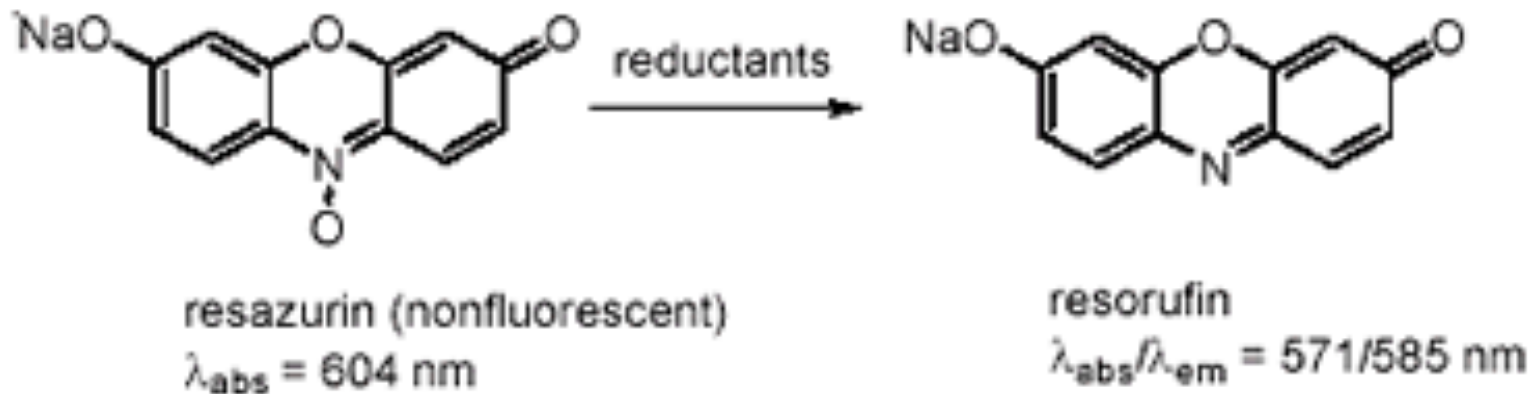


# **Alamar Blue assay for screening drugs on *Leishmania* promastigotes**

**Emna HANNACHI and Hasnaa MAKSOURI**

## Background

AlamarBlue detects cell viability by utilizing a nonfluorescent dye resazurin, which is converted to a fluorescent dye resorufin in response to chemical reduction of growth medium resulting from cell growth.



**Figure 1:** Reduction of the resazium

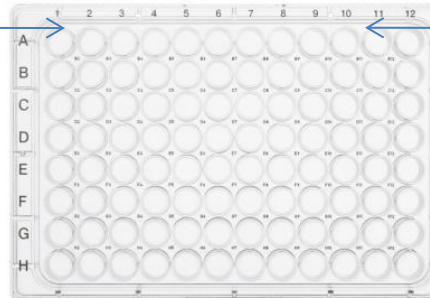
## Objective and Hypothese

➤ Study of the effect of Paromomycin on viability of *Leishmania* promastigotes and determination of the IC50.

■ Is Paromomycin highly sensitive to *Leishmania* promastigotes?

**Methods** : [Paromomycin]= 1 to 300  $\mu\text{M}$ , Vf/well=200  $\mu\text{l}$ , [parasites]= 2,5  $10^6/\text{ml}$

100  $\mu\text{l}$ /well Paromomycin  
[1]  $\mu\text{M}$  to [300]  $\mu\text{M}$



100  $\mu\text{l}$ /well promastigotes

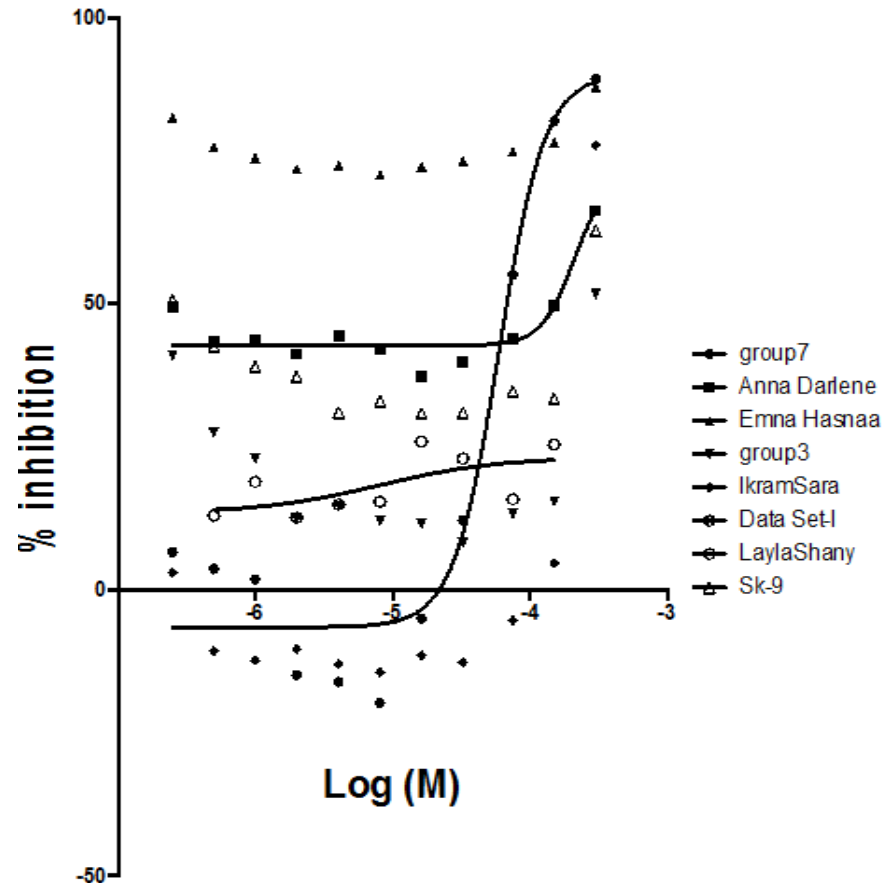
Incubation 48 hrs at 27°C

Alamar Blue

Incubation 24 hrs at 27°C

ELISA reader (570/620 nm)

# Results and Interpretation

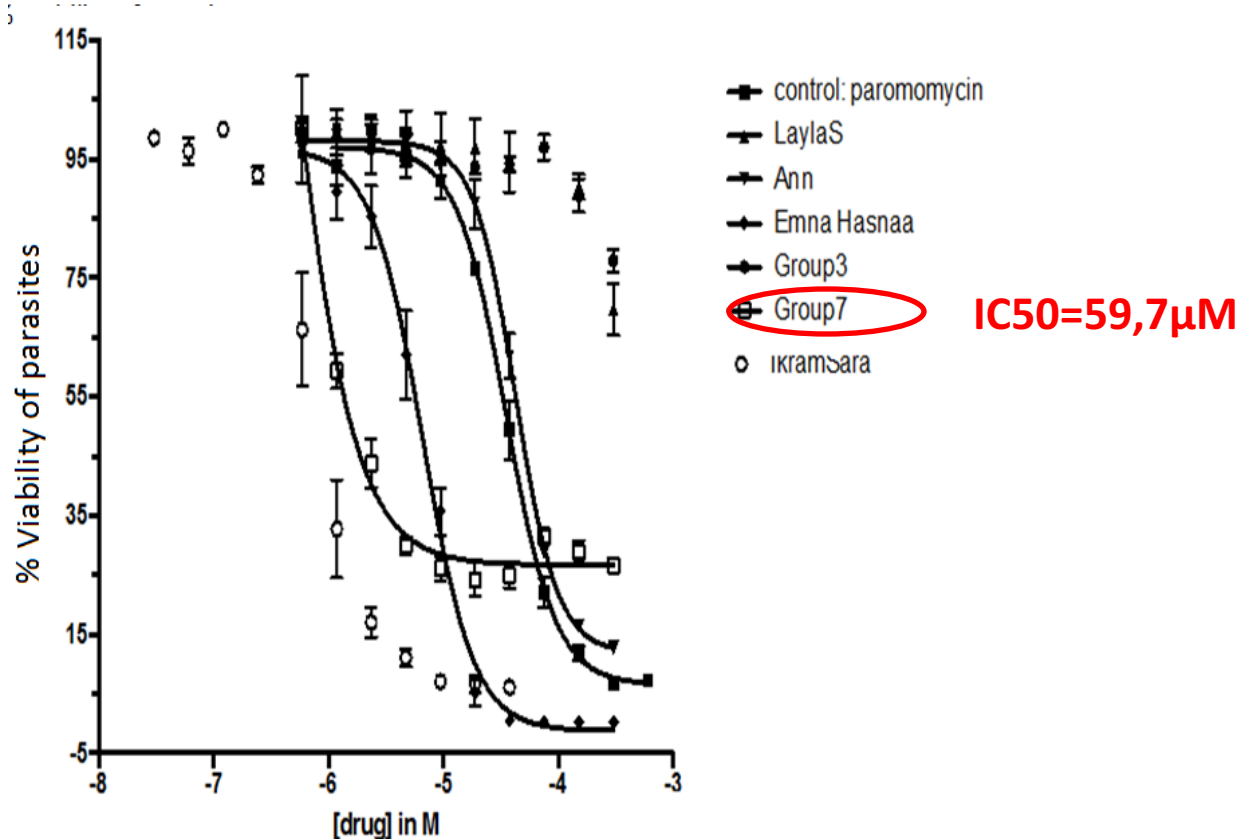


**Figure 2.** Percentage inhibition of *Leishmania* promastigotes with to Paromomycin

➤ From the preliminary results obtained, only group 7 gave an interpretable result:

Parasites in the Groupe 7 are sensitive to Paromomycin in dose dependent manner.

# Results and Interpretation



**Figure 3.** Percentage viability of *Leishmania* promastigotes with to Paromomycin

The viability of parasites in G7 decreases with increasing Paromomycin concentration in comparison with the control.

# Conclusion

- ❑ Paromomicyn is toxic to leishmania parasite, leading to mitochondrial permeabilization and dysfunction.
- ❑ We employed the Alamar Blue assay to measure parasite viability in the presence of increasing concentration of Paromomicyn.
- ❑ Our preliminary results demonstrate that promastigotes of Leishmania in G7 display a sensitivity to Paromomicyn compared to other groups.

# CHALLENGE

- Alamar Blue: cheap and quite sensitive if you seed the right amounts of cells and optimize detection times for each cell type.
- The manipulation must be repeated
- Effect of Promomycin on viability of amastigote form and other parasites
- Others drugs may used with assay
- What is the best in vitro assay? Alamar blue or other techniques :  
MTT, XTT: Each one has advantages and disadvantages. By combining and/ comparing both of them we will have a reliable results.



**THANK YOU FOR YOUR ATENTION**