

Discovery of a novel histone deacetylase 8 inhibitor by virtual screening

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Abstract In order to develop potent histone deacetylase inhibitors, a virtual screening approach was performed to discover novel lead structures. A commercial database containing about 167,000 molecules was in silico filtered by rule of five, zinc-binding groups, pharmacophore models, and binding pattern analysis. At last, three molecules were selected for enzyme inhibition assay, and one compound **02** has IC₅₀ of 1.6 μM against histone deacetylase 8 (HDAC8).

Keywords Histone deacetylase inhibitors · Virtual screening · Pharmacophore modeling · Docking

Introduction

Histone deacetylases (HDACs) are responsible for deacetylating the *N*-terminal tails of histones, and the corresponding chromatin alterations regulate transcription and other nuclear events (Giandomenico *et al.*, 2003; Gronroos *et al.*, 2002; Jin *et al.*, 2004; Minucci and Pelicci, 2006). Overexpression and aberrant recruitment of HDACs have a significant role in tumorigenesis. So far, 18 members of the HDAC family have been discovered and subdivided into 4 classes (De Ruijter *et al.*, 2003; Gregoretta *et al.*, 2004). Among these classes, class I HDACs (HDAC1, 2, 3, and 8) were proved to be the most correlative to the pathogenesis of cancer (Bernstein *et al.*, 2000; Foglietti *et al.*, 2006; Lagger *et al.*, 2002; Sun and Hampsey, 1999).

HDAC inhibitors (HDACis) have the effects of apoptosis, cell cycle arrest, and differentiation (Insinga *et al.*, 2005;

Johnstone, 2002; Marks *et al.*, 2001; Nebbioso *et al.*, 2005). Suberoyl anilide hydroxamic acid (SAHA) (Richon *et al.*, 1998) is the first HDACi approved by FDA for the treatment of cancer, and many promising HDACis are still under investigation in various stages of clinical trials (Zhang *et al.*, 2008).

Virtual screening is a powerful method for discovering novel HDACis (Liu *et al.*, 2010; Tang *et al.*, 2009; Thangapandian *et al.*, 2010). It is well known that the zinc-binding groups (ZBGs) in HDACis make significant contributions to the binding profile of HDACis around active site. In this article, a ZBG-based virtual screening was performed for searching novel HDAC8 inhibitor scaffold. The harvested candidates with reasonable physico-chemical properties were chosen for the further biological assay.

Materials and methods

Preparation of compound database

The SPECS chemical database (April 2009, downloaded from <http://www.specs.net>) was used in the current virtual screening process. All molecules in the database were converted to 3D conformations in UNITY database by CONCORD module in the SYBYL 7.3 software package. This database was filtered by “rule of five” (Lipinski *et al.*, 2001), in which molecules having >5 number of H-bond donors, >10 of H-bond acceptors, >500 molecular weight, and >5 calculated Log P were excluded.

ZBG-based virtual screening

Hydrosulfide group, hydrazide group, and several other groups were defined as ZBG. Subsequently, the druglike

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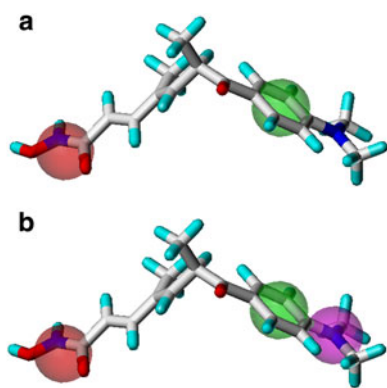


Fig. 1 The pharmacophore models used for virtual screening. The features were manually defined by UNITY in SYBYL7.3 software package. The molecular structure is TSN; the red ball is negative center; the green ball is hydrophobic center; the magenta ball represents H-bond acceptor site

database was screened by the defined ZBG criteria. As a result, the database was subdivided into several small databases. Obviously, these different sub-databases did not contain the same number of molecules. If there are more than 1,000 molecules in a sub-database, then this database will be screened by a pharmacophore model featured by a negative charge center and a hydrophobic point (Fig. 1a). If the number of molecules is still more than 1,000, then the refiltered database will be screened by a more restrict pharmacophore model (Fig. 1b) which contains three features (a negative charge center, a hydrophobic point, and an H-bond acceptor).

Docking study

The FlexX2 program (Rarey *et al.*, 1997; 1996) in the SYBYL 7.0 package was used for the docking analysis. The *holo*-form crystal structure of HDAC8 (PDB entry: 1T64) was used as the receptor (Somoza *et al.*, 2004), the derived sub-databases were docked into the active site of HDAC8, respectively. 30 docking patterns were generated

for each molecule, and CSCORE was applied to evaluate their binding qualities. The ligand TSN (Fig. 2) in the crystal structure of HDAC8 functioning as a positive control was also docked to the active site. The scored molecules of each database were analyzed by the manual examination compared to TSN.

Activity assay

The method for *in vitro* activity assay has been described in our previous study (Zhang *et al.*, 2010). In brief, the HDAC8 enzyme was expressed in *Escherichia coli*. Boc-Lys (acetyl)-AMC was used as the substrate of HDAC8. Vorinostat, the HDACi on market, was employed as a positive control. The compounds were serially diluted into six concentrations (100, 20, 4, 0.8, 0.16, and 0.032 $\mu\text{M/l}$) to probe their ability of inhibiting HDAC8 activity.

Result and discussion

Virtual screening

The number of molecules in the database was reduced from 167,475 to 157,822 by Lipinski's rule of five. Afterward, the refined database was filtered by the defined ZBGs. There are 5,227 molecules containing carboxylic acid moiety, 2433 molecules having amide group, and 1864 molecules possessing carbamido substructure, respectively (Table 1). To save computational time, sub-databases containing these three groups were refiltered by a two-feature pharmacophore model. After this filtration, there are 39 molecules having amide group, 63 molecules having carbamido group, and 3,812 molecules containing carboxylic acid group, individually. Consequently, the 3,812 carboxylic acid group containing molecules were filtered by a three-feature pharmacophore, and only 411 molecules were survived for the docking study.

Fig. 2 Structures of ligand TSN and molecule 01, 02, and 03

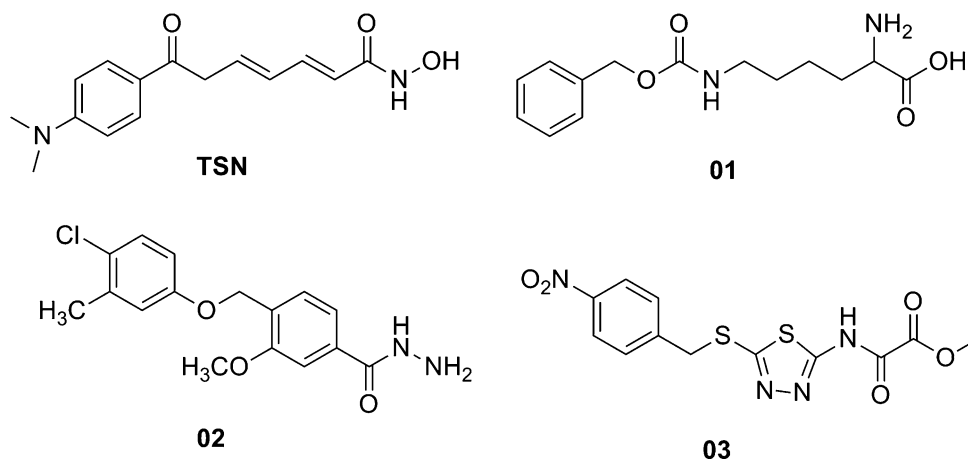
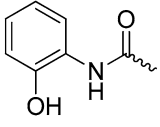
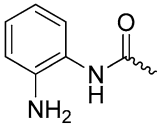
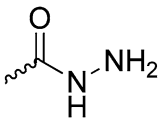
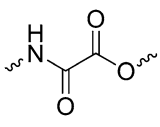
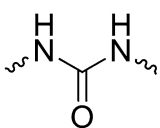
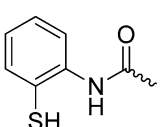
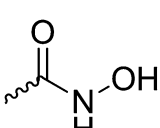
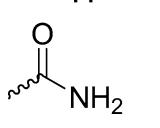
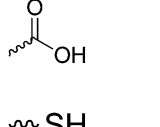
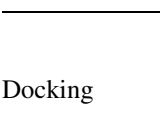


Table 1 Summary of the virtual screening results

ZBGs	Number of screened molecules	Number of molecules used for docking	Top N of TSN in the docking results
	53	53	1
	5	5	1
	103	103	1
	18	18	1
	1864	63	1
	5	5	1
	4	4	1
	2433	39	1
	5227	411	3
	731	731	1

Docking

Totally 1,432 molecules were in silico screened by ZBG- and pharmacophore-based screening, following by docking analysis (Table 1). The ligand TSN in the active site of HDAC8 was first docked to the crystal structure. The docked conformation sharing very low RMSD value (1.72 Å) with the crystal structure of TSN suggested that this molecular docking method was reliable (Fig. 3).

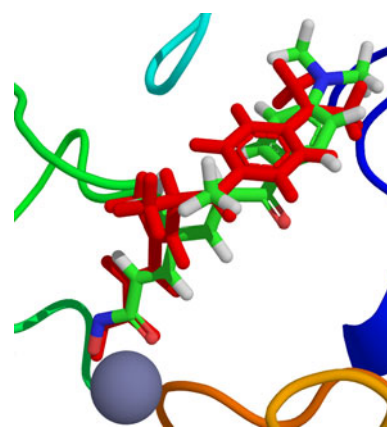


Fig. 3 Comparison of the docked pattern of TSN (colored by atom type) to the crystal structure of TSN (colored red) in the active site of HDAC8. The picture was generated by PyMOL (DeLano, W. L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, Palo Alto, CA, USA)

Only two molecules containing carboxylic acid groups had a higher score than the ligand TSN (Table 1). The top 100 scored molecules in the carboxylic acid group and hydrosulfide group databases were analyzed by visual examination. Our attention focused on three compounds **01** (database ID: AI/204-31755055), **02** (database ID: AK/968-41922428), and **03** (database ID: AA/516-31409020) (Fig. 2), although many molecules had good binding patterns in the active site of HDAC8. Figure 4 shows the location of these three molecules around the binding site. The carbonyl groups of **01** (Fig. 4a), the amino group and carbonyl group of **02** (Fig. 4b), and carboxyl group of **03** (Fig. 4c) stretched inside the channel to bind to the zinc ion. Multiple H-bond interactions can be found between the molecules and amino acid lined in the active site of HDAC8 (Fig. 5). Molecule **01** has H-bond interactions with residues Gly151 and Gly304 (Fig. 5a), in the meanwhile the amino group of molecule **02** functioning as an H-bond donor binds to His142 and His143 (Fig. 5b). The carbonyl group could contact Tyr306 as an H-bond acceptor. Moreover, molecule **03** has H-bond interactions with five residues (Fig. 5c). They are Asp101, His142, His143, Gly151, and Tyr306, respectively.

Activity assay

Three candidates were evaluated for their ability to inhibit HDAC8. Accordingly, only **02** showed potent inhibitory activity against HDAC8 with IC_{50} of 1.6 μ M in comparison to Vorinostat (1.48 μ M) (Fig. 6). This evidence demonstrates that the hydrazide group is one of the potent ZBGs. One case in point is molecule **02**, which would be a lead compound in further chemical modification and optimization.

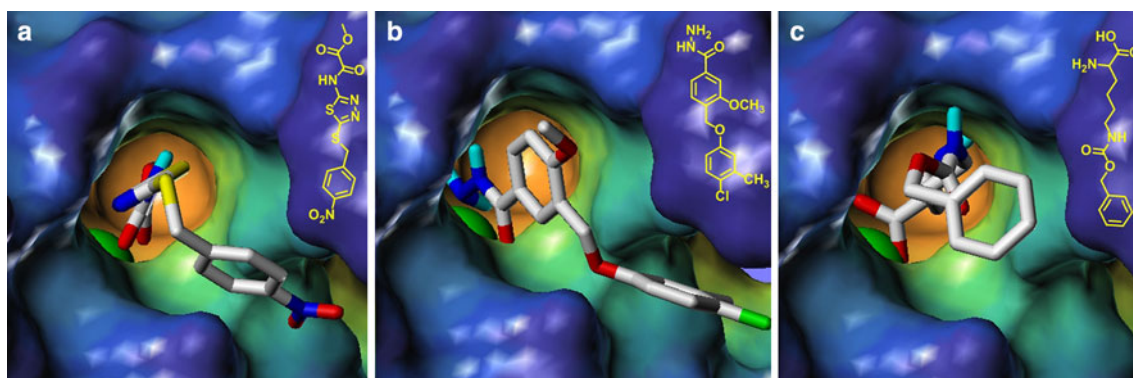


Fig. 4 Molecule **01** (a), **02** (b), and **03** (c) in the active site of HDAC8. The *green ball* is zinc ion. The surface was created by MOLCAD in SYBYL7.3

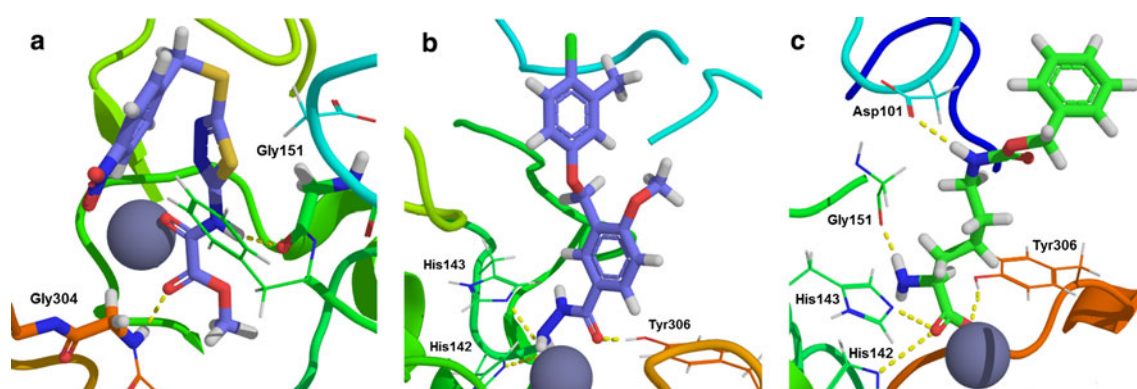


Fig. 5 Binding patterns of molecule **01** (a), **02** (b), and **03** (c) in the active site of HDAC8. The *gray ball* is zinc ion; the *dot lines* represent H-bond. The pictures were created by PyMOL

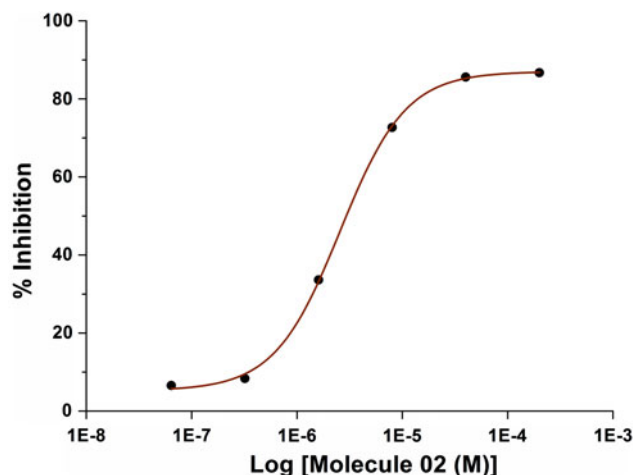


Fig. 6 IC₅₀ plot of molecule **02** against HDAC8. The X axis is log of molecule **02** at concentration of mol/l, and the Y axis is the percentage of inhibition

Conclusion

The current report disclosed a virtual screening approach for discovering novel HDACis. Various filter rules such as

ZBGs, pharmacophore models, and docking methods were applied to minimize the candidate pool. The activities of four molecules were determined by in vitro enzyme inhibition assay. One molecule, **02**, containing hydrazide group showed potent inhibitory activity against HDAC8. Our further study will be concentrated on chemical modification of this molecule.

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