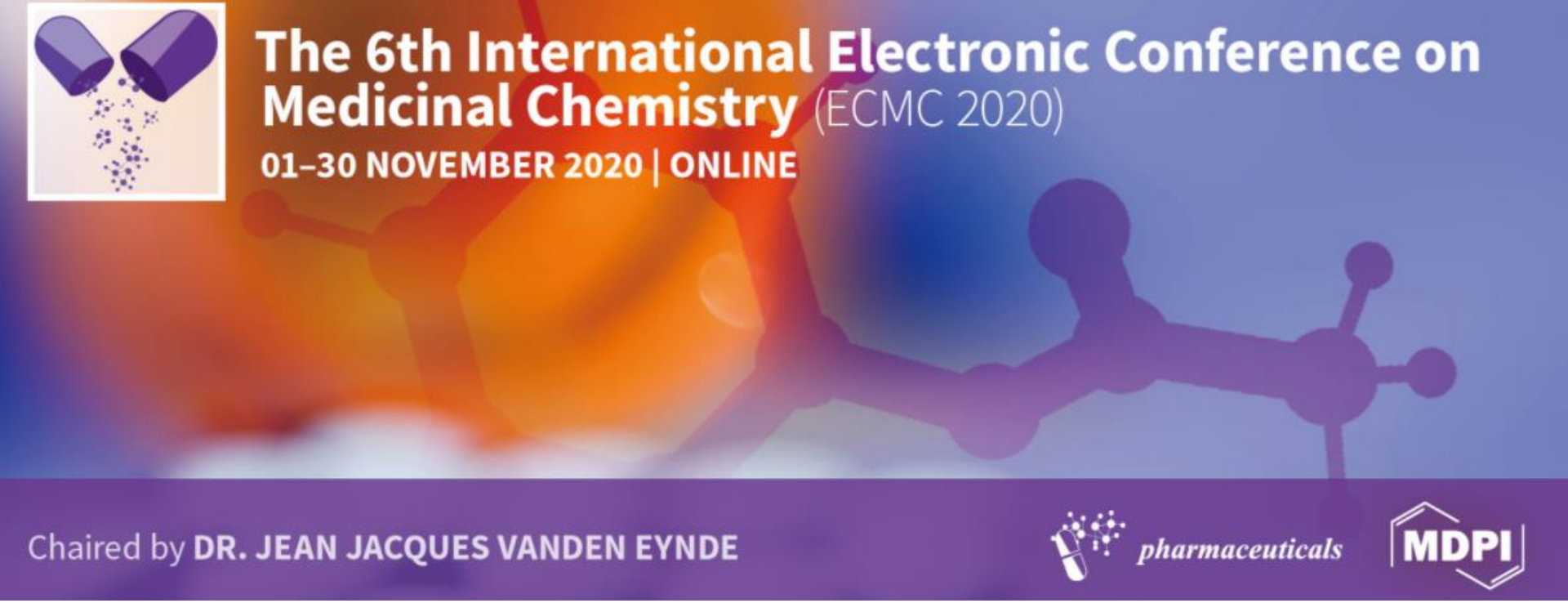


Advanced preclinical studies in Canine Leishmaniasis drug development.

Cintya Perdomo [a], Elena Aguilera [b], Ileana Corvo [a], Paula Faral-Tello [c], Elva Serna [d], Carlos Robello [c, e], Shane R. Wilkinson [f], Gloria Yaluff [d], Guzmán Alvarez [a],*

aLaboratorio de Moléculas Bioactivas, Universidad de la República, CENUR Litoral Norte, 60000 Paysandú, Uruguay.

bGrupo de Química Medicinal-Laboratorio de Química Orgánica, Facultad de Ciencias, Universidad de la República, Montevideo, C.P. 11400, Uruguay.

cUnidad de Biología Molecular, Institut Pasteur de Montevideo, Montevideo, C.P. 11400, Uruguay.

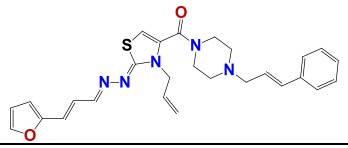
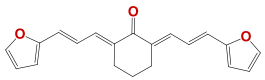
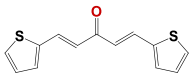
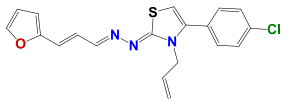
dDepartamento de Medicina Tropical, Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, San Lorenzo, C.P. 2169., Paraguay.

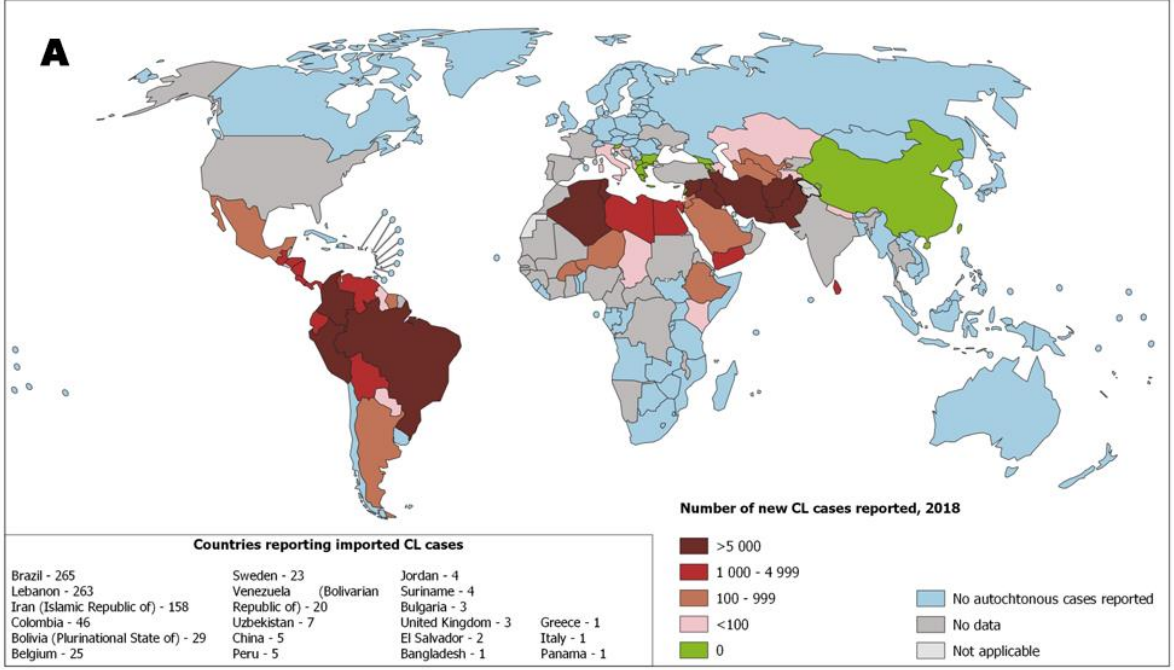
eDepartamento de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.

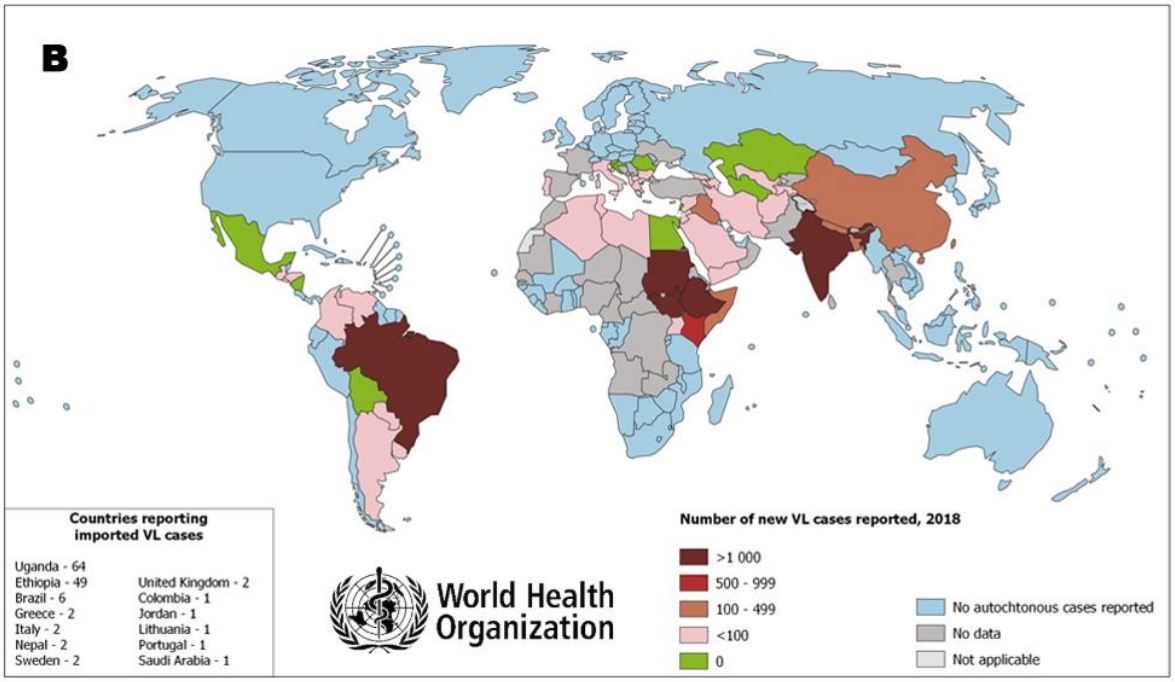
fQueen Mary Pre-Clinical Drug Discovery Group, School of Biological and Chemical Sciences, Queen Mary University of London, London, United Kingdom.

*Corresponding author. **Laboratorio de Moléculas Bioactivas, CENUR Litoral Norte, Universidad de la República, Ruta 3 (km 363), Paysandú, C.P. 60000, Uruguay. E-mail address: guzmanalvarezlqo@gmail.com (G. Alvarez).**

GRAPHICAL ABSTRACT

HIT314	HIT1019	HIT796	HIT266
			
Activity *in vitro* anti-*T. cruzi* (multiple strains)			
IC_{50} 0.72 μM amastigotes	IC_{50} 0.60 μM epimastigotes	IC_{50} 5.0 μM epimastigotes	IC_{50} >0.25 μM Amastigotes
Selectivity index >100 (IC_{50} mammalian cell/IC_{50} *T. cruzi*)			
Mechanism of action			
Cruzipain IC_{50} 4.3 μM	*Tc*TIM IC_{50} 86 nM	unknown	unknown
Stability *in vitro* (microsomal, plasma, other solutions)			
high	**low**	**Moderate**	**high**
Toxicology and efficacy			
Negative Ames Test (non-mutagenic) **Negative micronucleus test (non-genotoxic)** **LD_{50} 2000 mg/kg** **Full control of the parasitemia *in vivo* at 50 mg/kg in the murine model of Chagas disease**			





Canine Leishmaniasis have not :

A robust diagnostic method,

A efficient vaccine

A efficient drug

The control of the vector is not easy, it cannot be controlled by insecticides.

It is a zoonotic disease, when the first case of canine leishmaniasis arrive, few years later arrives the human cases.

A **dog culling** strategy is not supportable along the time for several reasons:

1) no reliable body of scientific evidence supports the effectiveness for disease control,

2) alternative reservoir hosts may play a role in maintaining the life cycle of *L. infantum*, like foxes, hares, armadillos, wild boars, cats and rabbits.

3) culled dogs are rapidly replaced with young dogs that are often more susceptible to primary infection.

4) serologic diagnostic tools often used for screening dogs as part of a culling program have limitations in terms of sensitivity and specificity.

5) dog culling is not a cost-effective, valid alternative from a socioeconomic perspective (e.g., drugs for euthanasia) to government institutions.

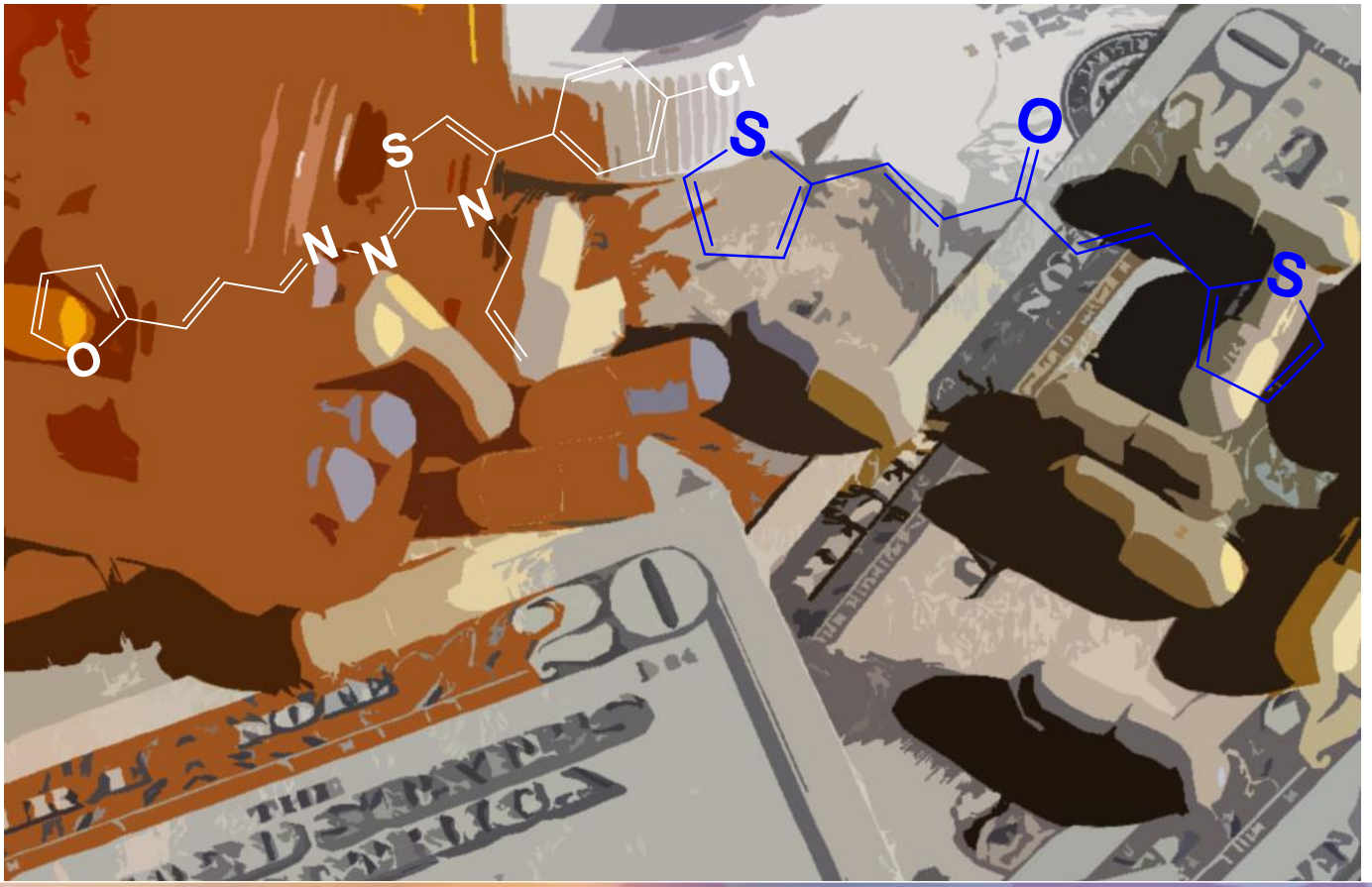
Then, the use of dog culling as a strategy to reduce the incidence of Visceral Leishmaniasis in humans cannot be justified and should no longer be used

it is successfully used for outbreak controls in short terms of time.

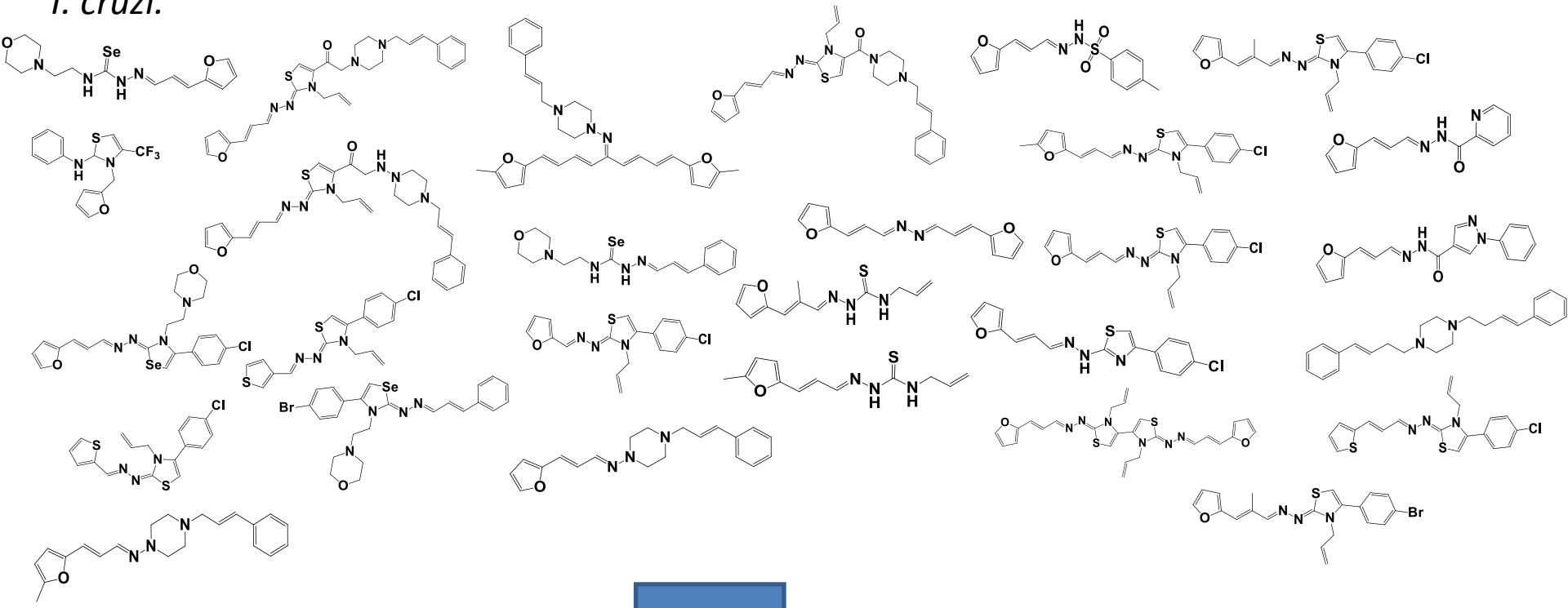
Low cost drug development

Because there is a neglected disease, and also because is for veterinary use.

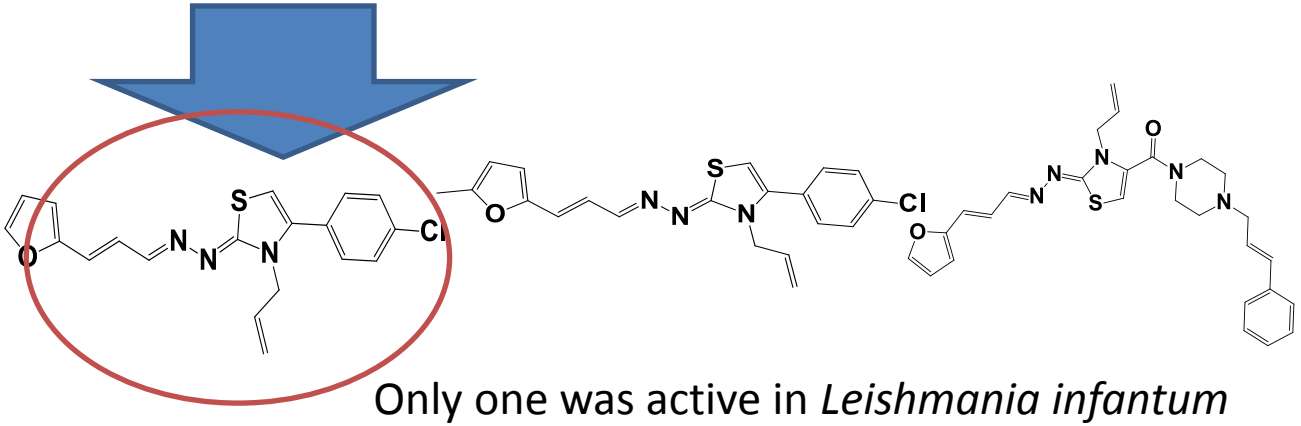
Then we are looking for low cost and easy production compounds:
THIAZOLIDENE HYDRAZINES and CURCUMINOIDS



29 hydrazines were characterized against *Leishmania spp*, selected from our background in *T. cruzi*.

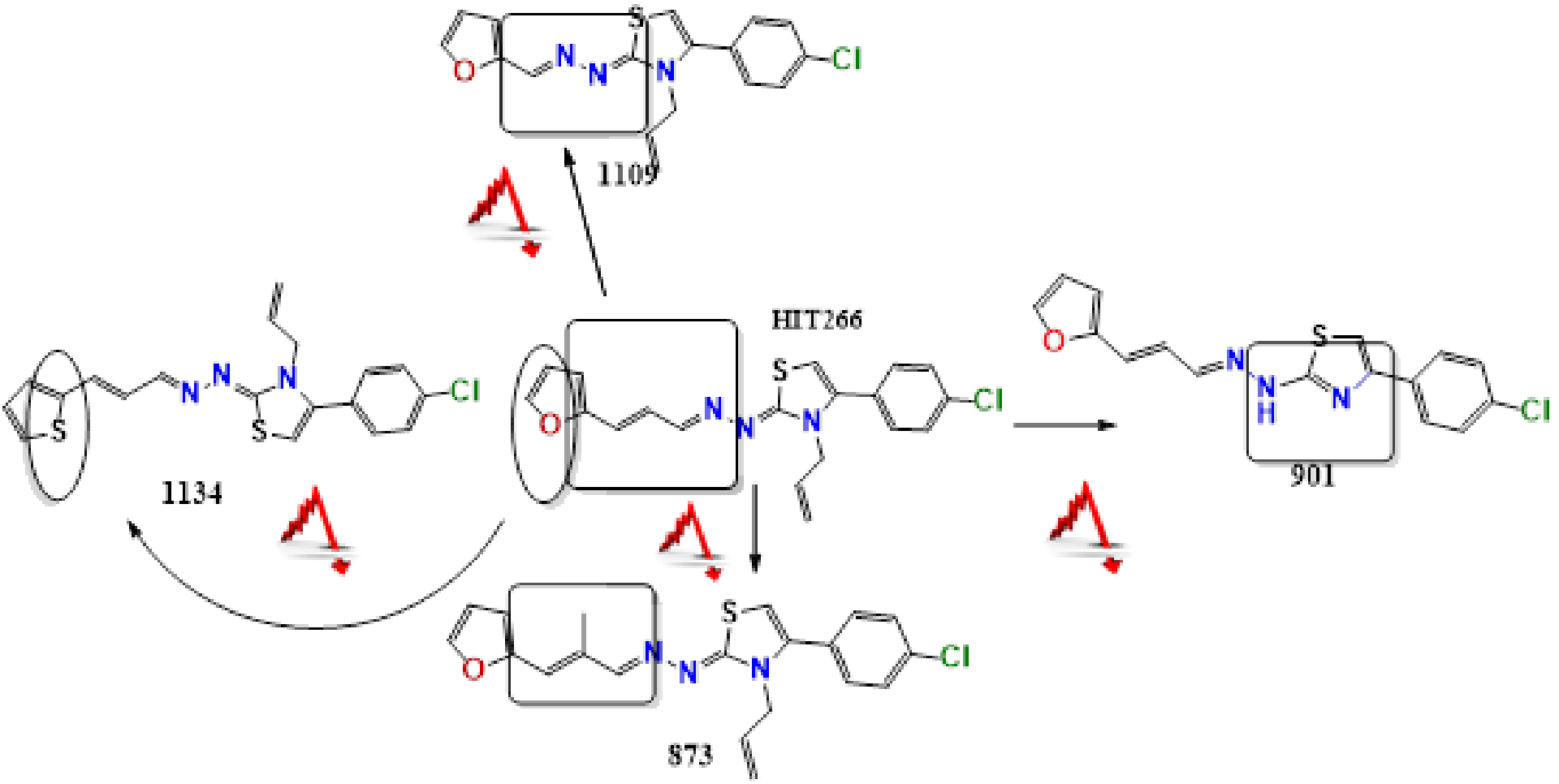


Only 3 were active (CI50 <10uM) against *T. cruzi, T. brucei* and *Leishmania spp*.

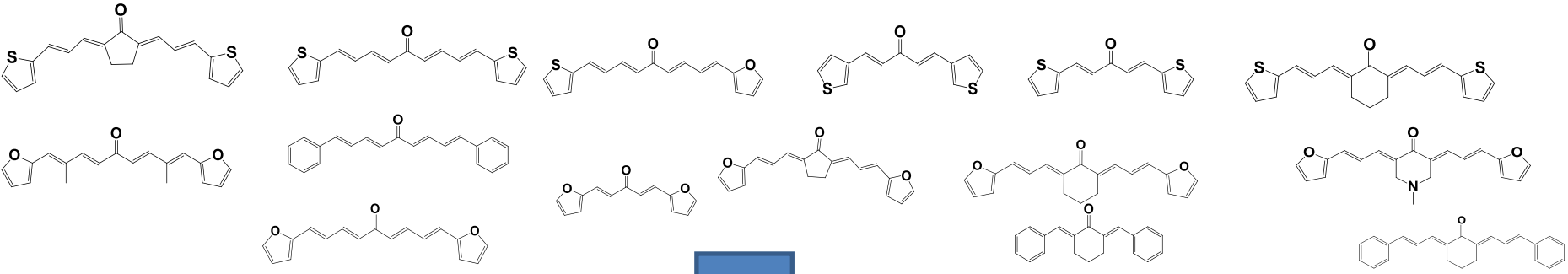


Only one was active in *Leishmania infantum* isolated from the last outbreak of canine leishmaniasis.

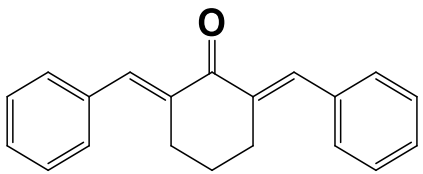
Structure-Activity analysis. Interpretation of the possible pharmacophore.

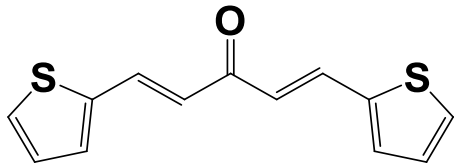


14 curcuminoids were characterized against *Leishmania spp*, selected from our background in *T. cruzi.*



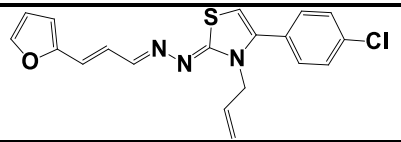
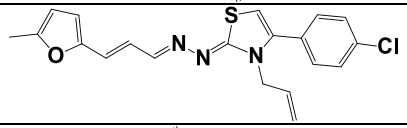
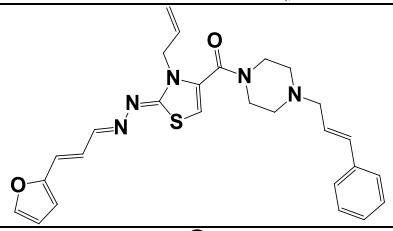
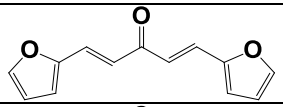
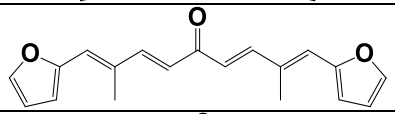
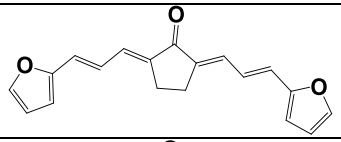
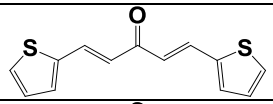
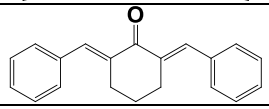
Only 2 were active (CI50 <10uM) against *T. cruzi, T. brucei* and *Leishmania spp*.





And also active in *Leishmania infantum* isolated from the last outbreak of canine leishmaniasis.

Toxicology background

Structure	Chemical collection code	IC50 ± %DE (μM) Macrophages	Ames Test	Genotoxicity in mice	LD50 in mice (mg/kg)
	**HIT266****	60±6	negative	negative	>2000
	**872**	66±7	negative	nd	nd
	**HIT314**	30±5	negative	negative	>2000
	**795**	115±2	positive	nd	Nd
	**809**	33±8	negative	nd	Nd
	**1223**	19±6	negative	nd	Nd
	**796****	38±7	negative	negative	>2000
	**799****	115±8	negative	nd	>2000

Pharmacokinetic background

Solubility (mg/mL)	Gastrointestinal absorption	BBB permeability	Penetrability of the skin (cm/s)	Bioavailability	Lipophilicity (LogP)	Stability in S9 rat fraction at 4hs
1.9×10^{-3}	low	no	-4.0	0.55	3.8	no
2.3	high	no	-7.2	0.55	0.5	yes
3.5×10^{-3}	high	no	-6.3	0.55	4.2	yes
2.2×10^{-3}	high	no	-5.2	0.55	4.8	yes
3.9×10^{-2}	high	yes	-5.3	0.55	3.7	yes
2.6×10^{-3}	high	yes	-4.4	0.55	4.5	no

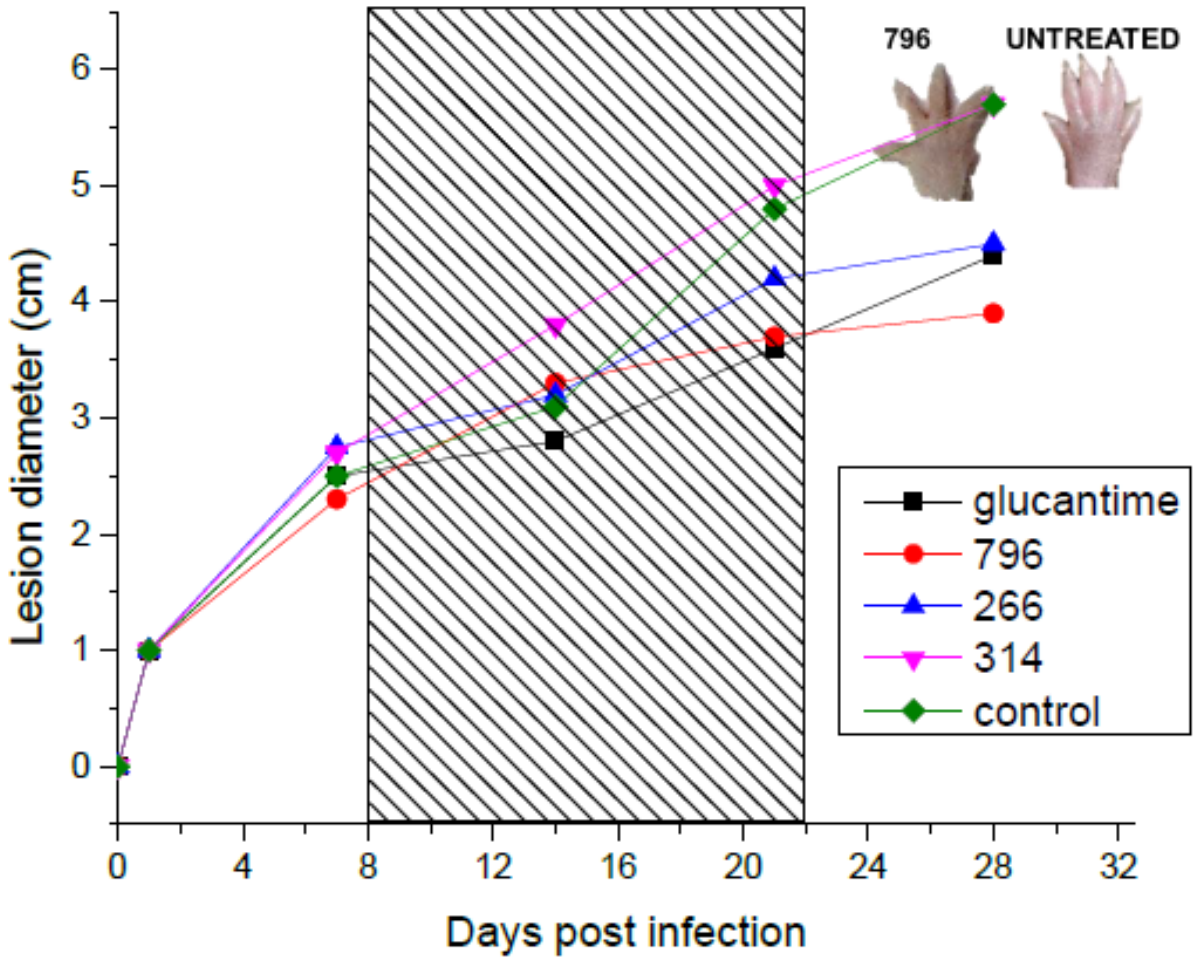
PROOF OF CONCEPT

In vivo test in the murine cutaneous leishmaniasis model for the lead compounds.

Compound	Doses mg/kg	Doses µmol/kg	Treatment duration in days	Administration	% parasite suppression*
796	50	203	14	Oral	55
314	50	102	14	Oral	10
266	50	135	14	Oral	30
796/314/266	5/25/25	20/50/68	14	Oral	43
Glucantime	100	273	14	Subcutaneous	40
Control	0	0	14	Oral	0

* percentage of parasites in the inoculated paw of the mouse at the end of the experiment, concerning the control mouse

Weekly evolution of the diameter of the infected paw of mice.



Conclusions

Of the 50 compounds evaluated from our chemical collection, we conclude that 3 of the leading compounds (**796**, **266**, **314**), were found to have good antiparasitic activity in different species of trypanosomatids. Furthermore, these molecules have low nonspecific cytotoxic effects and did not show genotoxic or mutagenic effects. In addition to these encouraging results, they are safe, showing 100% survival in the acute toxicity model *in vivo*.

Compound **796** was the most promising compound with effective control of the Leishmania parasite infection *in vivo*. Also, the synergic effect was observed between the two chemical groups (curcuminoids and thiazolidene hydrazines). Taken together, the results obtained encourage us to continue with the clinical phase of experimentation in dogs of our leading compounds.

Funding: This research was funded by CSIC (Comisión Sectorial de Investigación Científica) I+D 2016 program number ID435 (grant).

Acknowledgments: