2-Substituted Quinolines as Leishmanicidal Drugs

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Preclinical Research of New Treatments for Visceral Leishmaniasis
Promising Results and Ongoing Challenges
DNDi parallel session symposium: early-stage R & D effort
WorldLeish4 - Lucknow, India - 4 February 2009

IRD Institut de recherche pour le développement

CNRS Centre National de la Recherche Scientifique

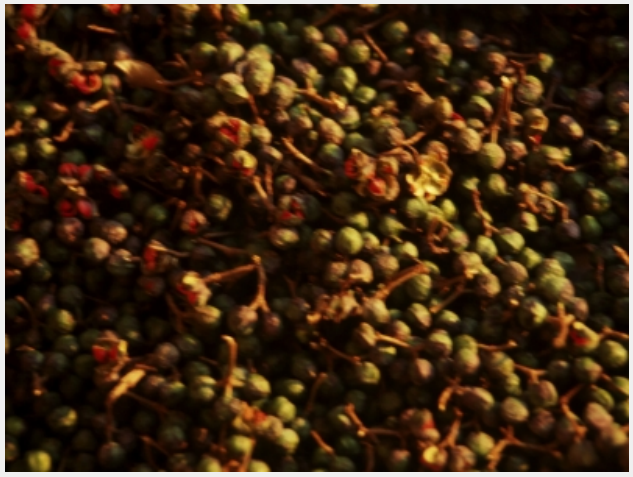
Université Paris-Sud 11

DNDi Drugs for Neglected Diseases *initiative*

Some Bolivian plants used in traditional medicine to treat the cutaneous leishmaniasis - IRD investigations with South American partners (Bolivia)

sebastian » (Oxalis sp.)

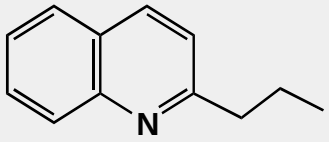
Chimanes



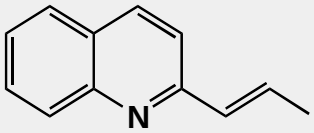
« apainiki » *Pera benensis* Rusby
(Euphorbiaceae)

Fournet A. et al. *J. Ethnopharmacol.* 1994, 41, 19-37.

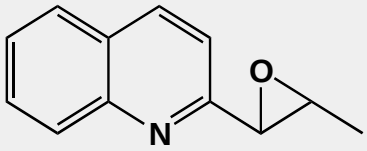
In vivo active quinolines alkaloids isolated from *G. longiflora*



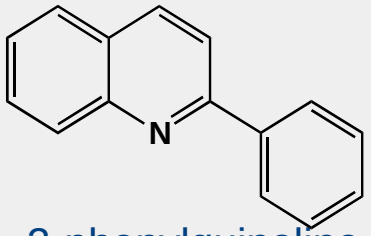
2-*n*-propylquinoline
Active against CL and VL



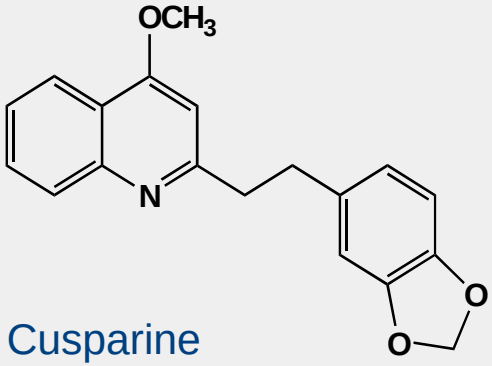
Chimanin B
Active against CL



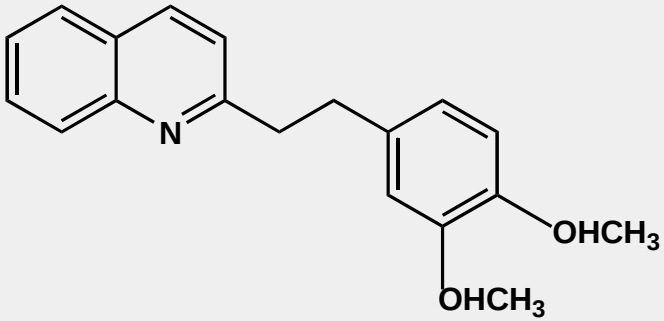
Chimanine D
Active against CL



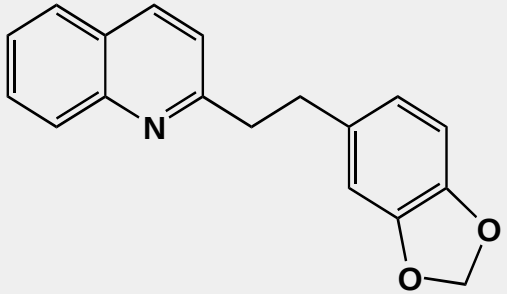
2-phenylquinoline
Active against CL



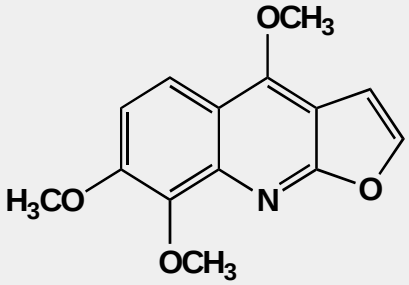
Cusparine
Active against CL



2-(3,4-dimethoxyphenylethyl) quinoline
Active against CL



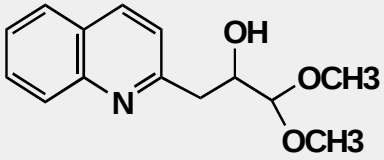
2-(3,4-methylendioxyethyl) quinoline
Active against CL

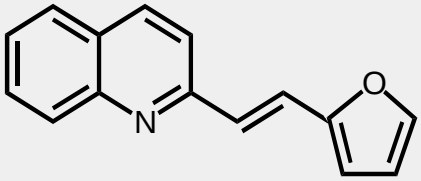


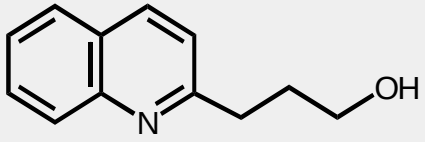
skimmianine
Active against CL

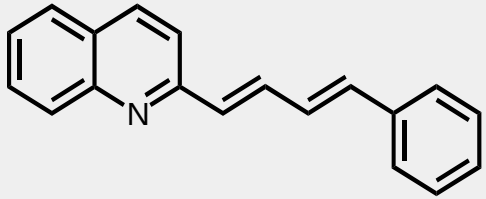
Fournet A. et al. *J. Antimicrob. Chemother.* 1994, **33**, 537-544.
Fournet A**.** et al. *Antimicrob. Agents Chemother.* 1993, **37**, 859-863
Fournet A**.** et al. *Antimicrob. Agents Chemother. 1996,* **40**, 2447-2451

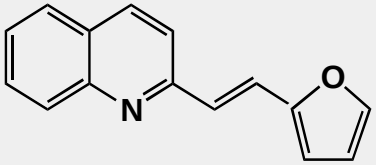
Pharmacomodulation of substituted quinolines











Fakhfakh M.A. *et al.*, *Tetrahedron Lett.* **2001**, 42, 3847

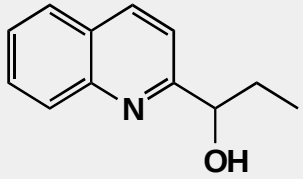
Fakhfakh M.A. *et al.*, *J. Organomet. Chem.* **2001**, 624, 131

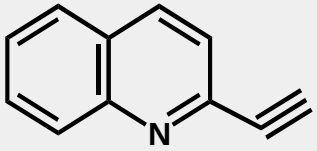
Fakhfakh M.A. *et al*, *Bioorg. Med. Chem.* **2003**, 11,5013

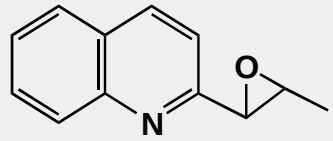
Quintin J. *et al.*, *Tetrahedron Lett.* **2002**, 43, 3547

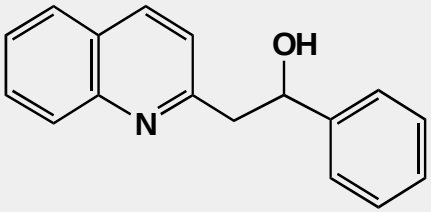
Seck M. *et al.*, *Tetrahedron Lett.* **2004**, 45, 1881

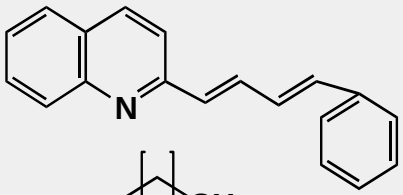
Dos Santos M. *et al.*, *Synlett.* **2004**, 15, 2697

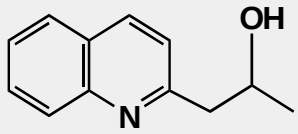


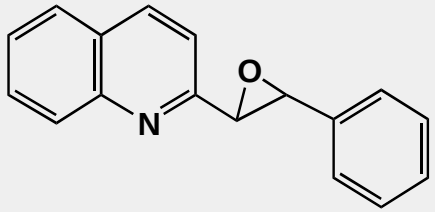


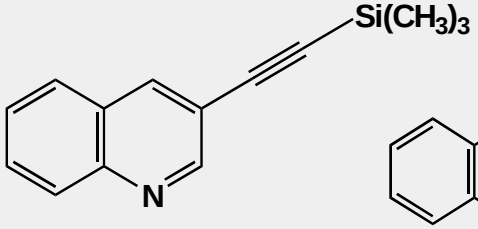


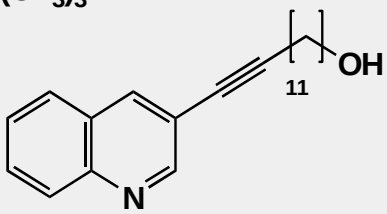


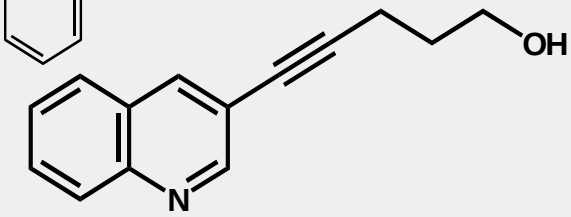


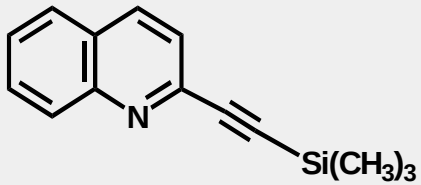






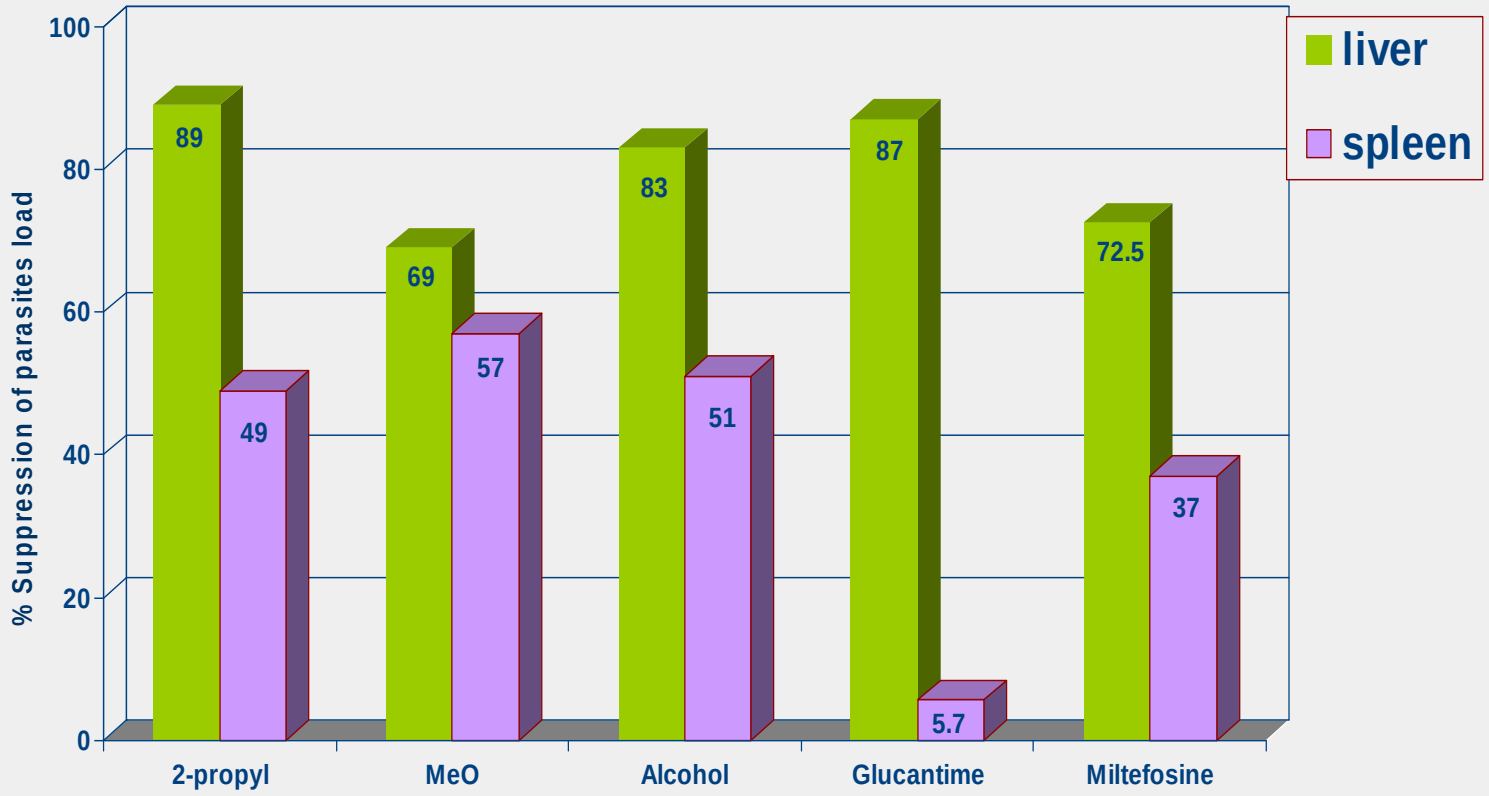






➢ Synthesis of more than 150 quinolines substituted on carbons 2 or 3

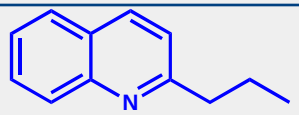
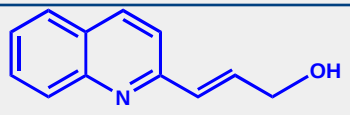
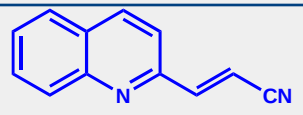
In vivo activity in experimental visceral leishmaniasis



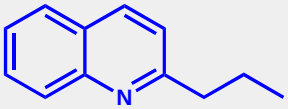
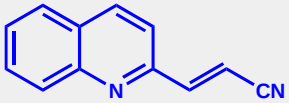
Efficacy of a 15-day treatment course with Glucantime® (28 mg of Sb^v per kg per day) or with quinolines (propyl, aldehyde, alcohol and nitril) administered orally at 12.5 mg/kg daily during the course of infection of BALB/c mice with *L.donovani*

Nakayama H., et al., 2005. *Antimicrob. Agents Chemother.* 49, 4950-4956.

Pharmacological evaluation

			
In vitro activity	**0**	**+++**	**+**
In vivo activity	**+++**	**+++**	**+++**
Analytical methods	Good resolution, retention time : acceptable		
Solubility pH 7,4	**1.5 mM**	**3.5 mM**	**0.6 mM**
pKa	**3.7**	**3.5**	**<2**
Log P at pH 7.4	**3.15**	**2.04**	**2.37**
Log P at pH 2	**- 0.62**	**- 1.20**	**1.46**
Plasmatical affinity proteins	**87.2%**	**53.2% at t0 32.1% at 24h**	**70.1%**
Albumin affinity	**79.4%**	**69.4% at t0 11.1% at 24h**	**62.7%**
Red blood cells affinity	**0**	**+++**	**+**
Reativity with SH	**0**	**+++**	**+**

ACUTE ORAL TOXICITY

Compound (mg/kg)	Dose	Apparent toxicity	Creatine (µM)	Cholesterol (mM)	AST (U/L)	ALT (UL)	Granulocytes (%)	Lymphocytes (%)
 Compound 1	1000	t_{15mn}- 3 lethargic T_{4h}- no signs	<18	3.8	422	199	25	75
	100	No signs	<18	4.1	515	168	34	65
	10	No signs	<18	3.9	410	215	35	65
Compound 2 	1000	t_{15mn}- 1 lethargic	21	3.4	388	260		
	100	No signs	<18	4.6	397	247		
	10	No signs	<18	3.9	337	223		
Control	CMC/ tween80	No signs	<18	3.5	456	304	27	72

n= 5

ASAT = aspartate amino transferase
ALAT = alanine amino-transferase

Campos Viera *et al*., *Biomed. Pharmacother*. 2008, 62, 684-689

INTERACTION BETWEEN LEISHMANICIDAL DRUGS AND 2-QUINOLINES (*IN VITRO LEISHMANIA DONOVANI*)

COMPOUND	PARTNER DRUG	FIC*	RESULTS
Compound 1	Compound 2	0.71 ± 0.21	Indifferent interaction
	Compound 3	0.57 ± 0.27	
	Sitamaquine	0.75 ± 0.19	
	Miltefosine	0.65 ± 0.26	
	Amphotericin B	0.52 ± 0.08	

*FIC = Fractional inhibitory concentration

- FIC < 0.5 = synergistic interaction
- $0.5 <$ FIC < 4 = indifferent interaction
- FIC > 4 = antagonistic interaction

IN VITRO BEHAVIOUR OF QUINOLINES WITH BIOLOGICAL ELEMENTS

Desrivot J, Herrenknecht C, Ponchel G, Garbi N, Prina E, Fournet A, Bories C, Figadère B, Hocquemiller R, Loiseau PM. Antileishmanial 2-substituted quinolines: *in vitro* behaviour towards biological components. *Biomedicine and Pharmacotherapy* 2007, 61, 441.

Erythrocyte membrane affinity *vs* antileishmanial activity

The three quinolines are all active *in vivo* but have different *in vitro* activities.

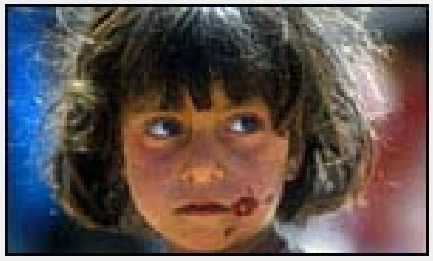
	In vitro activity on *L. donovani* promastigotes		Binding to erythrocyte membranes
	IC_{50}	MIC	
PROPYL (PQ)	> 100 µM		No
NITRILE (CN)	3.9 µM	8.9 µM	97% in 6 h
ALCOHOL	0.3 µM	1.0 µM	100% in 45 min

⇨ Alcohol is the most active *in vitro* and binds the most rapidly to membranes.

⇨ Propyl does not bind to membranes and does not show *in vitro* activity.

⇨ Is the affinity for the membranes related to antileishmanial activity ?

Red blood cells as vector ?



Hypothesis of quinoline targeting to the parasite

Erythrophagocytosis = phagocytosis of red blood cells by macrophages

Erythrophagocytosis of quinoline-loaded red blood cells:

Targeting of quinolines in macrophages, liver and spleen

Could protect the body against quinolines toxicity

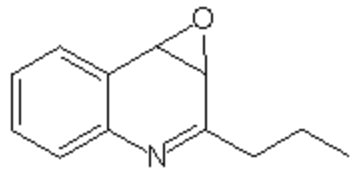
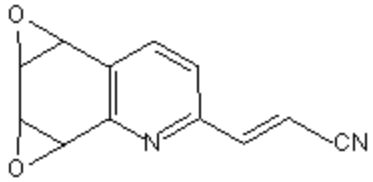
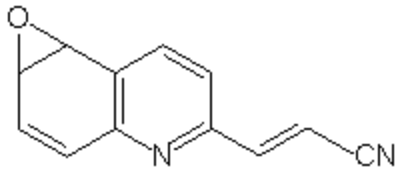
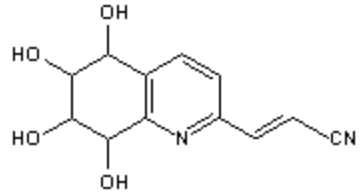
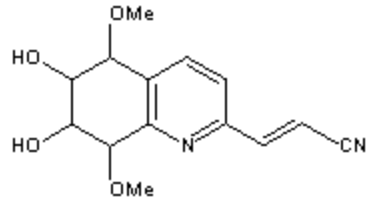
PRESENT STUDIES WITH SELECTED QUINOLINES

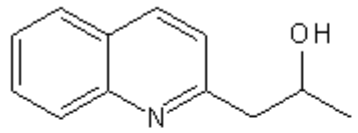
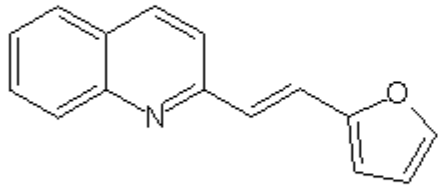
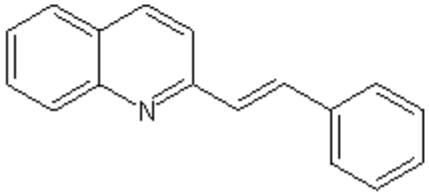
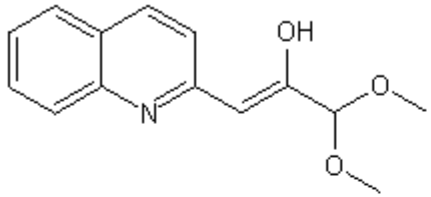
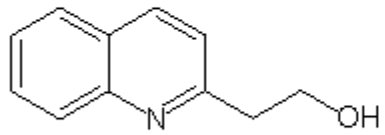
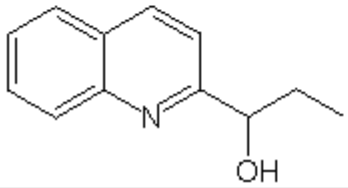
- Pharmaceutical drugability assessment
- Salt screening and active pharmaceutical ingredient recrystallization assays for quinolines
- Physicochemical profile and formulation for animal experiments
- Physical and chemical stability under stress of the selected compound and polymorphism
- Suitable physical forms (salts) for each active
- Relevant formulation
- Supplies of one useful salt of compounds for pharmacological evaluation
- Delivery of acceptable iv and po formulation to initiate *in vivo* experiments

Consortium « quinolines »

- IRD, CNRS, Paris 11 (France): Networks and hits supliers
- Advinus (India): Medicinal Chemistry, ADMET (administration, distribution, metabolism excretion and toxicology), safety evaluation
- CDRI (Central Drug Research Institute): anti parasitic tests (*in vitro* and *in vivo*)
- LSHTM (London School of Hygiene and Tropical Medicine): formation, bioassays
- Drugabilis (France): ad hoc preclinical preformulation
- Alpha Chimica (France): synthesis of metabolites

Synthesis of more than 180 quinolines substituted on carbon 2 prepared by Advinus (India)

LSHTM			In vitro activity against *L. donovani*						
Structure	Code	MW	40 µM	13.3 µM	4.4 µM	1.5 µM	IC_{50}	95% c.l.	CC50
	4a	187.2	30.33	18.58	9.56	3.83	**>40**		0
	2AC-NMH-4b	212.2	toxic/+	1.64	0.55	-1.09	**40>x>13.33**		>100
	2AC-NMH-3c	196.2	toxic/+	20.49	19.13	13.93	**40>x>13.33**		>100
	2AC-NMH-4c	248.2	toxic/+	11.75	3.28	2.19	**40>x>13.33**		0
	9a	276	toxic/+	42.01	30.90	32.29	**40>x>13.3**		

LSHTM			In vitro activity against *L. donovani*						
Structure	Code	MW	40 µM	13.3 µM	4.4 µM	1.5 µM	IC_{50}	95% c.l.	CC50
	BS-71	187	13.20	7.36	5.33	7.36	**>40**		>100
	BS-260F-4-6	221	12.69	10.15	5.58	4.82	**>40**		>100
	BS-38	231	15.74	3.55	0.51	0.25	**>40**		>100
	XF-751-755	244	12.18	8.88	5.33	4.57	**>40**		>100
	BS-321-F6-7	187	5.33	3.05	0.25	-0.76	**>40**		0
	BS-409	187	13.20	6.60	4.57	2.54	**>40**		0

The IRD and the Drugs for Neglected Diseases initiative (DNDi) have entered into two synergistic agreements to identify and develop new promising drug candidates against visceral leishmaniasis, Chagas disease and sleeping sickness.

This collaboration will allow the optimization and development of two chemical series of molecules first identified by IRD scientific teams :
quinolines for VL
canthin-6-one alkaloids for Chagas disease

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DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Partners since the beginning

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DNDi
Drugs for Neglected Diseases *initiative*

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