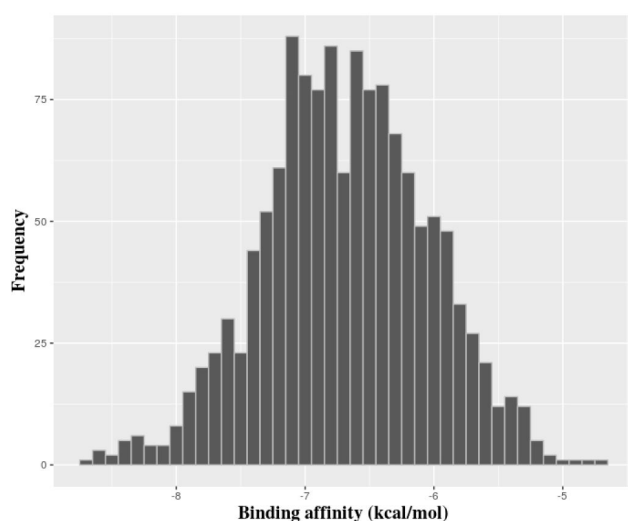

Fig. 6. Distribution of predicted binding affinities by molecular docking of potential inhibitors to apurinic/apyrimidinic endonuclease 1.

(2) partition coefficient log p ≤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
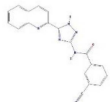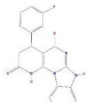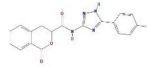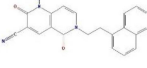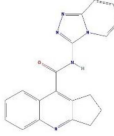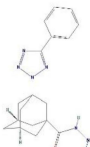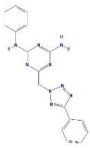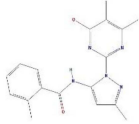
Structure	PubChem CID	Pharmacophore score 1	Pharmacophore score 2	Binding affinity (kcal/mol)
ZINC69565400	53580594	41.00639	43.377747	−8.7
ZINC20086366	51603501, 28611523, 25248999	40.193176	44.288307	−8.6
ZINC09087097	4909512, 40928637	40.654366	43.33812	−8.6
ZINC04624380	5299120, 7305393	40.906654	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

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 restrictedly 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
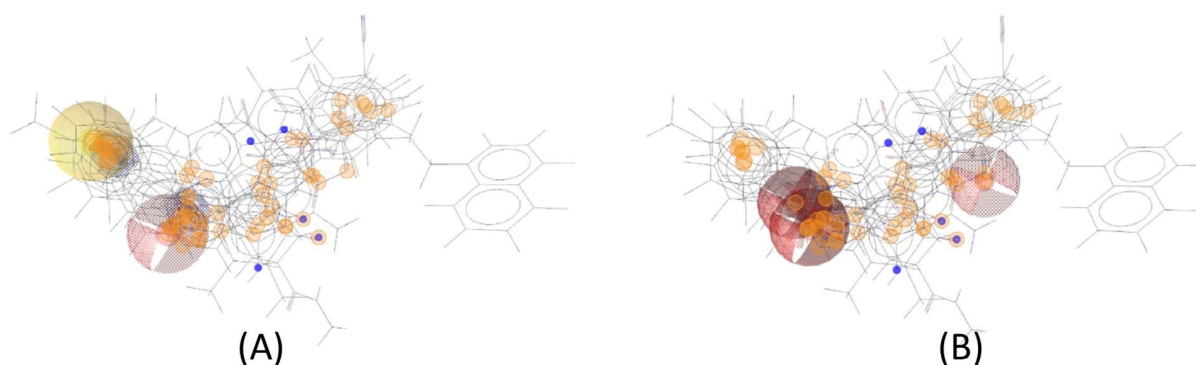

Fig. 8. Mapping of top 10 hits to pharmacophore models. (A) Model 1. (B) Model 2.

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

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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.

References

1. Bjorksten J, Acharya PV, Ashman S, Wetlaufer DB. Gerogenic fractions in the tritiated rat. *J Am Geriatr Soc* 1971;19:561-574.
2. Acharya PV. The isolation and partial characterization of age-correlated oligo-deoxyribo-ribonucleotides with covalently linked aspartyl-glutamyl polypeptides. *Johns Hopkins Med J Suppl* 1972;(1):254-260.
3. Barzilay G, Hickson ID. Structure and function of apurinic/apyrimidinic endonucleases. *Bioessays* 1995;17:713-719.
4. Xanthoudakis S, Smeyne RJ, Wallace JD, Curran T. The redox/DNA repair protein, Ref-1, is essential for early embryonic development in mice. *Proc Natl Acad Sci U S A* 1996;93:8919-8923.
5. Ludwig DL, MacInnes MA, Takiguchi Y, Purtymun PE, Henrie M, Flannery M, *et al*. A murine AP-endonuclease gene-targeted deficiency with post-implantation embryonic progression and ionizing radiation sensitivity. *Mutat Res* 1998;409:17-29.
6. Kelley MR, Logsdon D, Fishel ML. Targeting DNA repair pathways for cancer treatment: what's new? *Future Oncol* 2014;10:1215-1237.
7. Seiple LA, Cardellina JH 2nd, Akee R, Stivers JT. Potent inhibition of human apurinic/apyrimidinic endonuclease 1 by arylstibonic acids. *Mol Pharmacol* 2008;73:669-677.
8. Srinivasan A, Wang L, Cline CJ, Xie Z, Sobol RW, Xie XQ, *et al*. Identification and characterization of human apurinic/apyrimidinic endonuclease-1 inhibitors. *Biochemistry* 2012;51:6246-6259.
9. Naidu MD, Agarwal R, Pena LA, Cunha L, Mezei M, Shen M, *et al*. Lucanthone and its derivative hycanthone inhibit apurinic endonuclease-1 (APE1) by direct protein binding. *PLoS One* 2011;6:e23679.
10. Simeonov A, Kulkarni A, Dorjsuren D, Jadhav A, Shen M, McNeill DR, *et al*. Identification and characterization of inhibitors of human apurinic/apyrimidinic endonuclease APE1. *PLoS One* 2009;4:e5740.
11. Luo M, Kelley MR. Inhibition of the human apurinic/apyrimidinic endonuclease (APE1) repair activity and sensitization of breast cancer cells to DNA alkylating agents with lucanthone. *Anticancer Res* 2004;24:2127-2134.
12. Liuzzi M, Talpaert-Borlé M. A new approach to the study of the base-excision repair pathway using methoxyamine. *J Biol Chem* 1985;260:5252-5258.
13. Fishel ML, Kelley MR. The DNA base excision repair protein Ape1/Ref-1 as a therapeutic and chemopreventive target. *Mol Aspects Med* 2007;28:375-395.
14. Wilson DM 3rd, Simeonov A. Small molecule inhibitors of DNA repair nuclease activities of APE1. *Cell Mol Life Sci* 2010;67:3621-3631.
15. Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, Hersey A, *et al*. ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res* 2012;40:D1100-D1107.
16. Wang Y, Bryant SH, Cheng T, Wang J, Gindulyte A, Shoemaker BA, *et al*. PubChem BioAssay: 2017 update. *Nucleic Acids Res* 2017;45:D955-D963.
17. Wolber G, Langer T. LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. *J Chem Inf Model* 2005;45:160-169.
18. Teague SJ, Davis AM, Leeson PD, Oprea T. The design of lead-like combinatorial libraries. *Angew Chem Int Ed Engl* 1999;38:3743-3748.
19. Irwin JJ, Sterling T, Mysinger MM, Bolstad ES, Coleman RG. ZINC: a free tool to discover chemistry for biology. *J Chem Inf Model* 2012;52:1757-1768.
20. A decade of drug-likeness. *Nat Rev Drug Discov* 2007;6:853.
21. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 2001;46:3-26.
22. Mol CD, Izumi T, Mitra S, Tainer JA. DNA-bound structures and mutants reveal abasic DNA binding by APE1 and DNA repair coordination [corrected]. *Nature* 2000;403:451-456.
23. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient opti-

mization, and multithreading. *J Comput Chem* 2010;31:455-461.

24. Le Guilloux V, Schmidtke P, Tuffery P. Fpocket: an open source platform for ligand pocket detection. *BMC Bioinformatics* 2009;10:168.

25. Shityakov S, Förster C. *In silico* predictive model to determine vector-mediated transport properties for the blood-brain barrier choline transporter. *Adv Appl Bioinform Chem* 2014;7:23-36.

26. Fei J, Zhou L, Liu T, Tang XY. Pharmacophore modeling, virtual screening, and molecular docking studies for discovery of novel Akt2 inhibitors. *Int J Med Sci* 2013;10:265-275.

27. Wieder M, Garon A, Perricone U, Boresch S, Seidel T, Almerico AM, *et al*. Common hits approach: combining pharmacophore modeling and molecular dynamics simulations. *J Chem Inf Model* 2017;57:365-385.