Identification of Novel Small Molecule Inhibitors of *PMP22* for the Treatment of CMT1A

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Introduction

- Charcot-Marie-Tooth disease (CMT) is the most common inherited peripheral neuropathy, affecting ~1/2500 people.
- 52% of cases are the result of a duplication on chromosome 17 in the gene *PMP22*. This duplication is dominantly inherited and accounts for the most frequently diagnosed CMT neuropathy, Charcot-Marie-Tooth disease type 1A (CMT1A).[1]
- Overexpression of the duplicated *PMP22* gene disturbs Schwann cell maturation, leading to CMT1A symptoms; thus, **our aim is to identify small molecules with the capability to therapeutically reduce *PMP22* levels and treat the root cause of CMT1A.**[2, 3]
- Typical symptoms of CMT1A include progressive distal muscle atrophy, sensory loss, hyporeflexia, foot and hand deformities, and reduced compound muscle and sensory action potentials. [4, 5, 6]
- There is currently no treatment for the underlying cause of CMT1A.

High-Throughput Screening

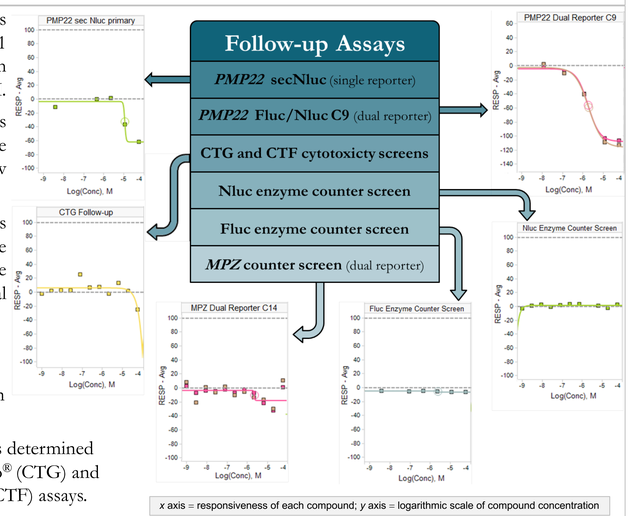
- The small molecule repository (SMR) of ~400K compounds and Sytravon library of ~40K compounds were screened using a quantitative high-throughput screen (qHTS) format.
- Following the qHTS, bioinformatics filters were applied to narrow the extensive library of compounds down to a few thousand candidates.
- The Sytravon and SMR primary screens were conducted at 5 conc. ranging from 57.5 μM to 3.7 nM and from 57.5 μM to 11.5 nM respectively.

SMR qHTS

- 417,916 Compounds
- Nluc inhibitors removed (published or previous NCATS data)
- 36,590 Compounds
- Reactive Compounds removed
- 32, 945 Compounds
- Cytotoxicity (kept compounds with <30% viability loss)
- 21,004 Compounds
- Nluc Assay Efficacy (> 50% inhibition)
- 15,101 Compounds
- Nluc Assay Potency (AC50 < 20 uM)
- 9,961 Compounds
- Nluc Assay Potency, Stringent Cut (AC50 < 5 uM)
- Follow-up Assays
- 1549 Compounds

Follow-up Assays

- All follow-up assays were screened at 11 conc. ranging from 57.5 μM to 0.97 nM.
- High potency is indicated by a curve occurring at low concentrations.
- High efficacy is indicated by the degree of response between horizontal asymptotes.
- Selectivity is determined by the *MPZ* counter screen assay.
- Lack of cytoxicity is determined by the CellTiter-Glo® (CTG) and CellTiter-Fluor™ (CTF) assays.
- All compounds were tested with both single and dual reporter assays[3] to eliminate false positives and select only *PMP22* inhibitors.



x axis = responsiveness of each compound; *y* axis = logarithmic scale of compound concentration

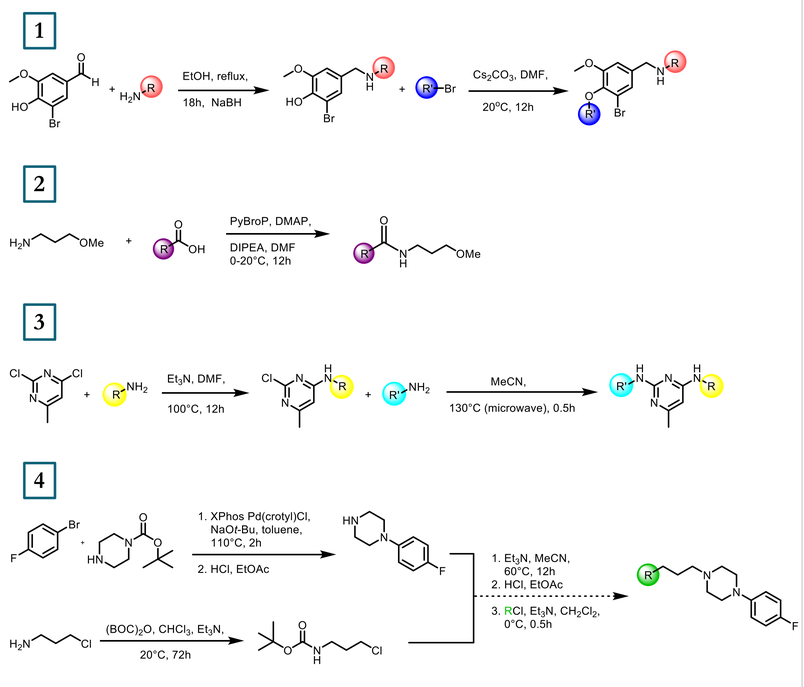
Hit Triage

- In total, 37 compounds have gone through qPCR (quantitative polymerase chain reaction) to confirm *PMP22* inhibition.
- 27 compounds were selected from the SMR and 10 were selected from the Sytravon library.
- 9 compounds were chosen to be re-synthesized or purchased and all will be subsequently purified to ensure purity prior to retesting.
- To date, 4 of the 9 compounds have been synthesized and their analogs are currently undergoing a battery of screens to verify their activity.
- Following verification, analogs will be synthesized with the goal of determining the structure-activity relationships of each chemotype.
- The synthesis of analogs of Compounds 1 and 3 has already begun. A more rigorous developmental process will commence once initial activity results are reported.

Chemotype*	Potency	Selectivity
1	1-3 μM	selective
2	0.1-1.0 μM	not selective
3	15-25 μM	not selective
4	5-10 μM	selective

* Please note that these structures are confidential.

Synthesis



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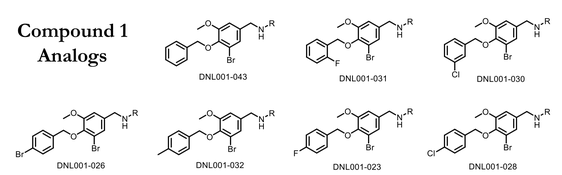
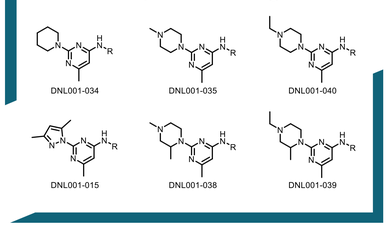
Structure-Activity Relationship Studies

Seven analogs of Compound 1 and six analogs of Compound 3 have been synthesized to date. Additional modifications will be made to improve potency, selectivity, and drug-like properties so that the project can move from "hit to lead" toward pre-clinical studies.

Compound 1 Analogs



Compound 3 Analogs



- Future groups for substitution on Compound 1 may include the amine R group and substituents on the two aromatic rings. On Compound 3, more work will be done to substitute the R' group and the R group may also be modified.
- SAR for Compound 2 and Compound 4 will also be pursued, most likely beginning with variations on the piperazine tail of Compound 4 and the R group of Compound 2.

Conclusion

- In conclusion, significant steps have been taken towards the identification and development of novel small molecules with the capability to therapeutically reduce *PMP22* levels specifically for the treatment of the CMT1A neuropathy.
- Once the activity of the lead compounds synthesized at NCATS is confirmed by biological assays and qPCR, analogs will be developed in a comprehensive manner. These analogs, like those already synthesized, will give insight into the mechanisms of *PMP22* regulation and aim to elucidate the structure-activity relationships, with hopes of eventually reaching a viable drug candidate to treat CMT1A.
- Future plans may also include screening the NCATS Genesis library of ~100K compounds in search of other promising putative *PMP22* inhibitors.

References

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(4) Steiner, I. et al. (2008). Increased severity over generations of Charcot-Marie-Tooth disease type 1A. J Neurol 255:813-819.

(5) Sames, L. et al. (2014). Recommendation to enable drug development for inherited neuropathies: Charcot-Marie-Tooth and Giant Axonal Neuropathy. F1000 Research '14, 3:83.

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Acknowledgments

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