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Drug Design and Optimisation at the Dihydroorotate Dehydrogenase (DHODH) Receptor using the Teriflunomide Scaffold as a Lead.

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INTRODUCTION

Mouse studies show that DHODH inhibition is a novel route for the treatment of epilepsy. Teriflunomide has been identified as a potent DHODH inhibitor with poor intra-cerebral penetration due to high polarity.

AIMS

To use the teriflunomide scaffold to identify DHODH inhibitors with improved non-polar characteristics, predisposing to better intracerebral penetration, by means of virtual screening and *de novo* drug design.

METHOD

Virtual Screening (VS)

Identification of PDB crystallographic depositions 1D3H[2] and 3U2O[3]. → **Modelling of teriflunomide, the biaryl analogue and their combined consensus pharmacophores in LigandScout®.** → **VS and filtering of hits for Lipinski Rule compliance in ZINCPharmer® and MONA®.** → **Docking of hits into the DHODH protomol in SYBYL-X®.** → **Identification of hits with the highest affinity for the DHODH receptor and a desirable clogP.** → **Toxicity prediction for the selected hits in Pro-Tox 3.0.**

De novo design

Modelling of a 2D topology map describing the ligand protein contacts between the DHODH receptor and teriflunomide in BIOVIA Discovery Studio®. → **Determination of clogP of teriflunomide.** → **Molecular growth of created seed structures in the DHODH ligand binding pocket using LigBuilder®.** → **Identification of molecules with the highest binding scores and a desirable clogP.** → **Toxicity prediction for the selected hits in Pro-Tox 3.0.**

RESULTS

Virtual Screening: 299 Lipinski Rule compliant hits.

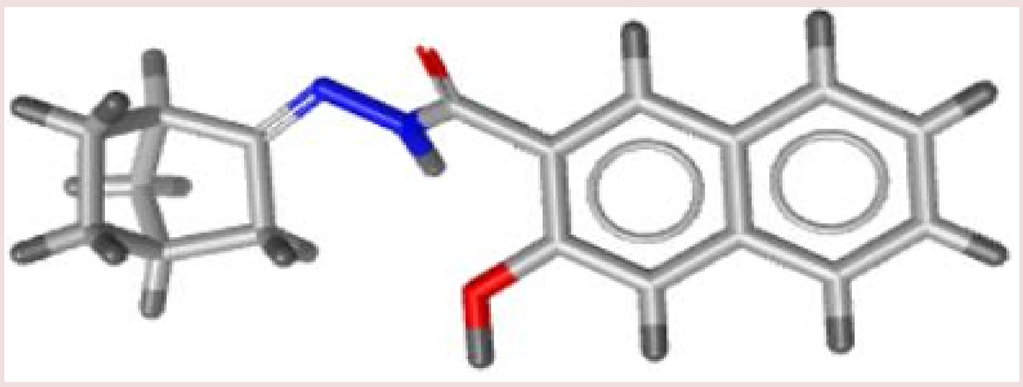

Figure 1.1: 3D structure of ZINC06536819, having the best properties of the VS cohort.

***De novo* design: 6 Lipinski Rule compliant hits.**

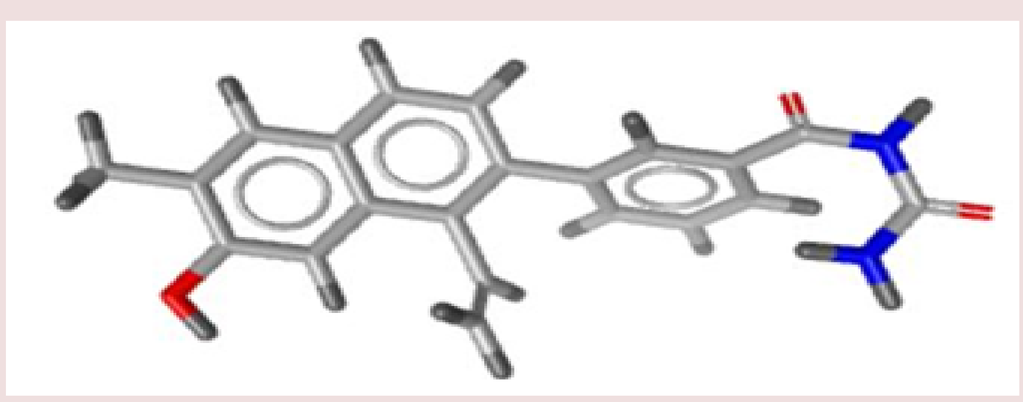

Figure 1.2: 3D structure of Seed30_Result195, having the best properties of the *de novo* cohort.

Table 1.1: Properties of the selected molecules.

	ZINC06536819	Seed30_Result19
clogP	3.45	4.96
Binding affinity	4.83 (Total Score)	8.45 (pKd)
Predicted toxicity class	4 (Harmful)	3 (Toxic)

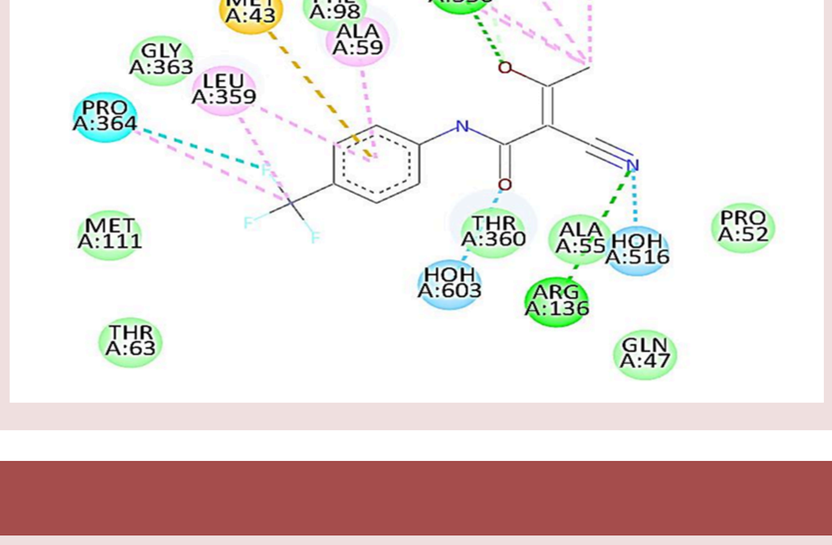

Figure 1.3: 2D topology map showing critical interactions between teriflunomide and the DHODH receptor.

CONCLUSION

The study produced molecules with improved non-polar characteristics (clogP of teriflunomide=2.13 vs. clogP of ZINC06536819=3.45 and clogP of Seed30_Result195=4.96), which predispose to increased intracerebral penetration. This allows the molecules to reach their site of the action in the brain and potentially exert an anti-epileptic effect. The VS molecules have affinities to the protomol and are more innovative than the *de novo* cohort. The *de novo* molecules have affinities to the ligand binding pocket and are more likely to be bioactive. All the molecules selected for further study require further optimisation to improve their toxicity profile and validation through molecular dynamics studies. The validated molecules can then be synthesized and tested *in vitro*.

REFERENCES

[1] Styr B, Gonen N, Zarhin D, Ruggiero A, Atsmon R, Gazit N et al. Mitochondrial Regulation of the Hippocampal Firing Rate Set Point and Seizure Susceptibility. Neuron. 2019;102(5):1009 1024. doi: 10.1016/j.neuron.2019.03.045.

[2] Liu S, Neidhardt E, Grossman T, Ocain T, Clardy J. Structures of human dihydroorotate dehydrogenase in complex with antiproliferative agents. Structure. 2000;8(1):25-33. doi: 10.1016/s0969-2126(00)00077-0.

[3] Erra M, Moreno I, Sanahuja J, Andrés M, Reinoso R, Lozoya E et al. Biaryl analogues of teriflunomide as potent DHODH inhibitors. Bioorg Med Chem Lett. 2011;21(24):7268-7272. doi: 10.1016/j.bmcl.2011.10.052.