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In vitro antileishmanial activity of tryptophanol derivatives

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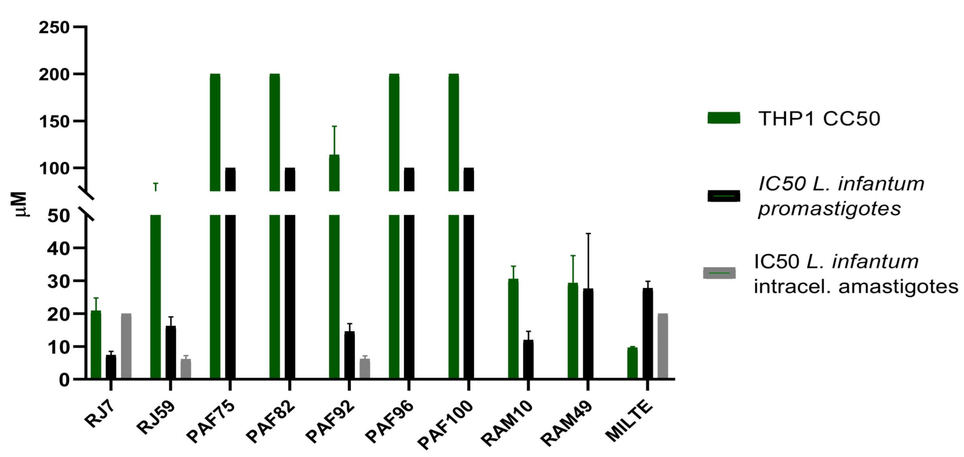
KEY FACTS

- Leishmaniasis is a vector-borne disease (VBD) caused by *Leishmania* sp. parasites and transmitted by sand flies
- Endemic in 98 countries, over a billion people at risk; more than 1 million cases/year worldwide
- Drugs for leishmaniasis are limited, toxic, and expensive, with declining efficacy and increasing parasite's resistance (Hendrickx et al, 2018)
- Urgent need for new therapeutic options
- Enantiopure tryptophanol-derived small molecules have been tested for different applications such as anti-cancer and ant-plasmodial activity (Barcherini et al, 2021, 2023; Pereira et al, 2015)

METHODS

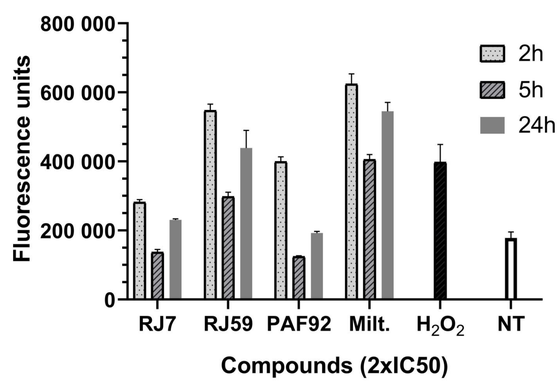
Compounds	**1st screening** → small lybrary of 9 tryptophanol derivatives. **2nd screening** → 10 new derivatives (ADTs, RJ89 & RJ90): Different linkers and sugar moieties were attached to the indole nitrogen of the selected tryptophanol derivatives (RJ59 and PAF92); SLMP53-1 is the same as RJ59; RJ88 from the same family of RJ59 without linker. Control drug: miltefosine	
***In vitro* cytotoxicity**	Host cytotoxicity	THP1 cell line (5 x 10^5 cells/ mL) → 48h treatment → MTT → CC_{50} (GraphPad Prism)
	Leishmania vector stage susceptibility	***L. infantum* (MHOM/ PT/88/IMT373)** (5 x 10^6 promastigotes/mL) → 48h treatment → MTT → IC_{50} (GraphPad Prism) Selectivity Index (SI) = CC50/IC50
	Leishmania host stage susceptibility (intracelular amastigotes)	24 h THP1 differentiation w/ PMA (2,5 x 10^5 cells/ mL) → Infection ratio 1:10 (macroph/promast.) for 24h → 48h treatment → Giemsa, microscopy (1000x) → IC_{50} (GraphPad Prism) SI
Reactive Oxygen Species (ROS) Detection	CM-H2DCFDA (Invitrogen); 2xIC_{50} concentration; H_2O_2 (1 mM) as positive control; 2h - 5h - 24h treatment; Fluorimeter/ Fluorescence microscopy (400x)	

1ST COMPOUNDS' SCREENING HIGHLIGHTS POTENTIAL OF RJ59 & PAF92 AS PROMISING SCAFFOLDS

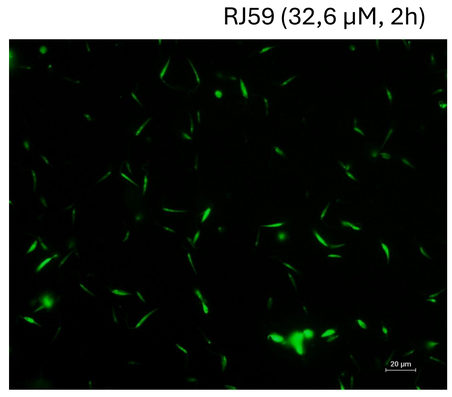


SCAFFOLD COMPOUNDS	THP1 (CC50, µM)	*L. INFANTUM* SUSCEPTIBILITY (IC50, µM)			
	THP1	Promastigotes		Amastigotes	
	Mean± SEM	Mean ± SEM	SI	Mean ± SEM	SI
RJ7	20,9±3,4	7,4±1,2	2,8	>20	< 1
RJ59	66,9±16,9	**16,3±2,7**	**4,1**	**6,1±1,1**	**11,0**
PAF92	114,1±30,3	**14,6±2,4**	**7,8**	**6,2±0,9**	**18,4**
MILTE	9,6±0,3	27,8±2,1	0,3	>20	

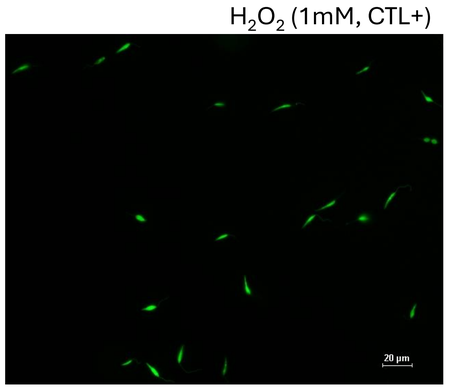
Thryptophanol derivatives induce oxidative stress



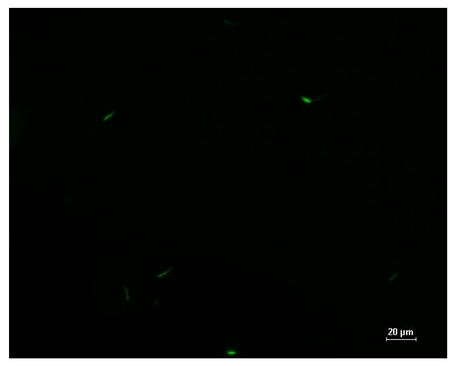
RJ59 (32,6 µM, 2h)



H_2O_2 (1mM, CTL+)



Non-treated (NT, CTR -)



Effect of selected tryptophanol derivative RJ59 (2xIC_{50} - 32,6 µM) on oxidative stress induction (generation of ROS) in *L. infantum* promastigotes; measured by fluorescence at 492–495/517–527 nm and microscopy (Nikon Eclipse 80i (400x).

2ND COMPOUNDS' SCREENING IDENTIFIES NEW TRYPTOPHANOL DERIVATIVES AS SAFE AND EFFECTIVE ANTI-LEISHMANIAL AGENTS

LEAD COMPOUNDS	THP1 (CC50, µM)	PROMASTIGOTES (IC50, µM)	
	Mean ± SEM	Mean ± SEM	SI
ADT 18.1	370,1±29,9	10,1±2,8	36,7
ADT 5.1	>400	12,5±3,9	>33
ADT 10.1	338,7±40,9	7,1±2,1	47,4
ADT 11.1	213,2±47,4	6,6±2,6	32,1
ADT 21.1	221,4±10,5	2,4±0,8	94,0
ADT 22.1	203,1±3	3,0±1,1	68,0
ADT 24.1	228,8±23,2	2,9±0,1	78,8
ADT 25.1	237,8±18,6	4,5±3,4	53,1
RJ 89	**284,2±75,4**	**1,6±0,5**	**172,6**
RJ 90	**221,4±32,9**	**1,4±0,5**	**153,4**
RJ 88	98,5±0,65	10,3±3,3	9,6
SLMP53-1	309,7±50,1	40,9±6,5	7,6
Milte	23,3±7,5	19,1±6,8	1,2

KEY RESULTS / TAKE HOME MESSAGES:

- ✓ RJ59 and PAF92 presented **moderate activity** on *Leishmania* promastigotes and intracellular amastigotes.
- ✓ **PAF92** presented the **best safety profile**
- ✓ RJ59 and PAF92 generate oxidative stress in an early treatment phase (2h)
- ✓ The optimized tryptophanol derivatives of the 1st screening were able to **increase activity and selectivity** against Leishmania parasites
- ✓ All **ADT compounds** presented much **higher activity** with **low cytotoxicity** (SI>32)
- ✓ **RJ89 and RJ90** presented the **best activity** with an IC_{50} of 1,6 µM and 1,4 µM, respectively, with **excelent safety profiles** (**SI 172,6 and 153,4**) → **lead compounds**

NEXT STEPS:

- Confirm results with other *Leishmania* strains / species
- Test the 10 new derivatives (2nd screening) in the intramacrophage host stage model
- Explore the mode of action of the best compounds
- Proceed for *in vivo* model of infection

References

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