ECMC-P 2025 Conference

The 1st International Electronic Conference on Medicinal Chemistry and Pharmaceutics

01–30 November 2025 | Online

MDPI

# HDAC4 PROTACs as a potential palliative care therapy for Spinal Muscular Atrophy: Impact of the various linkers on enzymatic activity

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## INTRODUCTION & AIM

HDAC4 is a class IIa member of histone deacetylases (HDACs), playing a role in regulating gene expression through chromatin remodeling, as well as in muscle alterations. It triggers the atrogin-1 and MuRF1 upregulation, muscle protein degradation, and slow atrophy progression, symptoms associated with Spinal Muscular Atrophy (SMA), for which it is considered an important target for corresponding palliative care therapeutic development.

## METHOD

In that sense, our interests were to develop selective PROTAC degraders of HDAC4 using known co-crystalized HDAC4 inhibitors (HDAC4Is) available at Protein Data Bank as starting points, in particular the truncated version of 6FYZ HDAC4I as a warhead, stripped of Cap moiety as instructed by the generated preliminary Py-CoMFA 3-D QSAR model (Figure 1).

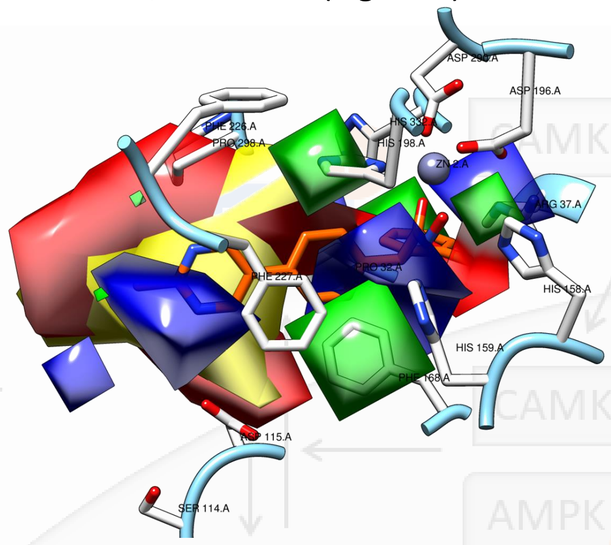


**Figure 1**. The Py_CoMFA 3-D QSAR model of HDAC4 inhibition derived from the CH1 probe by means of using www.3d-qsar.com

The primary objective during the initial profiling phase was to investigate their interactions with HDAC4 and assess how structural modifications influence potency. To that, the PROTACs were initially autonomously designed by means of Python's RDKit by merging the warhead with the *in-house* available linkers and either VHL-1 or CRBN E3 ligase ligands (Figure 2), respecting the desirable PK/PD profiles.

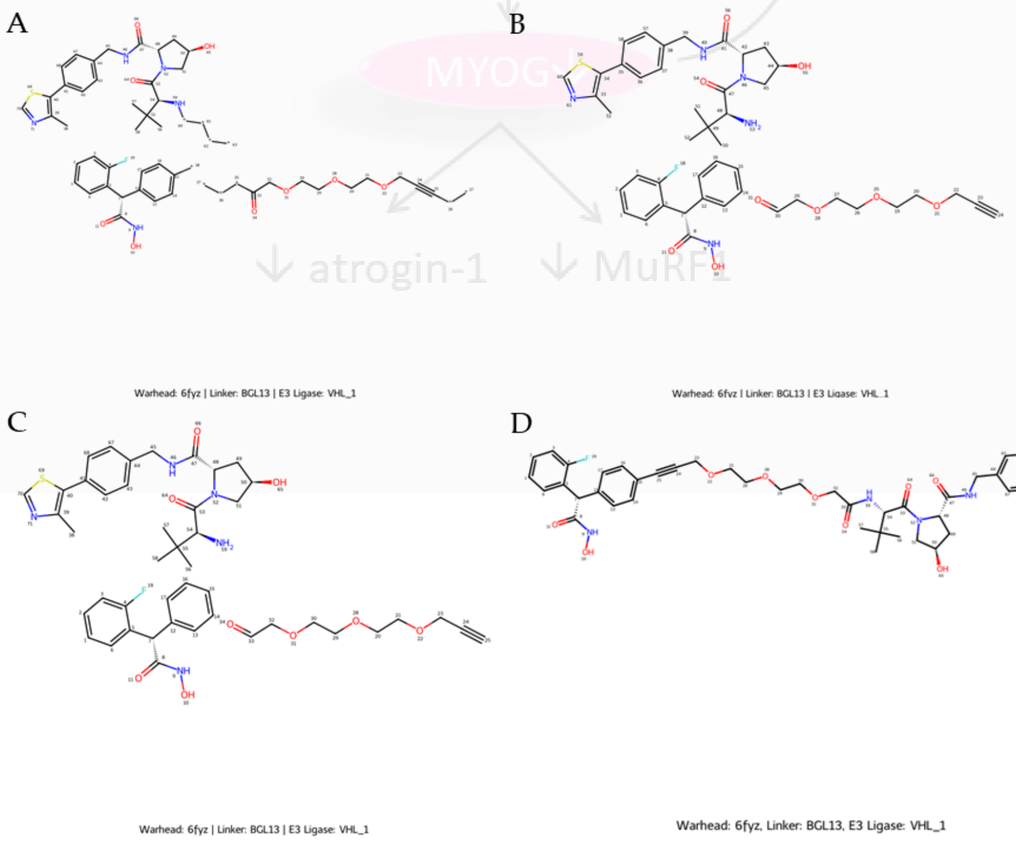


**Figure 2**. Intermediates of Py_AutoPROTAC_Designer workflow: (A) Calculated segment indices, (B) wildcards removal and neighbors identification; (C) original atom indices assignment; (D) designed PROTAC molecule.

## RESULTS & DISCUSSION

The ligand PJ- 618K was synthesized according to the literature procedure [1]. Sonogashira coupling of this compound with the appropriate alkyne, followed by hydrolysis in a basic medium, yielded the acid derivatives PJ-581 and TG-46. Amidation of these acids with CRBN and VHL ligands, followed by deprotection of the protected hydroxamic acid, afforded the molecules PJ-594 and TG-49.

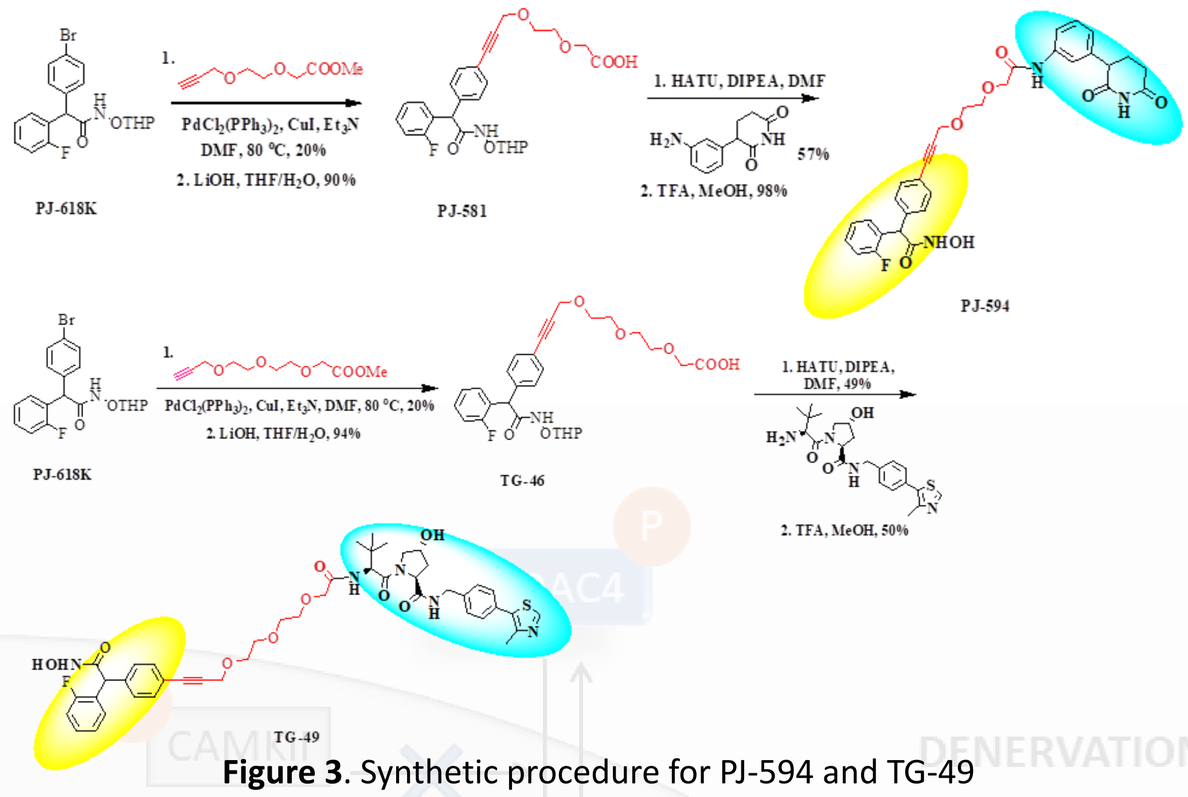


**Figure 3**. Synthetic procedure for PJ-594 and TG-49

Selected hits were promptly synthesized and submitted to enzymatic fluorogenic assaying, of which TG-49 and PJ594, as VHL-1-related PROTACs, showed remarkable $IC_{50}$s of 50 and 150 nM.

**Table 1.** Inhibitory activity of HDAC4 inhibitors and related PROTACs against the recombinant HDAC4, as determined by means of the fluorogenic assay

| Cmpd. | HDAC1 | HDAC4 | HDAC6 | HDAC7 |
|---|---|---|---|---|
| **PJ618-K** | > 10 ($IC_{50}$ μM) | 0.500 ± 0.235 ($IC_{50}$ μM) | 0.0051 ± 0.0012 ($IC_{50}$ μM) | 0.100 ± 0.092 ($IC_{50}$ μM) |
| | | **VHL-1-based PROTAC** | | |
| **TG-49** | > 10 | 0.05 ± 0.05 | > 10 | 0.001 ± 0.001 |
| | | **CRBN-based PROTAC** | | |
| **PJ594** | > 10 | 0.150 ± 0.114 | > 10 | 0.500 ± 0.268 |

## CONCLUSION

The synthetic and biochemical analysis revealed that even the minor changes in the linker structure resulted in significant variations in the inhibition of HDAC4, suggesting that the linker may play a critical role in mediating interactions with HDAC4. Yet, confirmation is pending with further ternary complex computational modeling and HDAC4 cellular degradation assays.

## FUTURE WORK / REFERENCES

1. Bioorg. Med. Chem. Lett**. 2019**, *29*, 83–88.

This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 7490, Artificial Intelligence-Guided Design, Synthesis, and Pharmacological Evaluation of Innovative PROTACs as Degraders of HDAC4, an Epigenetic Target for Spinal Muscular Atrophy - SM*AI*PROTACs

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