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SCARdock: A Web Server and Manually Curated Resource for Discovering Covalent Ligands

Qi Song,[#] Lingyu Zeng,[#] Qiang Zheng, and Sen Liu*

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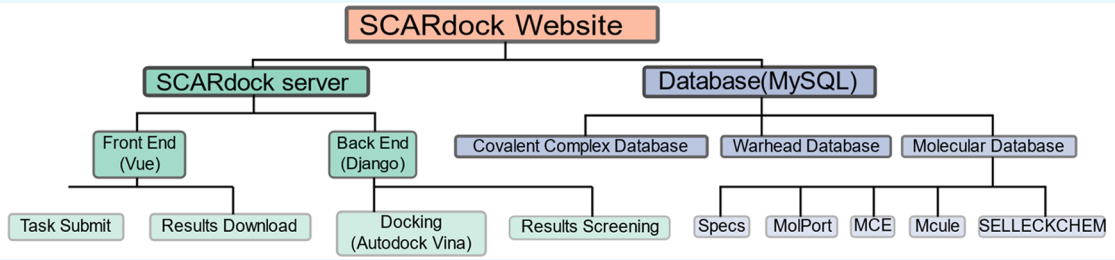

ABSTRACT: Background: Covalent drugs have been intentionally discarded historically due to the concern of off-target side effects, but the past decade has seen a fast resurgence of the discovery of covalent drugs. Compared to noncovalent ligands, covalent ligands might have better biochemical efficiency, lower patient burden, less dosing frequency, less drug resistance, and improved target specificity. Results: Previously, we proposed the steric-clashes alleviating receptor (SCAR) strategy for screening and repurposing covalent inhibitors. To help the discovery of covalent ligands targeting protein targets, we have developed a web server dedicated to providing the SCARdock protocol to general users. Along with this server, we presented three high-quality data sets for the discovery of covalent ligands: a manually curated data set containing 954 high-quality complex structures of covalent ligands and proteins, a manually curated data set of 68 experimentally confirmed covalent warheads targeting 11 different residues, and a prefiltered, classified, and ready-to-use data set of 690,018 entries of purchasable virtual compounds containing these experimentally verified warheads. Conclusions: The SCARdock server and the accompanied data sets would be of great value to the discovery of covalent ligands and are available freely at http://www.liugroup.site/scardock/ or https://scardock.com.

INTRODUCTION

Although small molecules can bind to protein targets either noncovalently or covalently, covalent drugs were intentionally avoided historically due to the concern of toxicity caused by nonspecific modifications of nontargeted biomolecules.[1] However, since the retrospective study revealed that the covalent mechanism is indispensable for many well marketed drugs including aspirin and penicillin,[2] the advantages of covalent drugs have been re-evaluated. It is now recognized that compared to noncovalent drugs, covalent drugs might have better biochemical efficiency, lower patient burden, less dosing frequency, less drug resistance, and sometimes improved target specificity.[1] This conceptual change has resulted in a huge boost in the discovery of covalent drugs in the past decade. Currently, over ten intentionally designed covalent drugs have been approved for clinical use,[3] among which are ibrutinib, a covalent inhibitor of Bruton's tyrosine kinase for treating B-cell cancers, and sotorasib, a covalent inhibitor targeting the once "undruggable" KRAS for treating nonsmall cell lung cancer. Covalent ligands are also attracting attentions in the development of PROTACs (proteolysis targeting chimeras) and protein−protein interaction regulators.[4−6]

The computational tools for discovering covalent ligands of proteins had also been underdeveloped for a long time. Previously, Abagyan et al. implemented covalent docking in ICM by converting ligands into pseudoligands covalently attached to the side chain of residue,[7] and Jones et al. developed GOLD with covalent docking functionality by using link atoms added to both ligand and receptor.[8] Nonetheless, covalent docking was not a focus of these computation-aided drug discovery (CADD) tools. With the escalating interests in covalent drugs, the number of CADD software for the virtual screening of covalent ligands has increased significantly in the past decade. Morris et al. designed AutoDock4 for docking covalent ligands with either a grid-based approach or an approach using flexible side chains.[9] Lawandi et al. updated FITTED to consider covalent and noncovalent docking simultaneously based on an automated identification of the

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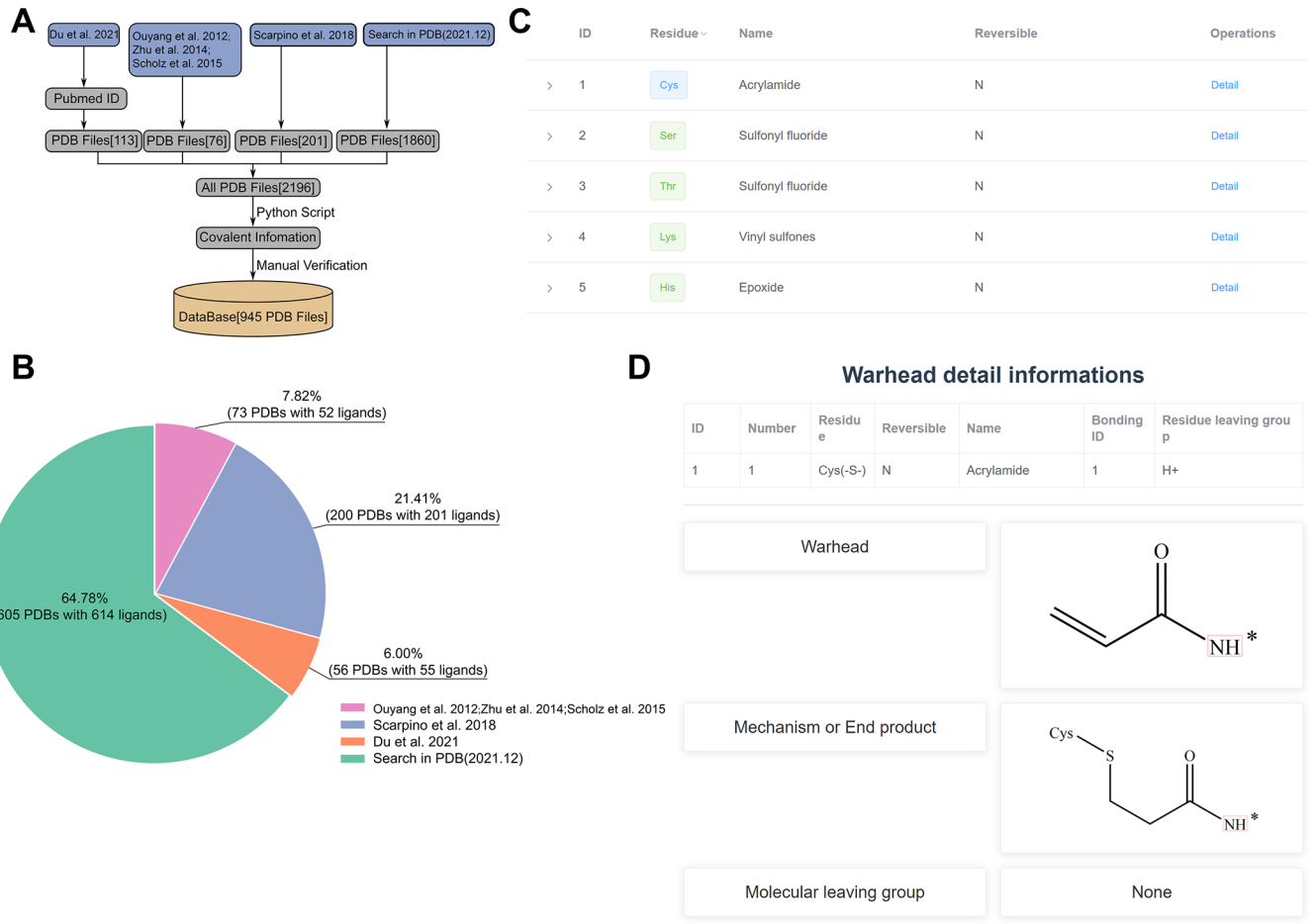

Figure 1. Data collection of covalent ligand−protein complexes and experimentally verified covalent warheads. (A) Collection pipeline of covalent ligand−protein complexes. (B) Statistics of the collected covalent complexes. (C) A screenshot of the warhead list on the SCARdock web server. (D) A screenshot of the warhead information page on the SCARdock web server.

ligand warhead atoms, and Labarre et al. introduced new features and data sets implemented in FITTED to broaden the ability of docking covalent inhibitors and metalloenzymes.[10,11] Ouyang et al. developed CovalentDock based on Autodock by utilizing a dummy atom to conceptually link the residue and the ligand.[12] Warshaviak et al. established CovDock-VS, a virtual screening version of the Schrodinger's CovDock[13] for docking covalent ligands.[14] London et al. created DOCKovalent by constraining predefined covalent bonds from the noncovalent docking program DOCK3.6 for screening electrophilic molecules.[15] Scholz et al. developed DOCKTITTE in the Molecular Operating Environment (MOE) by combining warhead screening, side chain attachment, pharmacophore-guided docking, and side-chain cleavage and pose rescoring.[16] Hoffer et al. presented Covadots, a covalent inhibitor design protocol by identifying chemical moieties to link noncovalent substructures with reactive protein residue.[17] Wei et al. implemented Cov_FB3D, a covalent inhibitor design protocol based on AutoDock v 4.2 by combining fragment-based drug design, molecular mechanism (MM) and quantum mechanical (QM) calculations.[18] Scarpino et al. proposed the covalent docking protocol WIDOCK by incorporating ligand reactivity in to AutoDock v 4.2.[19] Wu and Huang proposed the covalent docking protocol HCovDock by balancing the contributions between covalent and noncovalent interaction.[20] Wei et al. proposed Cov_DOX for covalent docking that achieved a high success rate of accurate binding structures of protein−ligand complexes.[21]

Although there are so many covalent docking tools now, free web servers for convenient covalent ligand screening are very limited. One web server is DOCKovalent (http://covalent.docking.org), but this server only has 12 warheads targeting limited residues such as Cys, Thr, and Ser. Another Web server is CovalentDock Cloud,[22,23] but this server is outdated and not reachable anymore. One successfully verified strategy in the design of covalent inhibitors is appending covalently reactive chemical groups (warheads) to a noncovalent inhibitor. This strategy supports the theory that the noncovalent binding step positions the warhead for covalent bonding. Based on this theory, we proposed the SCARdock protocol, which has been successfully applied in drug screening and repurposing recently.[24−27] To make computational screening of covalent ligands accessible more easily and widely, we set up a free web server of SCARdock. Along with this server, we collected and organized three useful data sets: a manually curated data set containing 954 published high-quality complex structures of covalent ligands and protein targets, a manually collected and curated data set of 68 experimentally confirmed covalent warheads targeting 11 different residues, and a prefiltered, classified, and ready-to-use data set of 690,018 entries of purchasable compounds containing these experimentally verified warheads. To our best knowledge, these data sets might be the most comprehensive and the largest ones of their kind thus far. Our server and data sets would be of great value to the discovery of covalent ligands.

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Table 1. Statistic Information of the Prepared Compound Dataset Filtered by Experimentally Verified Covalent Warheads

Source Library	Targeting Residue										
	Asp	Cys	Glu	His	Lys	Met	Pro	Pyr	Ser	Thr	Tyr
SPECS	9	1,733	12	442	44	8	2	130	5,521	1,658	0
MolPort	1,737	23,230	282	18,529	1,193	282	217	5,362	63,963	25,301	56
Mcule	12,196	106,952	3,563	53,700	27,058	3,562	5,081	32,100	188,217	100,661	4,349
MCE	19	359	1	133	62	1	5	296	253	155	1
SELLECKCHEM (Bioactive Compound Library)	8	214	2	70	48	2	2	119	107	146	1
SELLECKCHEM (FDA approved and in clinic)	7	101	1	48	5	1	2	83	69	48	0
SELLECKCHEM (Traditional Chinese Medicine Library)	0	40	0	18	14	0	1	24	17	19	0
SELLECKCHEM (Natural Product Library)	0	88	0	43	44	0	2	80	57	52	0
Total	13,976	132,717	3,861	72,983	28,468	3,856	5,312	38,194	258,204	128,040	4,407

CONSTRUCTION AND CONTENT

Data Collection and Manual Curation of High-Quality Complexes of Covalent Ligands and Proteins. A high-quality data set of covalent ligand−protein complexes is indispensable for developing, validating, and evaluating covalent ligand docking protocols. Previously, we[27] and other groups[12,13,16] used a data set containing 76 protein-inhibitor complexes for evaluating different computational tools. That data set was collected ten years ago[12] when the rational discovery of covalent drugs just started to gain attention. The past decade has seen fast growth of experimentally determined complexes of covalent ligands with different protein targets. Hence, it would be of great value to prepare an updated data set of covalent ligand−protein complexes to help the rational discovery of covalent ligands.

Recently, Scarpino et al.[19] and Du et al.[28] independently prepared two covalent ligand−protein complex data sets. Combining the reported data from these two papers and the data set we used previously, we obtained 390 complex structures. Additionally, we searched the PDB databank with the keywords "covalent" and "inhibitor" along with the settings "compatible with PDB format = Y" and "data collection resolution ≤2.5 Å". As of Dec 01, 2021, we obtained 2,006 structures from the PDB databank. Combining these entries and removing duplications, we finally obtained 2,196 covalent ligand−protein complexes with PDB structures. To confirm the covalent binding information in these collected structures, ligand IDs, covalent bonds, and references were retrieved from the PDB files via the "LINK" and "JRNL" fields. PDB files without covalent bonds were discarded. At last, all covalent bonds between ligands and proteins were visually confirmed in Pymol.[29] Finally, we obtained 954 covalent ligand−protein complexes with experimental PDB structures (Figure 1A,B, and Table S1). To get these files ready for virtual docking analysis, we kept one chain only if there were multiple chains in the PDB file and removed the ligands/atoms (such as waters, ions, and noncovalent ligands) not belong to the target chain(s) and the covalent ligand. General molecules such as cofactors and solutes were also discarded (Table S2). In total, these structures contain 935 ligands, and all ligands were validated via a PDB API (https://www.rcsb.org/ligand-vlidation/PDBID/ligandid).[30] We also collected the chemical features and DrugBank features using another PDB API (https://www.rcsb.org/ligand/ligandname).[30] The detailed information is provided as Table S1, including PDB IDs, covalent residues, ligand names, SMILES expressions, molecular weights, reference Pubmed IDs, etc.

Data Collection and Manual Curation of Experimentally Verified Covalent Warheads. During the design of covalent ligands, the selection of suitable warheads is critical. In our SCARdock protocol, warheads are used to filter compound libraries so that only ligands containing suitable warheads are used for docking. A warhead is a chemically reactive moiety, but its reactivity is also affected by the environment of the binding pocket of the protein. Meanwhile, the same warhead might have different reactivities on different molecular scaffolds. Furthermore, to limit off-target effects, not all chemically active warheads are suitable to be incorporated in covalent drugs. Therefore, a data set of valid warheads is highly valuable. To prepare this data set, we decided to only collect the warheads confirmed by reliable experimental assays. Therefore, a warhead was collected only when it was confirmed by at least one of the following types of experimental evidence: a complex structure, NMR data, and mass spectrometry. All data were confirmed by checking the original reports. After going through over 100 papers, we prepared a data set containing 68 unique warheads targeting 11 different residues (Table S3). A note is that one warhead could be used to target two or more residues. Additional information was also collected and reported for each warhead, including reversibility, targeting residues, leaving groups, bonding atoms, SMILES expressions, reaction mechanisms, references, etc. On the SCARdock server, the warheads were classified and ordered by their targeting residues. Users can easily locate the warhead of interest by the residues and the serial number of the warhead (Figure 1C). Each warhead has an information page displaying its generic name, leaving group, end product, reversibility, references, etc. (Figure 1D).

Data Collection and Preparation of Compound Data Set Containing Experimentally Verified Covalent Warheads. Virtual screening compound libraries are indispensable for *in silico* docking. Although some commercial ventures are starting to provide covalent compound libraries for virtual screening, the warhead numbers and the targeting residues are very limited. Based on our manually curated warhead data set, we compiled a screening data set of purchasable covalent compounds from several representative commercial ventures (Table 1). Currently, our data set contains 690,018 compounds targeting 11 different residues, among which there are 13,976 for Asp, 132,717 for Cys, 3,861 for Glu, 72,983 for His, 28,468 for Lys, 3,856 for Met, 5,312 for Pro, 38,194 for Pyr (pyruvoyl), 258,204 for Ser, 128,040 for Thr, and 4,407 for Tyr. A note is

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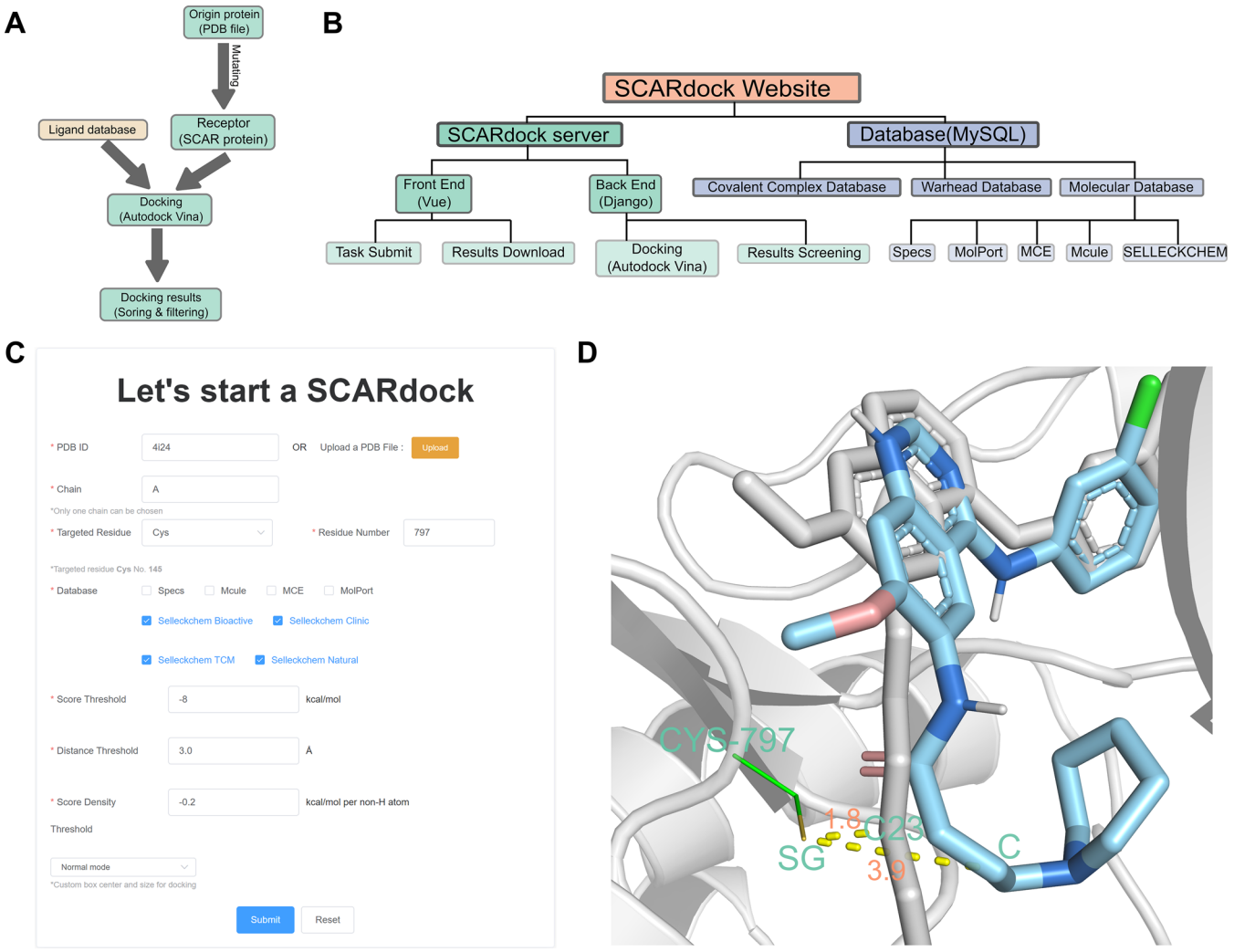

Figure 2. Pipeline and composition of SCARdock. (A) Pipeline of the SCARdock server. (B) Composition of the SCARdock Web site. (C) Task submission of SCARdock for 4I24 (PDB ID). (D) A comparison between a SCARdock result for 4I24 and the origin structure.

that if a compound contains more than one warhead or can target more than one residue, it is recounted upon each match. For each warhead, the bonding atom was identified and recorded for distance calculation later in the SCARdock protocol. The 3D conformations of all molecules were generated with RDKit[31] from SDF files. On the SCARdock web server, the prefiltered compound data sets are available for download in MOL and PDBQT files. The PDBQT files are ready for docking with Autodock Vina[32] locally or on the server.

SCARDOCK PIPELINE

Previously, we proposed the SCAR strategy and developed an experimentally verified pipeline for screening covalent inhibitors in drug screening.[27] SCAR is the first strategy that screens covalent ligands based on noncovalent docking and has been experimentally validated.[33,34] In the SCAR strategy, the covalent residue in the protein is mutated or removed before docking, and the distance between the bonding atoms is used for evaluating the possibility of covalent bonding.[27] To make our protocol more widely and easily accessible, we set up the SCARdock web server for screening covalent ligands (Figure 2A). Users need to designate the target protein by providing a PDB ID or uploading a PDB file directly, specify the targeting residue, and choose a ligand data set for docking. The server performs the docking process with AutoDock Vina v 1.1.2. As described in our previous work,[24−27] results are further filtered according to the docking scores, score density, and the distances between the assumed covalent atoms. At last, users are suggested to download the results, visually check the docked poses, and select ligands for experimental validation accordingly. The vendor IDs of the ligands are also provided so that users can inquire the purchasing information from the vendors.

The backend of the SCARdock server was developed with Django, and the front-end was built with Vue (Figure 2B). Web site data are stored and managed by MySQL. The visual screening pipeline of SCARdock was implemented with Python. For receptor preparation, after a PDB file is automatically downloaded from the PDB database or submitted directly, the specified chain is extracted and the specified targeting residue is mutated to Gly via PyRosetta. Then the mutated receptor is converted to a PDBQT file by MGLTools. The molecules in the selected data set matching the covalent residue are used in the docking progress. The default docking parameters can be used directly, or the coordinates and sizes of the grid box could be customized in the advanced mode. The ligands would be discarded if the score or the score density of the first pose is higher than the thresholds (for example: a score higher than −8 kcal/mol, and a score density higher than −0.2 kcal/mol per

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non-hydrogen atom). In addition, the distance between the bonding atoms in the targeting residue and the compound in each docked pose is calculated and recorded for filtering as well. The pose scores and atom distances are saved in the result report as a reference for manual validation. All SCARdock jobs and results are private and only available by the user.

UTILITY AND DISCUSSION

Case Study: An Example of Covalent Docking for EGFR. A structure of EGFR (PDB ID: 4I24) was used as an example to showcase how to screen covalent inhibitors using the SCARdock server. In the "Get Started" page, the receptor was automatically downloaded by providing PDB ID or uploaded with the PDB file. The chain identifier, target residue (name and number), and ligand data sets were selected (Figure 2C). The "advanced mode" allows users to manually set the grid box for docking with Autodock Vina. Then the job was submitted to the server. Once the task was done, results were checked and downloaded in the "My results" page. In this page, the settings used in the screening process are displayed. More details, such as parameters of the grid box, ligand information, and docking scores, are included in the downloadable files. In this example, SCARdock successfully retrieved the original ligands of 4I24 from the SELLECKCHEM library with poses consistent with the original structure, except that the remaining leaving group increased the distortion of the docked pose (Figure 2D).

RESULTS AND DISCUSSION

The past decade has witnessed a fast resurgence of the discovery of covalent ligands. A data set of high-quality covalent ligand−protein complexes will be of great value for the development and validation of covalent docking tools. A manually curated data set of covalent warheads will be of great importance for the design and optimization of covalent ligands. A data set of covalent compounds containing experimentally verified warheads will be highly useful for the *in silico* screening of covalent ligands. We provided these high-quality data sets along with the SCARdock web server to provide help to the discovery, design, and optimization of covalent ligands. The SCARdock server will allow users without coding skills to screen covalent ligands easily. In future, the covalent complex data set, the warhead data set, and the ligand data set will keep updating.

CONCLUSIONS

Based on previous studies and PDB, a data set of covalent complex structures and a data set of warheads were built, and all the data were supported by the experiments in the studies. Subsequently, several compound libraries were processed based on the warhead data set, and the data set of compounds is ready to be applied in the SCARdock pipeline. With the three data sets, SCARdock is accessible for users to screen covalent ligands for their target proteins, and the details of the ligands and warheads can be checked. In conclusion, the SCARdock web server will be a great resource to the researchers interested in the discovery of covalent drugs and chemical tools.

ASSOCIATED CONTENT

Data Availability Statement

The SCARdock server is freely available at http://www.liugroup.site/scardock/ or https://scardock.com.[35] The manually curated data set containing published high-quality complex structures of covalent ligands and protein targets, the manually collected and curated data set of experimentally confirmed covalent warheads, and the prefiltered and ready-to-use virtual data set of purchasable compounds are available on the SCARdock server.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c08147.

Data collection of covalent ligand−protein complexes; discarded general molecules such as cofactors and solutes; unique warheads targeting 11 different residues in biological sciences papers (PDF)

AUTHOR INFORMATION

Corresponding Author

Sen Liu − *Cooperative Innovation Center of Industrial Fermentation (Ministry of Education & Hubei Province) & Key Laboratory of Fermentation Engineering (Ministry of Education) and Hubei Key Laboratory of Industrial Microbiology & National "111" Center for Cellular Regulation and Molecular Pharmaceutics, Hubei University of Technology, Wuhan 430068, China*; orcid.org/0000-0001-5182-7241; Email: senliu.ctgu@gmail.com

Authors

Qi Song − *Cooperative Innovation Center of Industrial Fermentation (Ministry of Education & Hubei Province) & Key Laboratory of Fermentation Engineering (Ministry of Education) and Hubei Key Laboratory of Industrial Microbiology & National "111" Center for Cellular Regulation and Molecular Pharmaceutics, Hubei University of Technology, Wuhan 430068, China*

Lingyu Zeng − *Cooperative Innovation Center of Industrial Fermentation (Ministry of Education & Hubei Province) & Key Laboratory of Fermentation Engineering (Ministry of Education) and Hubei Key Laboratory of Industrial Microbiology & National "111" Center for Cellular Regulation and Molecular Pharmaceutics, Hubei University of Technology, Wuhan 430068, China*

Qiang Zheng − *Cooperative Innovation Center of Industrial Fermentation (Ministry of Education & Hubei Province) & Key Laboratory of Fermentation Engineering (Ministry of Education) and Hubei Key Laboratory of Industrial Microbiology & National "111" Center for Cellular Regulation and Molecular Pharmaceutics, Hubei University of Technology, Wuhan 430068, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c08147

Author Contributions

#Sen Liu conceived the idea and prepared the compound data sets. Qi Song set up the web server. Lingyu Zeng prepared the covalent ligand−protein data sets and designed the web pages. Qiang Zheng prepared the warhead data set. Qi Song and Lingyu Zeng contributed equally.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CADD: Computation-aided drug discovery
MM: Molecular mechanism
MOE: Molecular Operating Environment
PDB: Protein data bank
PROTACs: Proteolysis targeting chimeras
QM: Quantum mechanical
SCAR: Steric-clashes alleviating receptor strategy
SMILES: Simplified molecular input line entry system

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