Identification of New Macrofilaricidal Compounds for Treatment of Onchocerciasis

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Current efforts to control and eliminate onchocerciasis are hindered by the lack of compounds that target the adult worm stage. In a joint collaboration with DNDi, academia and Celgene, a pipeline was established to identify macrofilaricidal compounds. To date, more than 400 compounds have been screened *in vitro* against *Onchocerca gutturosa* adults, identifying 177 compounds with EC_{50} <1uM, 83 of which having EC_{50} <100nM. From this set of 400 compounds, a select set of 160 compounds were tested against both *Onchocerca lienalis* microfilariae and *Onchocerca gutturosa* adults, identifying 43 compounds with specific activity against the adult parasites *in vitro*. Active compounds with EC_{50} in the 0.015-1µM range and suitable pharmacological profiles were prioritized for *in vivo* testing. 23 lead candidates were tested by oral gavage in mice that harbored adult worms of the rodent filarial nematode *Litomosoides sigmodontis*. Compounds significantly reduced the *L. sigmodontis* adult worm burden by >90% after 10 days of BID and/or TID treatment. Presence of microfilariae in the treated animals suggest that the compounds do not have a strong microfilaricidal effect. Current efforts to further assess the impact of the compounds on microfilariae in the L. sigmodontis jird model are on going.

The current study demonstrates the successful establishment of a screening cascade which resulted in the identification of promising novel macrofilaricidal compounds. The identification of such macrofilaricidal compounds which lack microfilaricidal effects are ideal candidates for the treatment of onchocerciasis, as they have a reduced risk for microfilariae-driven adverse events.

Screening and Compound Progression

The primary *in vitro* screens target

1. *Brugia malayi and Litomosoides sigmodontis microfilaria* (5-day motility assay)
2. *Brugia malayi and Litomosoides sigmodontis adult* (5-day motility assay)
3. *Onchocerca lienalis* microfilariae, mf (5-day motility assay)
4. *Onchocerca gutturosa* adult worms (5-day motility /MTT assay)

Compounds with *in vitro* activity in the micromolar (µM) range against adult parasites are considered hits.

In vivo proof of principle is established in gerbils or mice infected with *Litomosoides sigmodontis* and is conducted on hits with good oral PK in the relevant host species. The dosing regimen is adjusted to reach plasma concentrations above EC_{50} for 24 hours. This model is regarded as a reasonable predictor of clinical efficacy.

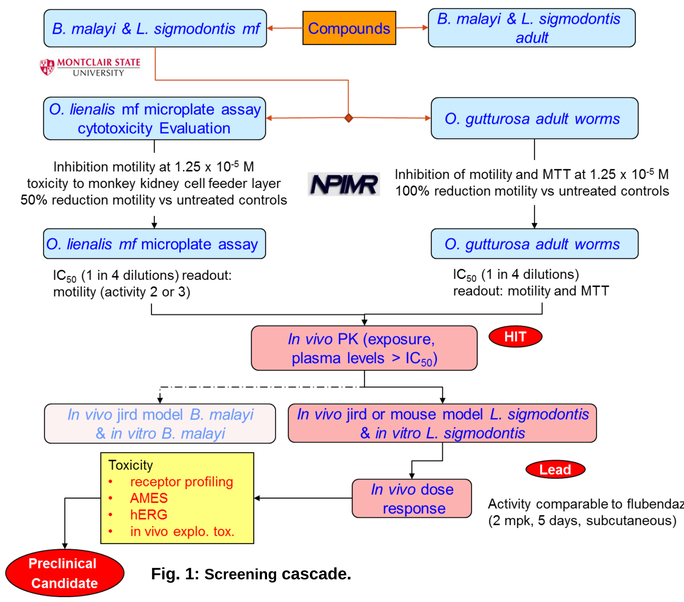

Fig. 1: Screening cascade.

Initially, compounds from multiple chemical series were progressed into the *in vitro* adult *O. gutturosa* and *O. lienalis* microfilariae motility assay. In this set of compounds, 43 hit compounds had specific activity against adult parasites. Compounds which show activity against *L. sigmodontis* and *B. malayi* trended towards activity against *O. gutturosa*, 86% had agreement amongst the parasites allowing for rapid triaging into the *O. gutturosa* assay. Multiple compounds from both series have demonstrated statistically relevant *in vivo* activity against *Litomosoides sigmodontis* in mice (**Figure 2**).

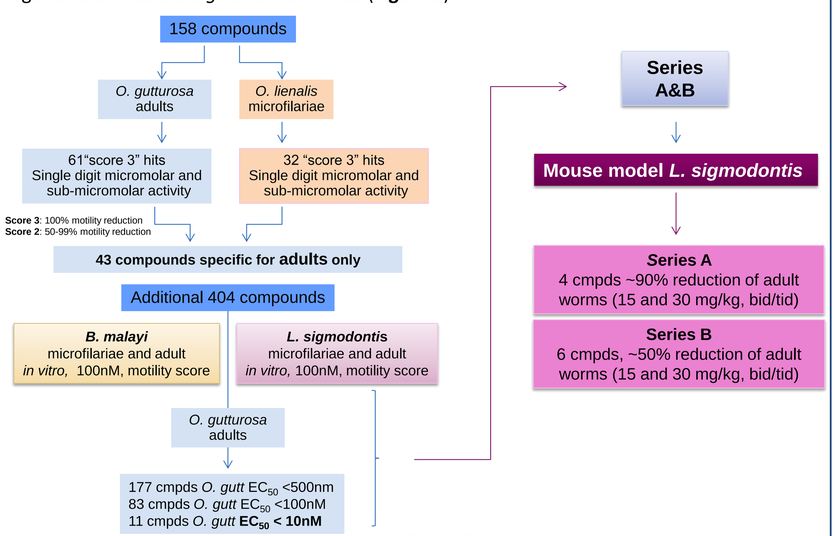

Fig. 2: Summary and current status

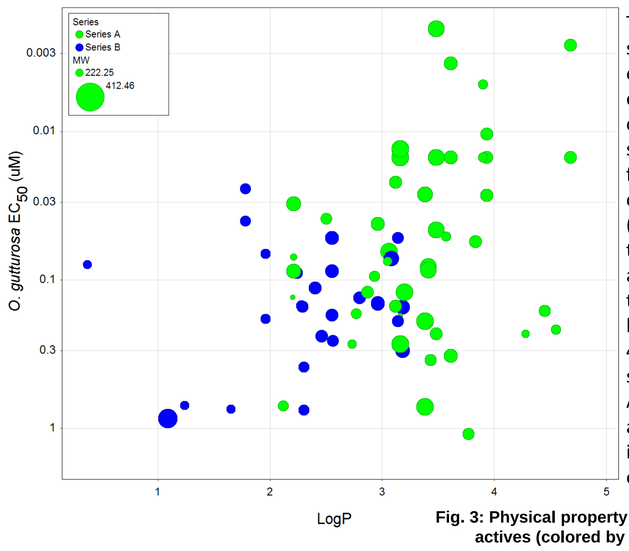

Fig. 3: Physical property analysis of *O. gutturosa* actives (colored by series and sized by MW)

Two distinct chemical series, A and B, emerged from this effort. The active compounds in both series, EC_{50} <1uM, have favorable physical chemical properties (**Figure 3**) with MW in the range of 200-400, and with the majority of the compounds having low lipophilicity, logP < 4. Additionally, both series have desirable ADME properties which allowed for progression into *in vivo* filarial efficacy models.

Cross species activity and stage selectivity was seen across the various parasitic nematodes within both chemical series. Although over 400 compounds have been tested against *adult O. gutturosa* and both *B. malayi* and *L. sigmodontis* adults and microfilaria, only 195 compounds have been tested concurrently against all three species and stages to date. From this set of 195 compounds, both series have specific compounds with activity favoring the adult stage of *O. gutturosa*, *L. sigmodontis* and *B. malayi* (**Figure 4**). Analogously, specific compounds also have overlap in both *B. malayi* and *L. sigmodontis* microfilaria in each series. Additionally, 13 compounds (series A) and 17 compounds (series B) have been identified as having **macrofilaricidal** selectivity in *B. malayi*.

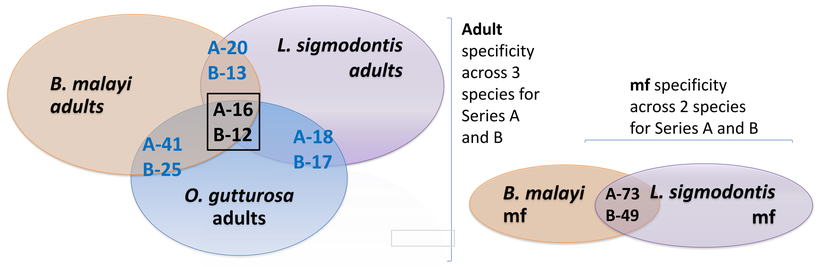

Fig. 4. Cross species and stage selectivities for Series A and B

Compound A: PoC Tool Compound

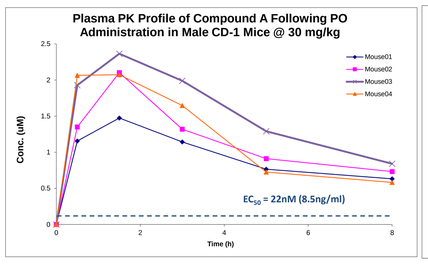

Fig. 5. Mouse PK, 30 mg/kg, p.o CMC/Tween suspension

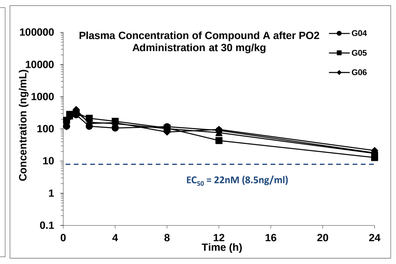

Fig. 6. Jird PK, 30 mg/kg, p.o CMC/Tween suspension

Table 1

Physical Properties	*in vitro* Efficacy			DMPK		*in vitro* Safety
Compound A	***O. gutturosa* adult EC_{50} (uM)**	***L. sigmodontis mf/adult*** motility score day 1-5 (100nM)	***B. malayi mf/adult*** motility score day 1-5 (100nM)	**S9 H/R % rem**	**C_{max} (µM) T_{max} (hr) AUC (µM·hr) (mouse)**	**THLE Kinase (3uM) Cerep (10uM) hERG**
MW = 381 logP = 3.5 TPSA = 91	0.022	4.0 - 0.3 mf 0.0 - 0.0 adult	2.5 - 0.5 mf 3.5 - 1.0 adult	100/85	2.0 1.75 12.8	20 uM 0 /72 12 / 80 (>70%) 0.542 uM

Motility Scoring: 4= rapid movement / largely coiled, 3 = moderate movement / uncoiled, 2 = slow movement / uncoiled, 1 = twitching movement / uncoiled, 0 = no motility (dead).

Based on the preliminary *in vitro* activity in *B. malayi*, *L. sigmodontis*, and adult *O. gutturosa* parasites, and favorable ADME properties compounds were prioritized for *in vivo* studies. Compound A demonstrated sustained exposures above the EC_{50} in both the mouse and in the jird (**Figures 5** and **6)** and was progressed into the *L. sigmodontis* efficacy studies. In the *in vivo* murine model of filariasis (**Figure 9**), Compound A showed reduction of adult worms at doses as low as 15mg/kg bid, 3days. Presence of microfilariae in the treated animals suggest that Compound A does not have a strong microfilaricidal effect and therefor it may demonstrate specific macrofilaricide activity. To assess true macrofilaricidal activity, *L. sigmodontis* infected jirds were treated with Compound A (**Figure 10**). Treated animals displayed microfilaremia up to 8wks post-treatment (wpt) suggesting that Compound A does not affect the microfilaria. The study is currently on going.

Upon the successful demonstration of efficacy with Compound A, lead optimization efforts were initiated with both series A and B. Compounds with EC_{50} ≤100nM and desirable physical chemical and pharmacokinetic properties (**Table 2**) were evaluated in the *in vivo* murine model of filariasis (**Figure 11** and **12**). A few compounds demonstrated statistically relevant *in vivo* activity against *L. sigmodontis* in mice and others demonstrated a trend towards activity providing SAR for emerging new analogs. Continued *in vitro* testing of additional analogs along with subsequent *in vivo* murine and jird studies are currently ongoing.

Litomosoides sigmodontis in vivo Filariasis Efficacy Model

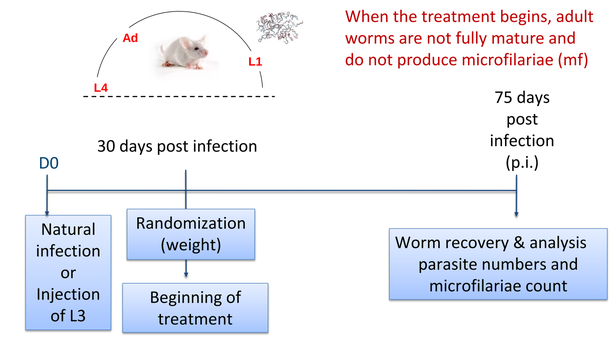

Fig. 8. Murine model of filariasis: *Litomosoides sigmodontis*

1. BALB/c are exposed to infected mites (*Ornithonyssus bacoti*), for 24 h
2. After inoculation of infective larvae (L3), migration occurs via the lymphatic system to the pleural cavity by day 2-6 post-infection (p.i.)
3. Between days 8-12 p.i, larvae molt into L4 stage, then mature to adults by day 30 p.i.
4. At day 30 p.i. animals are randomized, allocated in groups of 6 animals and treated.
5. At days 60-90 p.i., microfilariae are produced and released in the blood

In vivo Efficacy Results (*Litomosoides sigmodontis*)

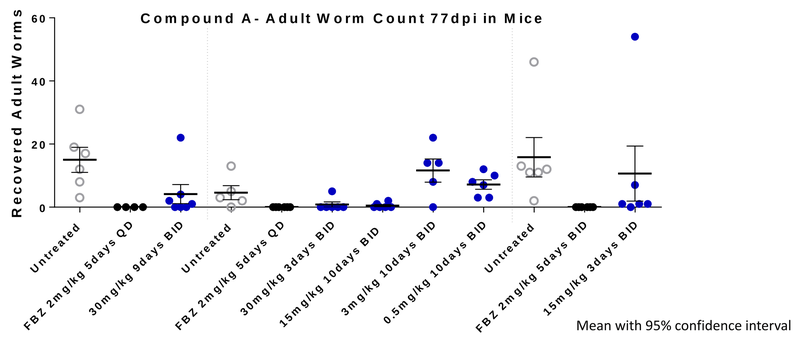

Fig. 9. Efficacy results, murine model of filariasis: *L. sigmodontis*

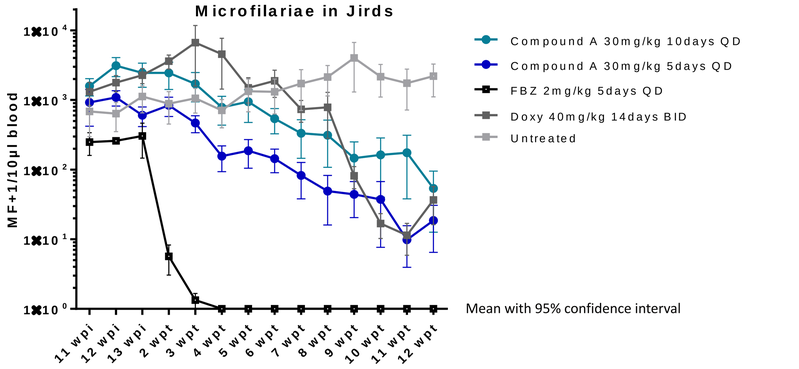

Fig. 10. Efficacy results, jird model of filariasis: *L. sigmodontis*

Table 2: Recently designed analogs

Compound	*O. gutturosa* adult EC_{50} (uM)	*L. sigmodontis mf/adult* motility score day 1-5 (100nM)	*B. malayi mf/adult* motility score day 1-5 (100nM)	% Red Adult Worms mean median	S9 H/R % rem
A2	0.055	3.0 - 0.0 mf 2.0- 0.0 adult	3.5 - 0.1 mf 1.0 - 0.0 adult	47% **83%**	100/100
B1	0.147	4.0 - 2.0 mf 4.0 - 3.0 adult	4.0 - 4.0 mf 4.0 - 3.5 adult	52% 35%	100/100
A3	0.029	3.0 - 0.0 mf 1.0 - 0.0 adult	3.0 - 0.1 mf 1.0 - 0.0 adult	47% 52%	56/61
B2	0.113	2.4 - 0.0 mf 4.0 - 3.0 adult	4.0 - 4.0 mf 3.5 - 1.0 adult	45% 52%	97/0
A4	0.042	4.0 - 0.0 mf 0.0 - 0.0 adult	2.5 - 2.3 mf 3.5 - 2.7 adult	**74% (83%, 10d)** **74%**	74/94
B4	0.031	2.0 - 0.0 mf 4.0 - 3.0 adult	4.0 - 3.0 mf 3.5 - 2.8 adult	**72%** **88%**	100/100
A5	0.062	4.0 - 2.8 mf 4.0 - 3.0 adult	4.0 - 4.0 mf 4.0 - 2.8 adult	32% 50%	94/97

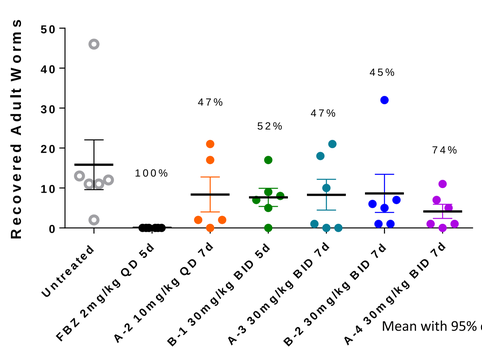

Fig. 11. Efficacy results, murine model of filariasis: *L. sigmodontis* (natural infection)

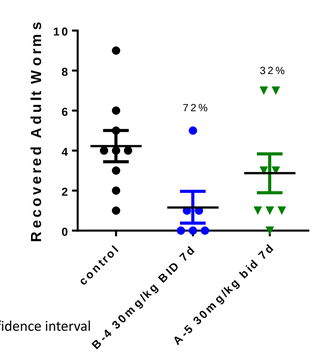

Fig. 12. Efficacy results, murine model of filariasis: *L. sigmodontis* (L3 inoculation)