Combining CBP Pharmacophore Construction and Molecular Docking to Search for Potential Competitive Inhibitors of Chitin Deacetylase

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Abstract: Chitin deacetylase (CDA) is a key enzyme for plant pathogens to evade host defense recognition. However, in the study of CDA inhibitors, only chitin deacetylase from colletotrichum lindemuthianum (CICDA) was found to participate in the reverse hydrolysis reaction in sodium acetate to acetylate free amino sugar residues into N-acetylated forms. Based on this, we selected 10,632 small molecules from the DrugBank database for computer virtual screening to find new potential CDA inhibitors. First, we use the CBP model with ROC = 0.800 to coarsely screen small molecules. Then we use the LibDock and CDOCKER programs in Discovery Studio 2016 (DS 2016) to dock the best-matched small molecules to identify interactions with key residues on the active site of CICDA. Finally, we found two potential compounds with good adaptability, high docking score and important interactions with protein active sites. And we confirm that their structures are stable and have multiple non-bonding interactions with important amino acid sites such as ASP50, TYR145, HIS206 and ZN1255 by MD simulations. Therefore, we conclude that the selected compounds are likely to be new inhibitors of CDA. In this research could provide a valuable resource and guidance for CDA-related inhibitors development.
Keywords: chitin deacetylase; colletotrichum; virtual screening; docking.

Introduction

In the long struggle between plants and pathogens, plants have evolved a highly efficient and complex immune system, and pattern recognition receptors located on plant cell membrane epitopes play an important role in sensing the presence of pathogens and activating immunity. Chitin is one of the important components of fungal cell wall, and chitin released by pathogenic fungi in the process of infecting host will be recognized by host membrane receptors to induce immune response[1-3]. However there is cumulative evidence that fungi evade plant defense mechanisms by partially deacetylating either their exposed cell wall chitin[4-6]. In this cases, the resulting partially deacetylated oligomers are not well recognized by the specific plant receptors reducing or preventing the elicitation of the defense responses[7]. Currently, antifungals targeting cell walls include β-D-glucan synthase inhibitor, chitin synthase inhibitors and glycosyl-phosphatidyl Inositol (GPI) anchor pathway inhibitor[8, 9]. Thus, CDA represents a promising target for antifungals.

Chitin deacetylase is one of the members of Carbohydrate esterase 4 superfamily, which can hydrolyze acetyl groups of N-acetylglucosamine units of chitin and chitin oligosaccharides, thus producing acetic acid and chitosan, the poor substrates of chitinase[10]. As important enzyme catalyzing the conversion to chitin to chitosan, chitin deacetylase plays a very important role in agriculture and drug discovery. We can seek chitin deacetylase inhibitors to block the deacetylation modification of chitin by chitin deacetylase, tear apart the cunning camouflage of pathogenic fungi, expose their true state, and make the organisms play a therapeutic role in their own prevention and treatment.

Colletotrichum is a genus of soil-borne plant fungi widely distributed in tropical, subtropical and temperate regions, which often infects crops and induces serious economic losses[11]. In a vote organized by Molecular Plant Pathology magazine in 2012, the pathogenic fungi of the genus Anthrax were promoted as the eighth most important phytopathogenic fungi in the world according to their scientific significance and economic importance[12]. Among the numerous studies on chitin deacetylase, some researchers have found that in 3.0 mol/L sodium acetate, ClCDA can participate in the reverse hydrolysis reaction, acetylate the free amino sugar residues into N-acetylated form, and some studies have confirmed that acetate plays a competitive inhibitory role in this process. This makes it possible to control plant pathogens by inhibiting chitin deacetylase[13-15].
With the development of computer-aided drug design, structural biology, protein crystallization and resolution technology (X-ray diffraction, nuclear magnetic resonance). The use of computational techniques in drug discovery and development has become the most effective method. Ligand-based virtual screening of drugs can efficiently screen potential compounds from a large number of compounds through the interaction between proteins and small molecule compounds, avoiding blind screening, thereby reducing human, financial and time costs[16]. This study will use DS2016 software to further explore competitive inhibitors of chitin deacetylase on the basis of docking acetate and other molecules with chitin deacetylase. The virtual screening flow chart is shown in Fig. 1.

### Fig. 1. Schematic representation of the virtual screening process implemented in the identification of CICDA inhibitors. 2iw0: crystal structure of CICDA; ACT: acetate ion; NAG: chitin monomer form; NAG2: chitin dimer form; NAG3: trimeric form of chitin.

### Materials and Methods

#### Data Collection and Preparation

The X-ray crystal structure of CICDA (PDB ID: 2iw0) was downloaded from the RCSB Protein Data Bank (www.rcsb.org). According to the relevant literature, it is known that the CICDA catalytic subunit is generated from zinc-binding triplets, which are composed of two histidine (His104, His108) and aspartate (Asp50)[17]. Therefore, after removing the original ligand from the complex, ASP50, HiS104, HiS108 and Zn1255 were set as active sites and the active radius was set to 10Å. Then 2iw0
was docked with acetate ion (ACT), chitin monomer form (NAG), chitin dimer form (NAG$_2$) and trimeric form of chitin (NAG$_3$) respectively for LibDock molecular docking. Finally, the docking results of 2iw0-ACT was used as receptor, and three forms of N-acetylglucosamine chitosan were used as ligands for LibDock molecular docking respectively.

**Fig. 2.** Crystal structure of chitin deacetylase (2iw0).

**Pharmacophore Model Generation**

The highest scoring NAG$_2$-2iw0 conformation was selected according to the results of LibDock docking to construct a receptor-ligand complex based pharmacophore model (CBP). The pharmacophore model generates crystal complexes utilizing known CICDA (PDB ID: 2iw0) and ligand (NAG$_2$), and chooses both as receptors and ligands, respectively, to build the CBP model within the protocol of ‘receptor-ligand pharmacophore model generation’ in DS. Specifically, the maximum hydrophobic distance was set to 5.5 Å and the maximum hydrogen bond distance was set to 3.0 Å. Other parameters such as ‘mode’ and ‘docking’ were set as ‘fast’ and ‘rigid’, respectively[18]. In order to verify the selectivity of the obtained pharmacophore model, ACT, NAG and NAG$_3$ were used as active ligands of CBP for model validation, and 38 compounds in the CICDA-bait set were randomly selected as inactive ligands. The optimal model was selected and virtual screening was performed using the Drugbank database to identify new potential chitin deacetylase inhibitors[16, 19].
Molecular Docking

Because the number of small molecules in DrugBank database is too large, after screening with pharmacophore, there are 7794 remaining small molecules, we use the LibDock molecular docking method to conduct the next round of screening. In this round of screening, the receptor is ClCDA (2iw0), the ligand is Best fit view small molecule obtained after the last round of pharmacophore screening, ASP50, HiS104, HiS108, Zn1255 are set as active sites, and the active radius is set as 10 Å. Virtual screening was carried out by docking all the prepared ligands at the defined active site using Libdock. Based on the Libdock score, all the docked poses were ranked and grouped by name. All compounds were ranked according to their Libdock score.

LibDock is a fast rigid docking method using the hot zone map of the active sites of receptor molecules, while CDOCKER is an implementation of a CHARMm based docking tool. The receptor is held rigid while the ligands are allowed to flex during the docking process. For each complex pose, the CHARMm energy (interaction energy plus ligand strain) and the interaction energy, which indicate ligand binding affinity, are calculated[20]. The combination of LibDock and CDOCKER can make up for the shortcomings of both sides and screen target small molecules quickly and efficiently. Therefore, we used the screening method of CDOCKER molecular docking to screen the small molecules screened by LibDock for another round of screening.

CDOCKER module of Discovery Studio was used for molecular docking study. The CHARMm forcefield was used for receptors and ligands. The binding site spheres of ligands and receptors were defined as the regions that come within radius 10 Å from the geometric centroid of the ligands ASP50,
HiS104, HiS108 and Zn1255, respectively. During the docking process, the ligands were allowed to bind to the residues within the binding site spheres[20].

*Molecular Dynamics Simulations*

Molecular dynamics (MD) simulations were performed using DS 2016 Standard Dynamics Cascade and Dynamics package. Samples of ligand-receptor complexes were applied with the CHARMM polar hydrogen force filed and solvated by applying explicit periodic boundary in a solvation model before running MD simulations. MD simulations were conducted under the setting parameters, which were listed as follows: steepest descent of energy minimization was 500, steps of conjugate gradient minimization were 500, the system was heated from 50K to 300K within 2 ps, and steps of equilibration were 1000. The simulations were performed with a total production time of 200 ps. For other parameters, we adopted default setting values. We used the functions of Analyze Trajectory to analyze root mean square deviations (RMSDs) of protein-ligand complexes and ligands, total energies and potential energies of protein-ligand complex, after MD simulation[21].

**Results**

*Identification of Substrate-Binding Region*

CICDA is a member of the Carbohydrate esterase 4 superfamily, whose members share a conserved region in their primary structure that is recognized as a catalytic subunit[22]. It is known that the CICDA catalytic subunit is generated from a zinc-binding triad consisting of two histidines (His104, His108) and aspartate (Asp50)[22]. Therefore, we used the zinc-binding triplet as the active center for LibDock docking with the substrates of CICDA (NAG, NAG$_2$, NAG$_3$) and competitive inhibitors (ACT). At the same time, in order to intuitively demonstrate the mechanism of action of the inhibitors, we docked the substrates again while retaining the docking of the inhibitors. The docking results are shown in Table 1 and 2 below.

**Table 1.** Molecular docking results based on active groups generated by catalytic subunits.

| Name | ligand non-bond monitor | Interaction | Absolute Energy | LibDock Score |
|------|-------------------------|-------------|-----------------|---------------|


| NAG₃ | ASP50; ASN78; TRP79; HIS108; Ala107; TYR145; Zn1255 | CoH-B; M-A; CaH-B; UnM-D | 85.9766 | 169.116 |
|---|---|---|---|---|
| NAG₃ | TYR145; TYR173; His206 | CoH-B; Pi-Si; Pi-D | 76.0685 | 139.62 |
| NAG₃ | ASP50; TYR145; TYR173; His206 | CoH-B; CaH-B; UnA-A | 81.9908 | 112.738 |

| NAG₂ | ASP50; TYR145; His206; His209; Zn1255 | CoH-B; M-A; CaH-B; UnA-A | 47.6782 | 136.954 |

| NAG₂ | ASP49; ASP50; ASN78; TRP79; His206; His209; Zn1255 | CoH-B; M-A; CaH-B; UnA-A; UnD-D | 37.8813 | 118.084 |

| NAG₂ | ASP50; TYR145; His206; His209 | CoH-B; CaH-B | 45.8682 | 103.574 |

| NAG₂ | ASP50; His206; Zn1255 | CaH-B; UnA-A; Un-B | 43.2916 | 81.1415 |

| NAG | ASP49; ASP50; TYR145; His206 | CoH-B; CaH-B | 24.2754 | 97.3587 |

| NAG | ASP49; ASP50; TYR145; His206 | CoH-B; CaH-B | 24.8467 | 95.2109 |

| NAG | ASP49; ASP50; TYR145; His206; Zn1255 | CoH-B; CaH-B; M-A | 18.2063 | 93.0567 |

| NAG | ASP49; TYR173; His206 | CoH-B; CaH-B; Pi-D | 28.9975 | 92.939 |

| ACT | TYR145; Zn1255 | CoH-B; M-A; A-C | 0.666834 | 41.9592 |

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**Table 2.** Molecular docking results based on the active group generated by adding ACT1256 catalytic subunit.

138 CoH-B: Conventional Hydrogen Bond; M-A: Metal-Acceptor; CaH-B: Carbon Hydrogen Bond; UnM-
139 D: Unfavorable Metal-Donor; Pi-Si: Pi-Sigma; Pi-D: Pi-Donor Hydrogen Bond; UnA-A: Unfavorable
140 Acceptor-Acceptor; UnD-D: Unfavorable Donor-Donor; Un-B: Unfavorable Bump; A-C: Attractive
141 Charge.
| Name | ligand non-bond monitor | Interaction | Absolute Energy | LibDock Score |
|------|-------------------------|-------------|-----------------|---------------|
| NAG₃ | ASP50; ASN78; TRP79; Ala107; | vDW; CoH-B; CaH-B; UnD-D; | 67.0774 | 137.545 |
|      | HIS108; **TYR145; His206** | Un-B | | |
| NAG₃ | ASP50; TRP79; Ala107; **TYR145**; | CoH-B; CaH-B; UnD-D | 73.7395 | 132.644 |
|      | **TYR173; His206; ACT1256** | | | |
| NAG₃ | ASP50; **TYR145; His206** | CoH-B; CaH-B; Pi-D | 72.5358 | 125.602 |
| NAG₂ | ASP50; TRP79; HIS108; **TYR173**; | CoH-B; CaH-B; Pi-D | 29.6294 | 91.8633 |
|      | **ACT1256** | | | |
| NAG₂ | ASP50; ASN78; **TYR145**; | CoH-B; P-LP; CaH-B; UnA-A; | 36.4111 | 91.253 |
|      | **TYR173; His206; His209** | UnD-D | | |
| NAG₂ | ASP50; **ASN78; TYR145; His206**; | CoH-B; CaH-B | 34.9803 | 90.6119 |
|      | His209 | | | |
| NAG₂ | ASP50; ASN78; **TYR145**; | CoH-B; CaH-B | 39.0936 | 90.0171 |
|      | **His206; His209** | | | |
| NAG | ASP50; ASN78; TRP79; **TYR145**; | CoH-B; CaH-B; UnD-D | 14.1647 | 89.6868 |
|      | **His206** | | | |
| NAG | ASP50; **ASN78; His206; His209** | CoH-B; CaH-B | 27.6388 | 85.2853 |
| NAG | ASP50; **His206; His209** | CoH-B; CaH-B; UnA-A | 24.4608 | 83.7609 |
| NAG | ASP50; **ASN78; TRP79; His209** | CoH-B; CaH-B | 18.1118 | 82.7795 |

CoH-B: Conventional Hydrogen Bond; M-A: Metal-Acceptor; CaH-B: Carbon Hydrogen Bond; UnM-D: Unfavorable Metal-Donor; Pi-Si: Pi-Sigma; Pi-D: Pi-Donor Hydrogen Bond; UnA-A: Unfavorable
Acceptor-Acceptor; UnD: Unfavorable Donor-Donor; Un-B: Unfavorable Bump; A-C: Attractive Charge; vDW: van der Waals; P-LP: Pi-Lone Pair.

From the docking results, it is noteworthy that ASP50, TYR145, His206, Zn1255 appear at high frequency in the docking of NAG, NAG₂, NAG₃ (Table 1). These groups are likely to be important sites for the binding of CICDA and chito-oligosaccharides, which means that if these sites are bound by other substances, they will competitively inhibit chito-oligosaccharides and then inhibit CICDA. This is of great breakthrough significance in inhibiting the deacetylation modification of chitin in the cell wall of pathogenic fungi, enabling the chitinase secreted by host cells to successfully recognize and hydrolyze chitin, thus controlling the infection of plant pathogenic fungi, and finding new inhibitors.

By comparing the docking results of whether the catalytic subunit contains ACT or not, it was found that the docking results of NAG, NAG₂ and NAG₃ after adding ACT were not as good as before. Absolute Energy and LibDock Score scores were lower than previous values. LibDock score is a comprehensive representation of van der Waals forces, hydrogen bonds, PI interactions, and other parameters. Higher the LibDock score and absolute energy means a high chance of ligand-protein binding[23, 24]. Analysis of the interaction groups revealed that none of the conformations had an effect on Zn1255 after the addition of ACT. According to the data, chitin deacetylases are representative members of the CE-4 family, which usually rely on metal-dependent mechanisms for acid/base catalysis[17]. It is inferred that Zn1255 plays an important role in deacetylation of chitin deacetylase-bound substrates, and the addition of ACT may block the interaction between Zn²⁺ and substrates, perhaps, Zn²⁺ chelators could act as inhibitors of CDA[25]. This provides important site information for searching for new inhibitors.

Generating Receptor-ligand Pharmacophores

Studying intermolecular interactions is important for structure-based drug design. Molecular docking is one of the commonly used methods, but in traditional docking methods, the accuracy of docking is often discounted because these programs can place compounds anywhere in the binding site, and the corresponding scoring equation often cannot find the most likely binding site. But in most cases, for a given binding site, which interaction plays a key role in ligand-receptor interaction is often known[26]. Because, from the complex structure, we can get the groups and their spatial distribution which contribute greatly to the activity of the inhibitors. In this case, the experience-based discovery of binding sites and known binding modes can be considered in the docking process to create a
pharmacophore model for docking. This will lead potential inhibitors to bind to known, energetically favorable interactions.

As far as the LibDock docking results of the catalytic subunit are concerned (Table 1), the first conformation of NAG₂ is linked to all the groups recurring at present, and the higher scores of Absolute Energy and LibDock Score are only second to highest scores. This means that the concept is well integrated with CICDA. Therefore, it is excellent to construct a pharmacophore model (CBP) based on receptor-ligand complexes using this conformation.

A total of 10 pharmacophore models were generated by CBP operation based on NAG₂ conformation. Fifty-six features were found in the ligands: HB_ACCEPTOR: 31, HB_DONOR: 25, and eight features of matching receptor-ligand interaction: AAAAADDD. The top 10 models with the highest Selectivity Score were retained after combining their permutations, as shown in Table 3. In combination with several eigenvalues of Sensitivity, Specificity, ROC, Selectivity Score and Feature Set, Pharmacophore_01 pharmacophore model is the best, with equal number of Character Set hydrogen acceptor donors and the best sensitivity and specificity. Although the ROC value is only 0.800 and less than 0.822, it is also a good score, and its Selectivity Score is far superior to other pharmacophores. Therefore, Pharmacophore_01 pharmacophore was selected as a model for the next potential inhibitor screening.

| Pharmacophore     | Number of Features | Feature Set | Sensitivity | Specificity | ROC | Selectivity Score |
|-------------------|--------------------|-------------|-------------|-------------|-----|-------------------|
| Pharmacophore_01  | 6                  | A₁A₂A₃D₄D₅D₆D₇ | 0.66667     | 0.93333     | 0.800 | 12.274            |
| Pharmacophore_02  | 6                  | A₁A₃A₄A₅D₆D₇   | 0.66667     | 0.93333     | 0.822 | 11.361            |
| Pharmacophore_03  | 6                  | A₁A₂A₃D₄D₅D₆D₇ | 0.66667     | 0.93333     | 0.822 | 11.361            |
| Pharmacophore_04  | 5                  | A₁A₃D₄D₅D₆D₇   | 0.66667     | 0.73333     | 0.744 | 10.760            |
| Pharmacophore_05  | 5                  | A₁A₃D₄D₅D₆D₇   | 0.66667     | 0.73333     | 0.656 | 10.760            |
| Pharmacophore_06  | 5                  | A₁A₂A₃D₄D₅D₆D₇ | 0.66667     | 0.73333     | 0.722 | 10.760            |
| Pharmacophore | 6  | A1A2A3A5D6 | 0.66667 | 0.93333 | 0.822 | 10.447 |
| Pharmacophore_08 | 5  | A1A2D6D7 | 0.66667 | 0.66667 | 0.756 | 9.8460 |
| Pharmacophore_09 | 5  | A2A3A5D8 | 0.66667 | 0.86667 | 0.767 | 9.8460 |
| Pharmacophore_10 | 5  | A1A2A5D6D7 | 0.66667 | 0.73333 | 0.767 | 9.8460 |

A = Acceptor; D = Donor.

Fig. 4. (A) Chitin deacetylase crystals (PDB code: 2iw0) with interaction diagram of co-crystalline ligand NAG2 and pharmacophore_01, (B) co-crystalline ligand NAG2 and pharmacophore_01, (C) pharmacophore_01.

**CBP Pharmacophore Model-Based Virtual Screening**

Fit value is an index to measure the overlap between pharmacophore characteristics and molecular chemical characteristics, which is helpful to understand the chemical significance of pharmacophore hypothesis[16, 17]. We achieved 10,632 small molecules from the DrugBank database for virtual screening. Finally, 49 HIT compounds mapped to the pharmacophore model Pharmacophore_01 were retrieved according to Best Fit Value in the docking results. The obtained compounds matched well with the CBP model.

**Molecular Docking**
The key characteristic of a good docking program is its ability to reproduce the experimental binding modes of ligands. To test this, a ligand is taken out of the X-ray structure of its protein–ligand complex and docked back into its binding site. The docked binding mode is then compared with the experimental binding mode, and a root-mean-square distance (RMSD) between the two is calculated; a prediction of a binding mode is considered successful if the RMSD is below a certain value (usually 2.0 Å) [27]. In this study, docking analysis of the active site of CICDA was performed using DS 2016. Ligands in protein 2iw0 were extracted. The docking method adopts two docking methods, LibDock and CDOCKER. Subsequently, the binding positions of the docked compounds were compared with the ligands in the crystal complexes, and the RMSD values deviations were calculated to be 0.2587 and 0.3410, respectively. It can be seen from Fig. 5 that the ligands docked by these two docking methods are well aligned with the ligands in crystal complexes, which proves the accuracy and reliability of these two docking methods.

![Fig. 5](image)

**Fig. 5.** Alignment of the docked ligands with the ligands in the crystallographic complex. (A) The ligand by the LibDock docking method; (B) The ligand by the CDOCKER docking method.

Receptor-based virtual screening of 49 molecules recovered after pharmacophore-based screening was performed using the LibDock method. Compounds with successful docking were selected, and the selected screened hits were then docked accurately in the CDOCKER module. Finally, the CDOCKER module calculated 44 HIT compounds as targets. We selected the top 20 molecules with the highest scores of LibDock Score and CDOCKER ENERG, respectively, and found seven common small molecules from them. The docking results of 7 small molecules are as follows: Table 4, Fig. 6.

**Table 4.** Docking results of 7 potential chitin deacetylase inhibitors.
| Name    | Structural Formula | LibDock Score | -CDOCKER ENERG | Fit Value |
|---------|--------------------|---------------|----------------|-----------|
| ACT     | ![ACT Structure](image)  | 41.9592       | 42.7319        |           |
| DB02470 | ![DB02470 Structure](image) | 111.272       | 39.7564        | 3.62138   |
| DB02824 | ![DB02824 Structure](image) | 112.136       | 64.0163        | 2.83566   |
| DB03227 | ![DB03227 Structure](image) | 112.370       | 36.5474        | 2.80273   |
| DB03846 | ![DB03846 Structure](image) | 124.264       | 37.1136        | 2.88979   |
DB04603

DB05446

DB11296

227
Fig. 6. The receptor-ligand interaction of screening compound with the 2iw0 active site. (A) DB02470 Receptor-ligand interaction with 2iw0 active site. (B) DB02824 Receptor-ligand interaction with 2iw0 active site. (C) DB03227 Receptor-ligand interaction with 2iw0 active site. (D) DB03846 Receptor-ligand interaction with 2iw0 active site. (E) DB04603 Receptor-ligand interaction with 2iw0 active site. (F) DB05446 Receptor-ligand interaction with 2iw0 active site. (G) DB11296 Receptor-ligand interaction with 2iw0 active site. (H) ACT Receptor-ligand interaction with 2iw0 active site.
It is not difficult to find through the receptor-ligand interaction of screening compound with the 2iw0 active site that only DB02470 and DB03227 interact with the active groups ASP50, TYR145, His206, Zn1255. Compound DB02470 formed generated hydrogen bond with ASP50, TYR145, and HIS206 and generated metallic bond interactions with ZN1255 as depicted in Fig. 6(A); Compound DB03227 formed π-π stacking interactions with TYR173, generated hydrogen bond with ASP50, TYR145, and HIS206 and generated metallic bond interactions with ZN1255 as depicted in Fig. 6(C).

**Molecular Dynamics Simulations**

Molecular dynamics is the pivotal theoretical approach which can be utilized to gain molecular insight into the stability of the binding pose of the screened molecules in the active site[28]. MD simulations yield energetically favorable conformations by optimizing a protein-ligand complex, which is needed to understand protein–ligand interactions and ligand binding induced structural changes[29]. So, according to the results of molecular dynamics simulation, the binding stability of the selected compounds can be verified. The RMSD is commonly used as an indicator of convergence of the structure towards an equilibrium state and is most meaningful for low values. To evaluate the stability of 2iw0 ligand complexes under dynamic conditions, we performed molecular dynamics (MD) simulations using DS. Preliminary conformations were obtained by CDOCKER molecular docking experiments. We sampled 100 data points by setting a regular interval from the 200ps simulation trajectory. RMSDs of protein-ligand complexes are shown in Fig. 7. The average value of RMSD for each ligand was calculated over the simulation trajectory. The average RMSD value of protein-ligand complex with DB03227, DB02470 or 2iw0 was 1.11528Å, 0.988519Å and 0.97396 Å, respectively. The RMSD trajectory of the complex was more equilibrium after 100ps, compared with 2iw0. The total energies and potential energies of ligand-protein complexes were approximately identical to each other for the 200ps simulation (shown in Fig. 8 and Fig. 9). Among them, DB03227 has the lowest total energy and potential energies with the receptor. In addition, through molecular dynamics simulation, DB02470 and DB03227 both formed hydrogen bonds with water molecules (shown in Fig. 10). These hydrogen bonds may contribute to the stability of the complexes. Combined with each evaluation index, these two compounds may interact stably with 2iw0 and have potential negative regulatory effects on 2iw0.
Fig. 7. The RMSD values of protein-ligand complexes during MD simulation.

Fig. 8. Potential energies of protein-ligand complexes during MD simulation.
**Fig. 9.** Total energies of protein-ligand complexes during MD simulation.

![Graph showing total energies of protein-ligand complexes during MD simulation.](image)

**Fig. 10.** The compounds were screened for receptor-ligand interaction with the 2iw0 active site after MD simulation. DB02470 on the left and DB03227 on the right.

![Molecular structures of DB02470 and DB03227 with interactions highlighted.](image)

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**Discussion and Conclusions**

In pathogenic plants, CDA is a heavily glycosylated secreted enzyme allegedly playing a role in the host-pathogen interaction, deacetylating the chitin oligomers resulting from the activity of plant chitinases on the fungal cell walls, thereby evading plant immune defense[7]. Therefore, to find an inhibitor of CDA to weaken its activity is a promising target to resist the infection of phytopathogenic fungi. However, there have been few reports on CDA inhibitors. Only 1 inhibitor, acetate ion (ACT), the ligand selected as the reference in this study, has been part of relatively mature research until now.
In this study, 10,632 small molecules taken from the DrugBank database for virtual screening, was followed by CBP pharmacophore, LibDock, CDOCKER and molecular dynamics simulation. LibDock and CDOCKER scores unfolded degree of energy optimization and stability of the conformation. High LibDock and CDOCKER score compounds illustrated better energy optimization and a stable conformation than lower score achievers[30]. After calculation by the LibDock and CDOCKER module, 44 compounds showed to be capable to bind stably with CICDA. Besides, among these ligands, 7 compounds had higher LibDock and CDOCKER scores than ACT (LibDock score: 41.9592; CDOCKER score: -42.7319), indicating that these 7 compounds could form a more stable complex with CICDA with better energy optimization compared with ACT.

Then, the chemical structures of the 7 compounds were analyzed by molecular structure inspection. The 7 complexes that CICDA combined with 7 candidacies have more chemical bonds than ACT (show in Fig. 6), which again indicates that these 7 compounds could bind with CICDA at active site more stably. In addition, DB02470 and DB03227 can interact with all the active groups ASP50, TYR145, His206, Zn1255, which again demonstrates the reliability of again demonstrates the reliability of the above suspected active sites, and they may contribute to competitive inhibition of activity of CICDA.

Finally, their stability in the natural environment were assessed performing molecular dynamics simulation, and it is computational results showed that RMSD, potential energy and total energy of these ligand-CICDA complexes tend to stability with time (show in Fig. 7, 8 and 9), which suggested that these 2 complexes could exist in the natural environment stably. Molecular dynamics module computation confirmed that RMSD of DB03227 and DB02470 were obviously lower than the reference ligand ACT, which demonstrates these 2 compounds may have a higher stability with CICDA compared with ACT.

Based on these results, drugs designation and development, such as modification and refinement, could be prospectively carried out to make combination of ligand and receptor more stable[30]. Since these compounds were virtual screening and their inhibitory activities have not been reported, we will conduct experiments such as IC50 and EC50 measurements in further studies to detect their biological activities[21].

In this study, 4 modules of discovery studio 2016, including CBP pharmacophore, LibDock, CDOCKER and molecular dynamics simulation, were used to screen and analyze the biochemical structure characteristics of novel potential compounds. Molecular conformation, binding affinity and
stability of the selected compounds were calculated and analyzed to determine their advantages over the
control compound act. A series of high-tech computational studies indicate that these 2 compounds may
have potential effects in inhibitors of CICDA. Furthermore, our study provides guidance for the screening
of lead compounds with potential inhibitory effects. Through this method, more leading compounds
could be screened out, so as to improve the current inhibitor development and improve the efficiency of
inhibitor development.

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