Application of molecular modeling in predicting palliative features of HDAC4 PROTACs against Spinal Muscular Atrophy

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Abstract: Developing HDAC4-targeting PROTACs could offer a cost-effective strategy for SMA palliation by promoting selective HDAC4 degradation, reducing atrogin-1 and MuRF1 upregulation, and thereby slowing muscle protein loss and atrophy progression. The design of HDAC4-targeting PROTACs in this study relied on the irreversible structure-based alignment of co-crystallized HDAC4 inhibitors (HDAC4Is) to identify optimal zinc-binding groups, which were then used as anchoring moieties for autonomous fragment-based PROTAC construction with linkers and VHL-1 or CRBN ligands, using the in-house Py_AutoPROTAC_Designer workflow. Modeled HDAC4:PROTAC:E3 ligase ternary complexes revealed, by applying a rank-by-rank strategy, high-affinity and synthetically tractable candidates, exhibiting predicted potencies in the picomolar range, ready to be promptly synthesized and submitted to enzymatic assays, cellular (*in vitro*) models, and animal (*in vivo*) studies.

Keywords: SMA, HDAC4, PROTACs, covalent docking, autonomous design

1. Introduction

Spinal muscular atrophy (SMA) is a rare neurodegenerative disorder caused by SMN1 loss, in which disease progression triggers HDAC4 overexpression that promotes muscle protein degradation *via* a myogenin-dependent pathway [1]. This study introduces a computational workflow using Py_AutoPROTAC_Designer to design HDAC4-targeting PROTACs from HDAC4Is ZBG fragments, linkers, and E3 ligase ligands, combining protein–protein docking and structure-based alignment to build ternary complexes and predict high-affinity candidates.

2. Methodology

2.1. Irreversible structure-based alignment assessment

Irreversible SBAA of HDAC4Is was performed with Vina, by covalently linking ZBGs to Zn^{2+}, and docking of water molecule while treating Zn^{2+}–HDAC4I as flexible.

2.2. Py_AutoPROTAC_Designer workflow

Annotated SMILES of warheads, linkers, and E3 ligase ligands were joined *via* a custom RDKit workflow, then filtered by PAINS and ADMET to prioritize drug-like PROTACs.

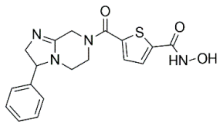
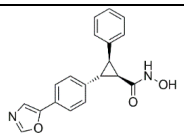
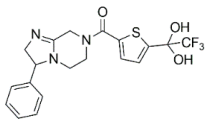
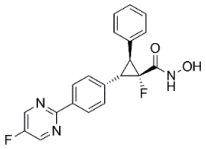
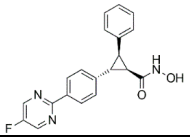
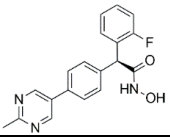
2.3. HDAC4:PROTAC:E3 ligase ternary complex modelling

HDAC4*PROTAC:*/VHL-1 or CRBN complexes were generated with HADDOCK3/Vina.

3. Results and Discussion

To the best of the authors' knowledge, there are only 9 experimental HDAC4 structures in complex with HDAC4Is, primarily belonging to two classes: hydroxamic acids (PDB entry codes: 2VQM, 2VQV, 4CBT, 4CBY, 5A2S, and 6FYZ) and trifluoromethyl ketone derivatives (PDB entry codes: 2VQJ, 2VQQ, and 2VQO) (Table 1). Thereafter, distinct ligands were used for irreversible structure-based alignment assessment (SBAA) procedures (Table 2) to reveal the best-performing docking program for subsequent cross-docking of the here-reported PROTACs.

Table 1. PDB Codes, Ligand Structures, and Potencies of Histone Deacetylase 4 Inhibitors (HDAC4Is) Co-crystalized within either Wild Type (WT) and Mutated (MUT) protein.

PDB ID (Type)	Ligand Structure	IC_{50} (nM)	PDB ID (Type)	Ligand Structure	IC_{50} (nM)
2VQM (WT[a]) 2VQV (MUT[b])		978	**4CBY (CMUT)**		20
2VQJ (WT) 2VQO (MUT) 2VQQ (MUT)		367	**5A2S (CMUT)**		10
4CBT (CWT)		60	**6FYZ (CWT)**		36

[a]Co-crystalized in wild-type HDAC4; [b]Co-crystalized in mutated HDAC4.

3.1. SBAA

Molecular docking program Vina, with the vina_config setup exerted high docking

accuracy (DA) in both experimental conformation (EC) and randomized conformation (RC) re-docking (RD) and cross-docking (CD) stages (Figure 1). Due to their synthetic feasibility, (S)-2-(2-fluorophenyl)-N-hydroxy-2-phenylacetamide from 6FZY as ZBG was selected for further autonomous PROTAC design.

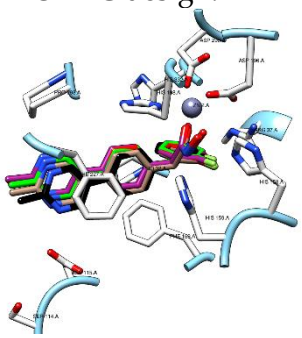


Figure 1. Irreversible SBAA of 6FYZ. EC pink, ECRD green, ECCD black, RCCD tan.

3.2. PROTACs autonomous design

Thus generated SMILES of warheads were alongside the available linkers and E3 ligase ligands incorporated in Python's RDKIT module for automated PROTAC generation, for which the SMILES placeholders were replaced with wildcards for RDKit processing, fragments (warhead, linker, E3LL) were concatenated into indexed combined molecules, and boundary indices were defined for region-specific analysis and atom-level tracking (Figure 2). Each designed PROTACs was evaluated through a multi-stage *in silico* pipeline integrating descriptor-based screening, PAINS and substructure filtering, and predictive models for ADMET, enabling rapid prioritization of drug-like, synthetically feasible candidates.

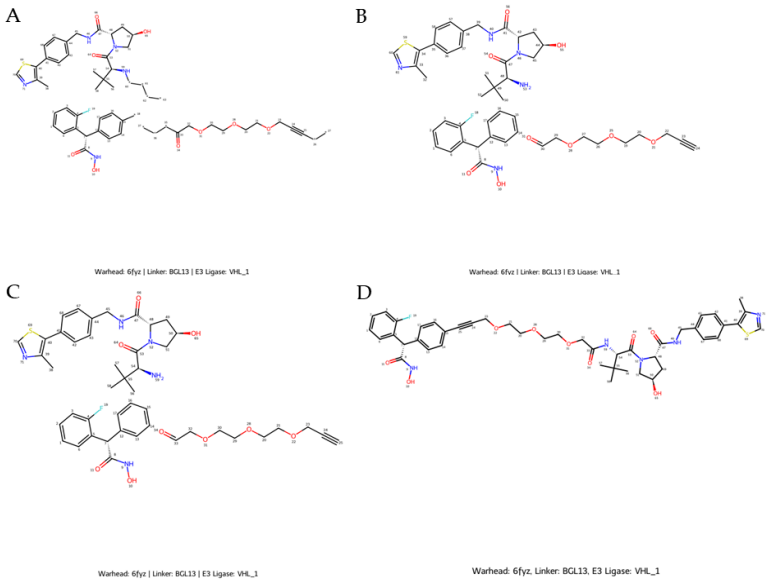


Figure 2. Intermediates of Py_AutoPROTAC_Designer workflow: (A) Calculated segment indices, (B) wildcards removal and neighbours identification; (C) original atom indices assignment; (D) designed PROTAC molecule.

3.3. HDAC4:PROTAC:E3 ligase ternary complexes

Ternary complexes modeling revealed several high-affinity PROTAC molecules having Gibbs free energy of binding in the range below -11 kcal/mol. By converting the scoring function outcome into the potency, all of the molecules were predicted to have EC_{50} values in pM range, distinguishing perhaps PROTAC internally called TG-49 with EC_{50} equal to 121 pM.

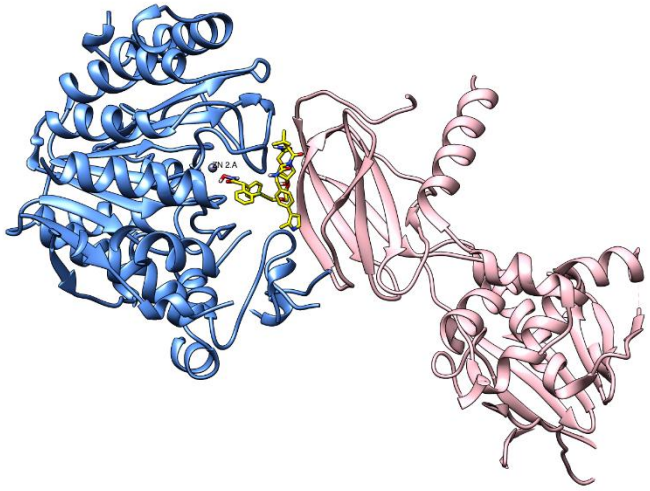


Figure 3. HDAC4:TG-49:VHL-1 ternary complex. PROTAC molecule is depicted in yellow, HDAC4 in cornflower blue ribbon, VHL-1 E3 ligase in plum ribbon.

4. Conclusions

The enclosed methodology shows strong potential for autonomous PROTAC design and affinity prediction.

Acknowledgment

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References

[1] N. Tomašević, *Histone deacetylase 4 (HDAC4), an epigenetic target for spinal muscular atrophy*, Biologica Serbica, 45 (2023) 52-64.