Techniques used to Study MetalloDrugs-DNA interactions

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Techniques used to Study MetalloDrugs- DNA interactions

- ***Experimental :***

- **Interaction of Metal complexes with hematin.**

Maribel Navarro, William Castro

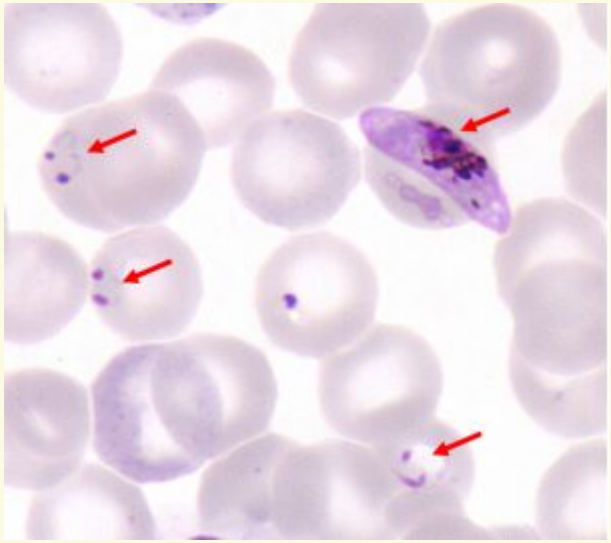
Fármacos Inorgânicos contra la

Malaria

Vector: Anopheles

Malaria

Parasites



Plasmodium :

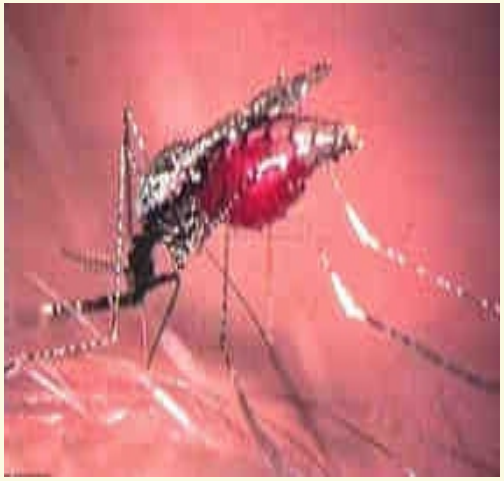
P. Falciparum

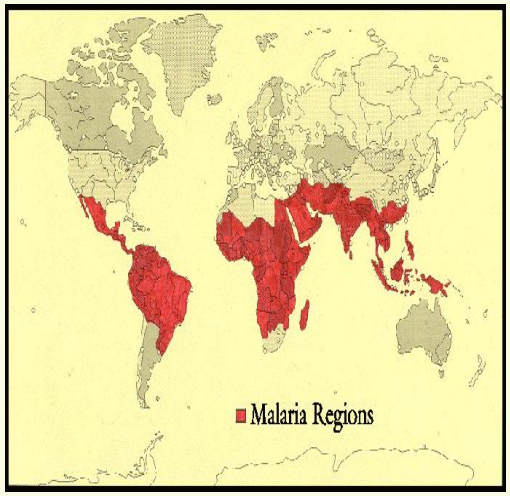
P. Vivax

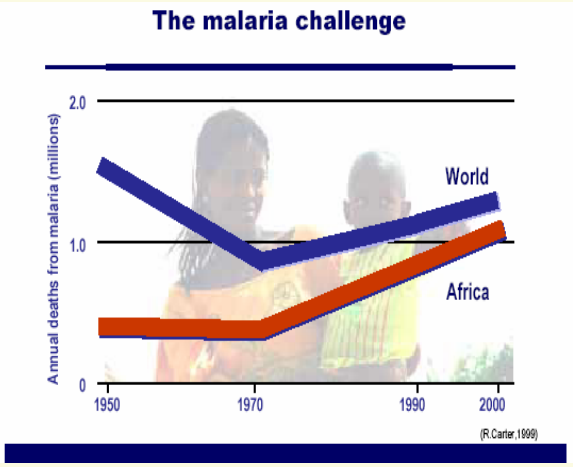
P. Ovalae

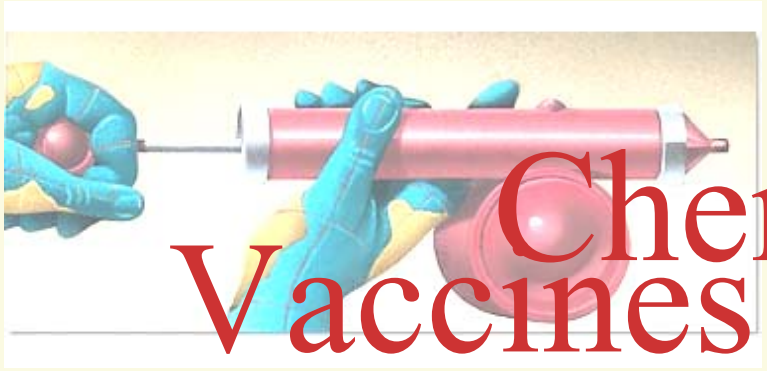
P. Malariae

Malaria is a very old disease, which is still an important cause of illness and death in children and adults in tropical countries. Affects an estimated 400 million people and is threatening more than one billion people around the world in recent years.





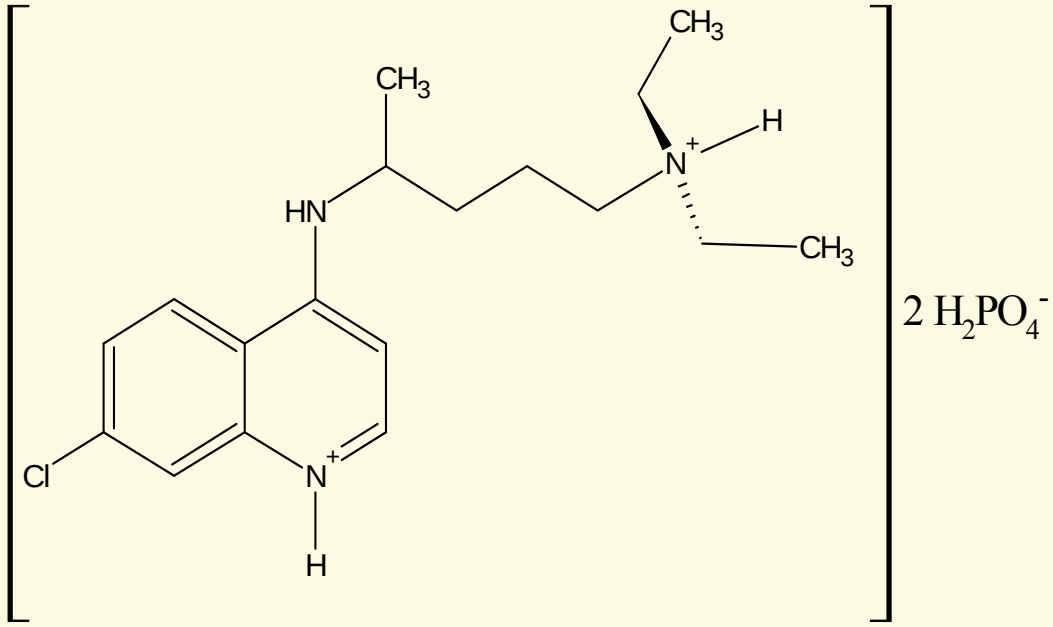




Vaccines

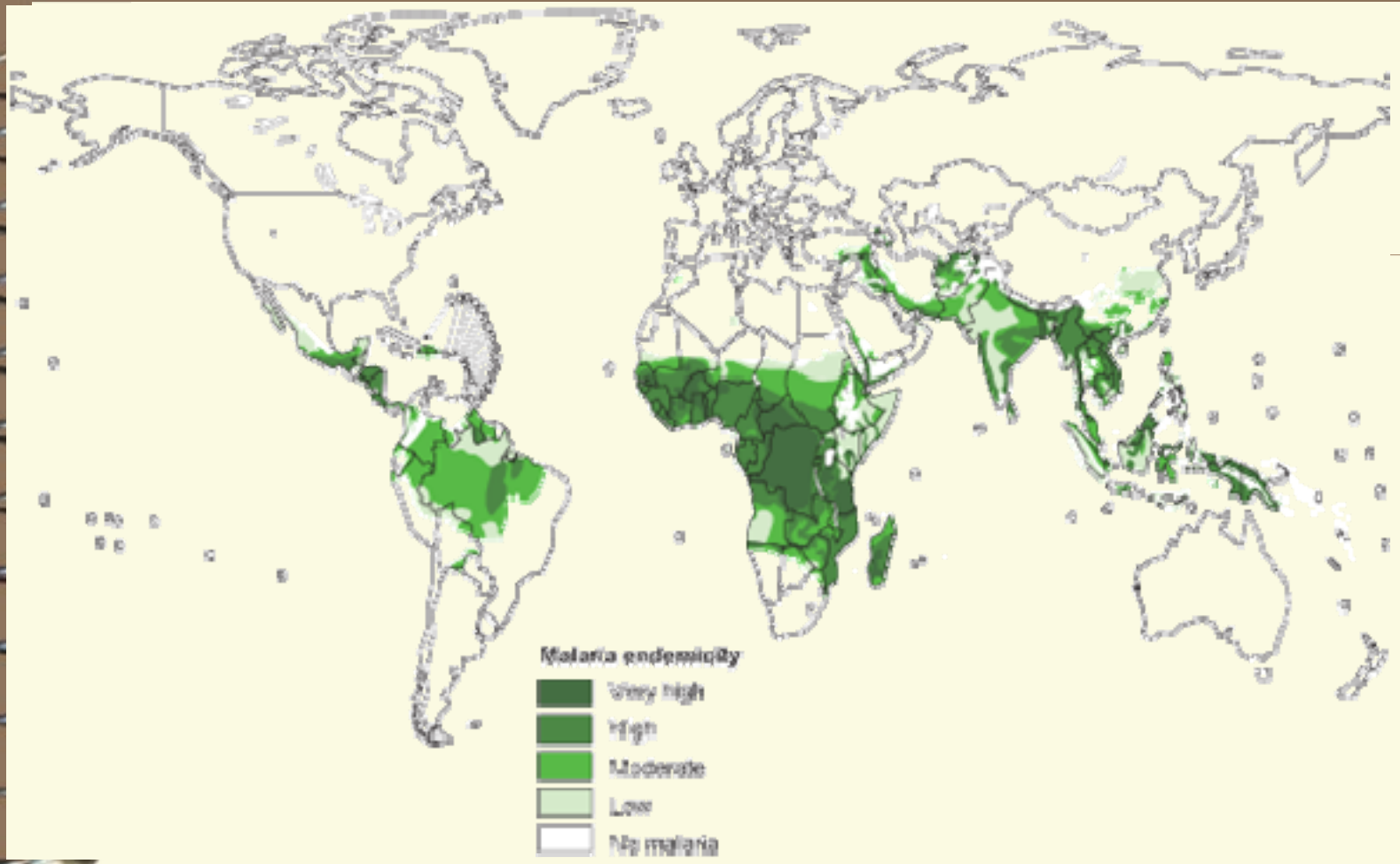
Chemotherapy

Chloroquine Phosphate USP ®



Global Pharmaceuticals

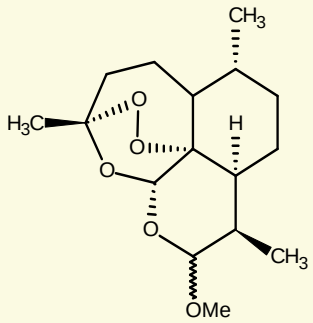
Cloroquine Diphosphate



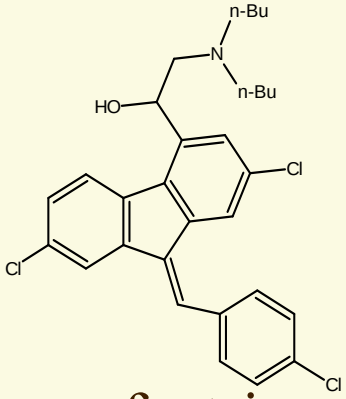
The World Malaria Report 2009

Half of the world's population is at risk of malaria, and an estimated 243 million cases led to estimated 863 000 deaths in 2008

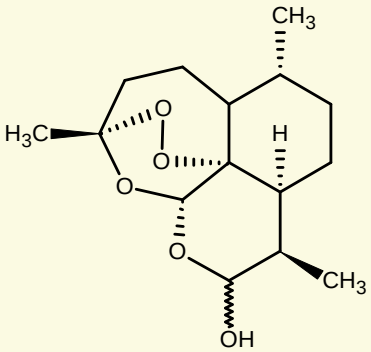
Actual Treatment



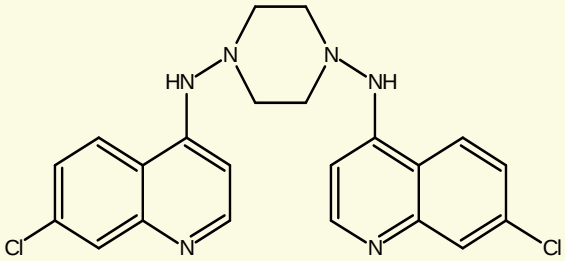
Artemeter



Lumefantrine

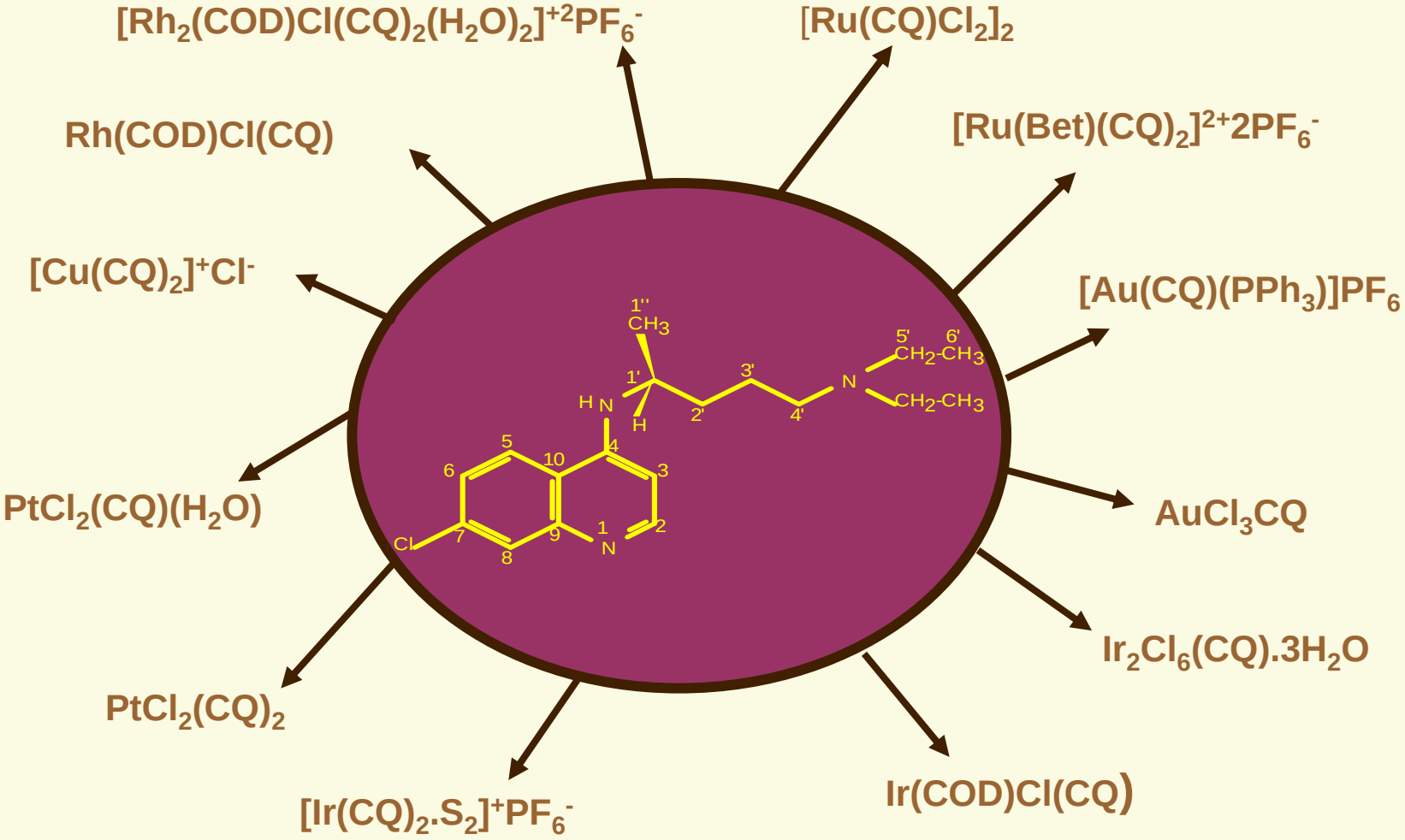


Dihidroartemisine



Piperaquine

Metal-CQ complexes

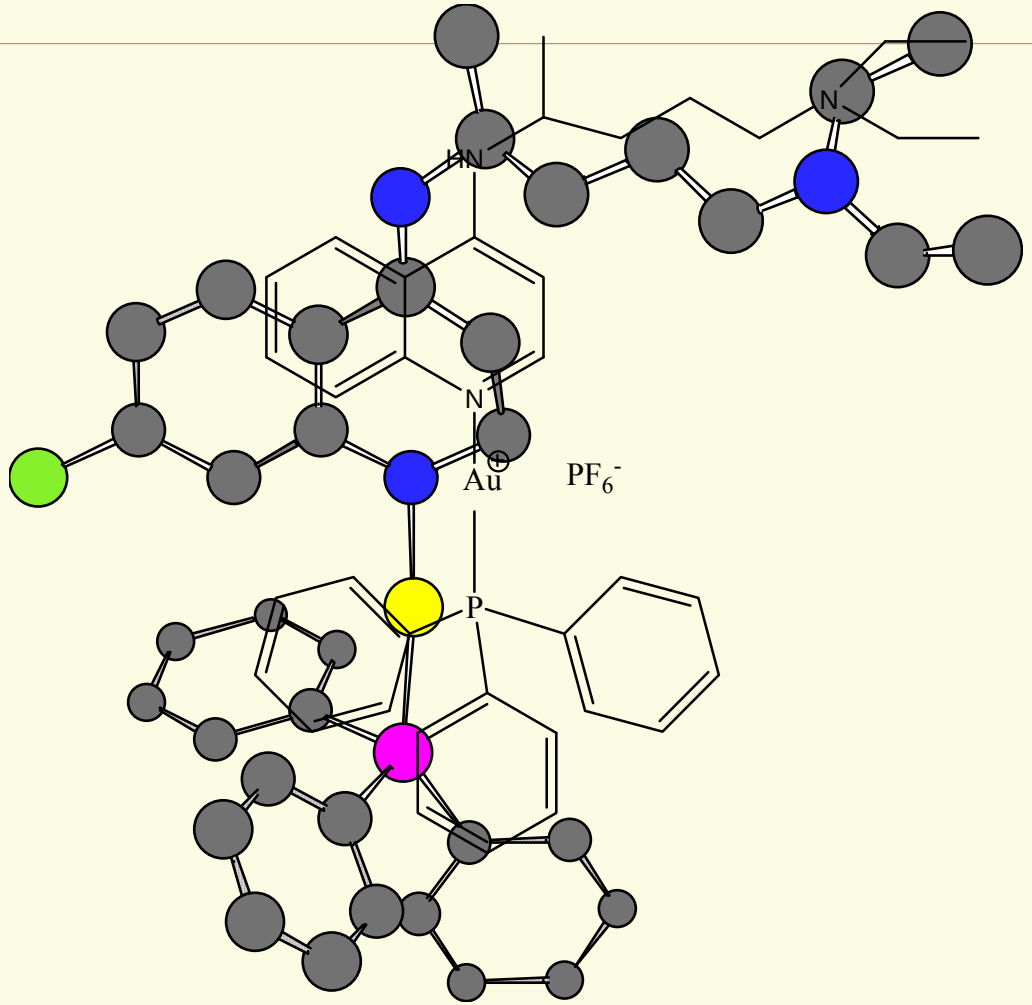


Effect of CQDP and their metal complexes on the in vitro growth of strains *of the P. berghei*

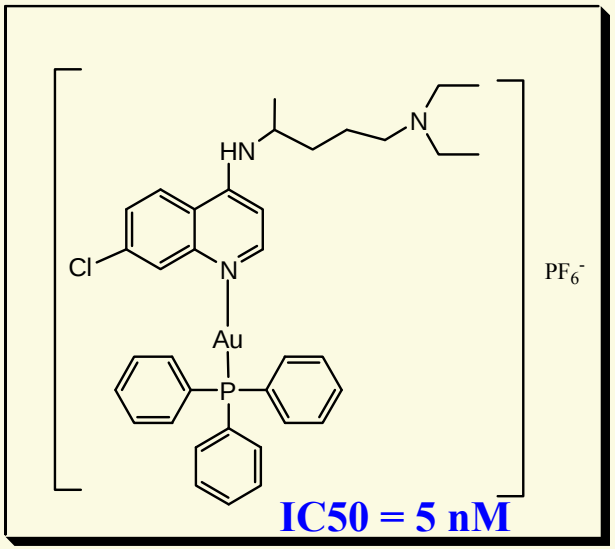
Compuesto	IC_{50} (nM)	Relación $IC_{50complejo}/IC_{50CQDF}$
CQDF	72	
$Rh(COD)(CQ)Cl$	73	1.1
$[Rh(COD)(CQ)_2(H_2O)]^{2+}2PF_6^-$	263	0.3
$[RuCl_2(CQ)]_2$	18.3	4.0
$[AuPPh_3(CQ)]^+PF_6^-$	3.3	21.8
$Ir_2Cl_6(CQ).3H_2O$	59	1.2
$Ir(COD)(CQ)Cl$	72	1.0
$[Ir(CQ)_2(S)_2]^+PF_6^-$	126	0.6
$[Cu(CQ)_2]^+Cl^-$	33	2.2
$Pt(CQ)_2(H_2O)_2$ (trans)	1175	0.006
$Pt(CQ)_2(H_2O)_2$ (cis)	617	0.12

Revista de la Sociedad Venezolana de Química, Vol. 20 No 1 Enero-Marzo 1997

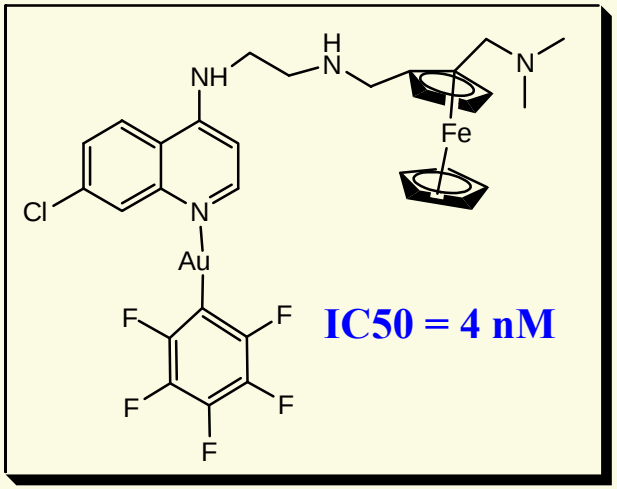
Leader Complex



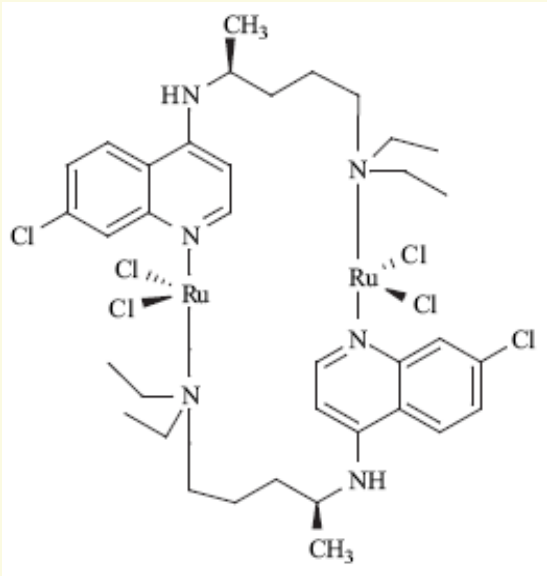
Metallo-antimalaric drugs more Promising



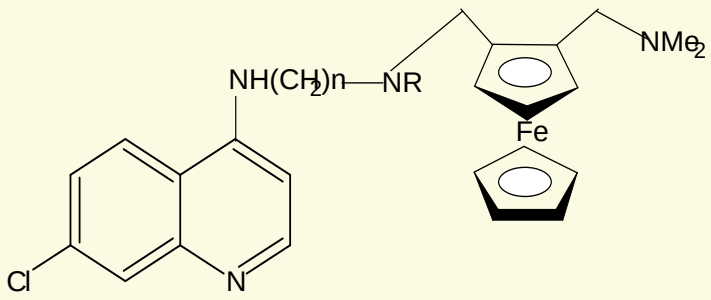
IC50 = 5 nM



IC50 = 4 nM



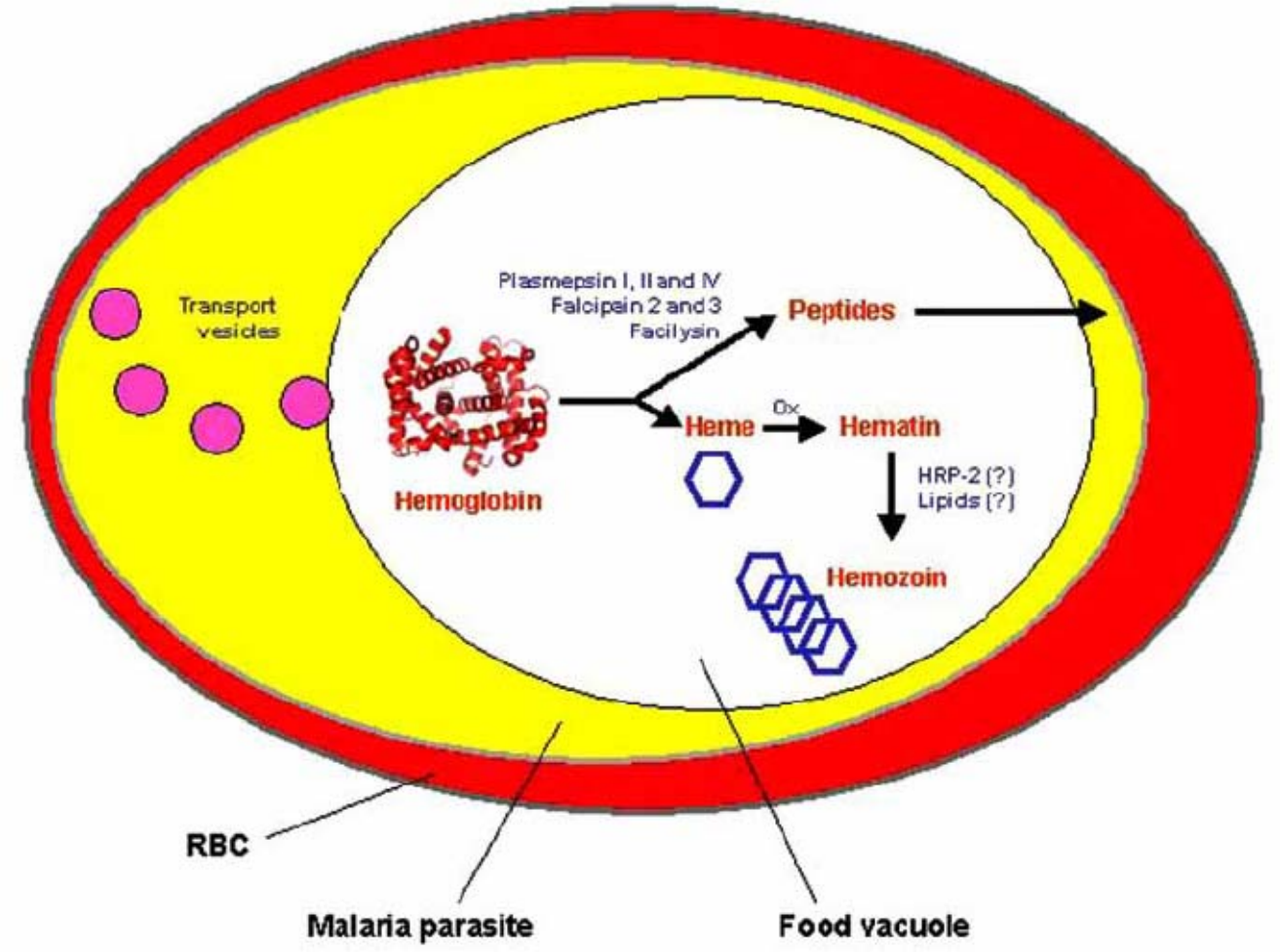
IC50 = 10nM



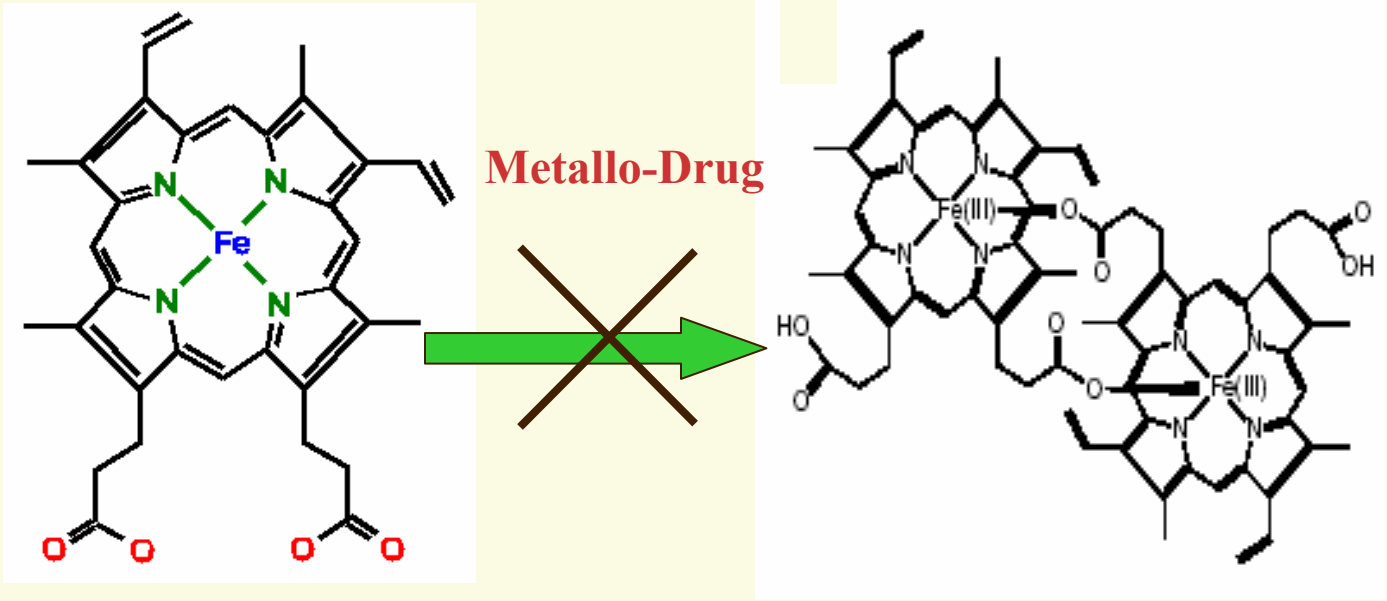
IC50 = 5 nM

Sanofi-aventis and entered phase II clinical trials in September 2007

Schematic representation of hemoglobin catabolism in Plasmodium falciparum.



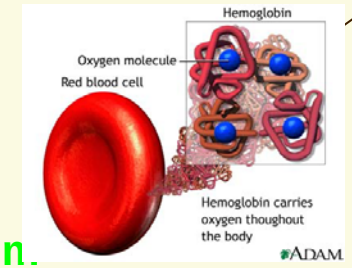
Malarial's Drugs Action Mechanism



Interference of the drug with the detoxification of heme

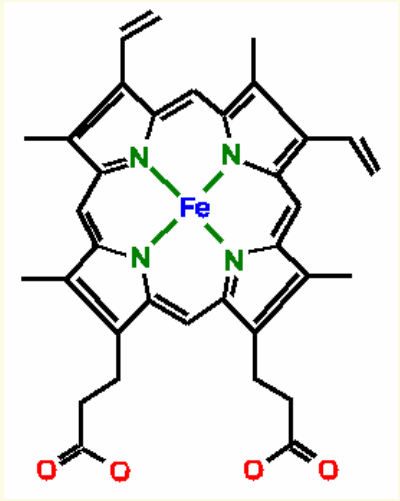
Food Vacuole

PEPTIDS



Plasmodium

Digestion (Proteasas)



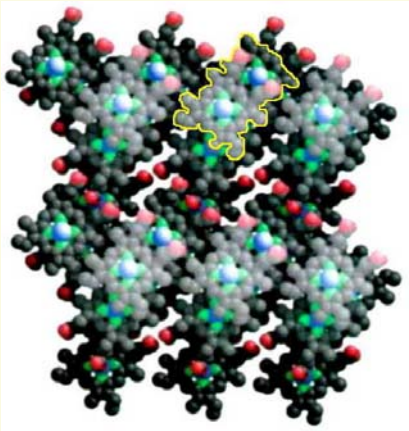
Oxidation

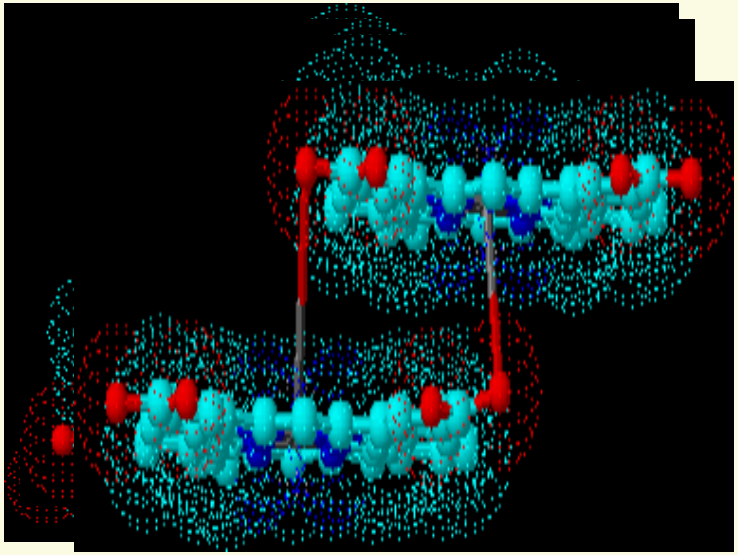
HEMATIN

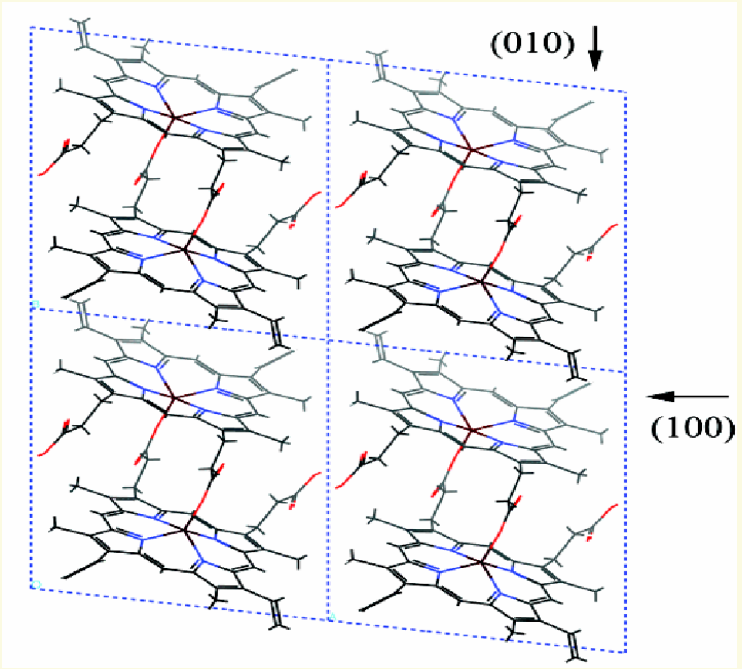
$H_20/OH^-Fe(III)PPIX$

Hemozoín

Structure of Hemozoín

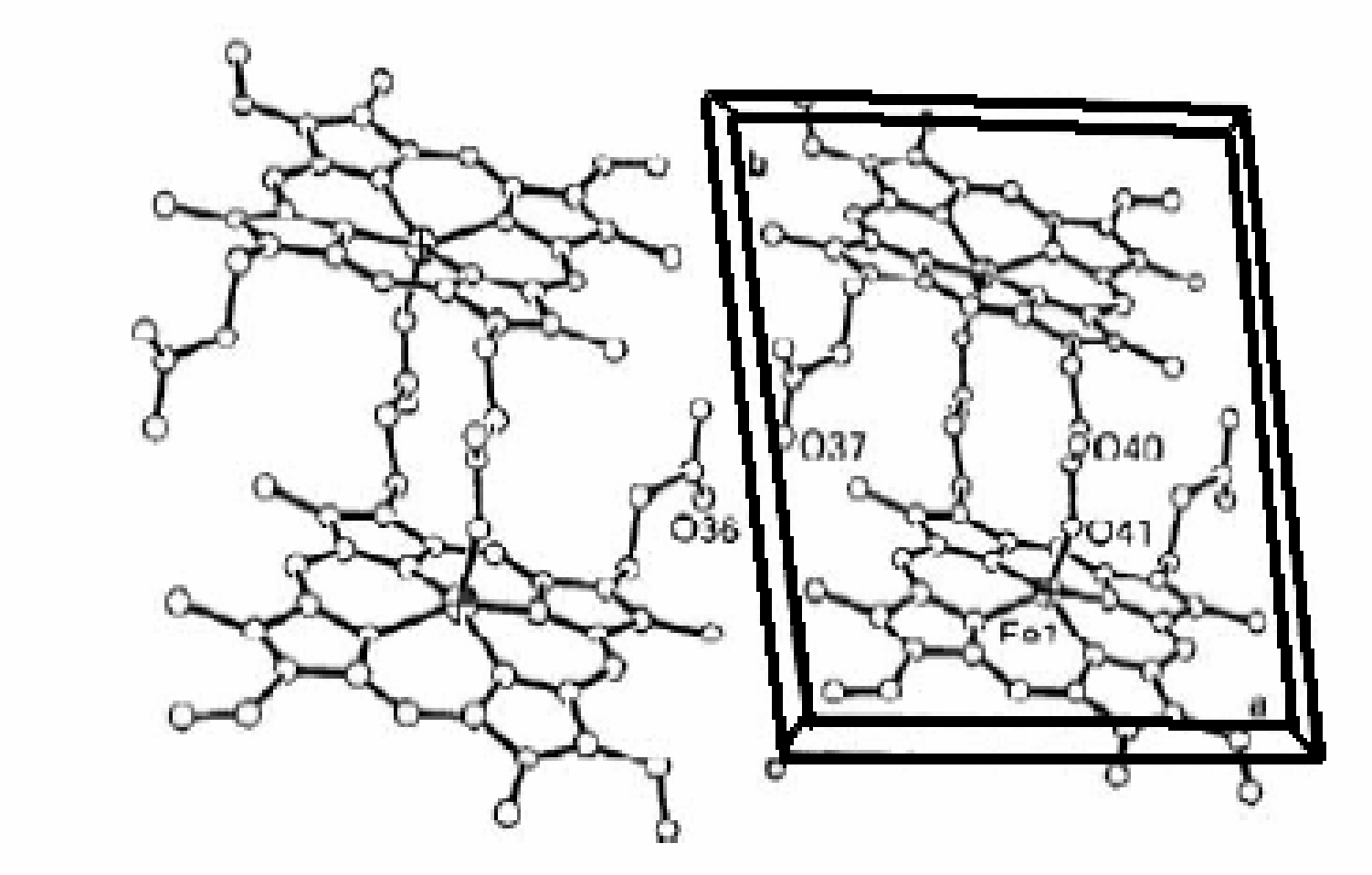






Nature. **404**, 307, (2000)

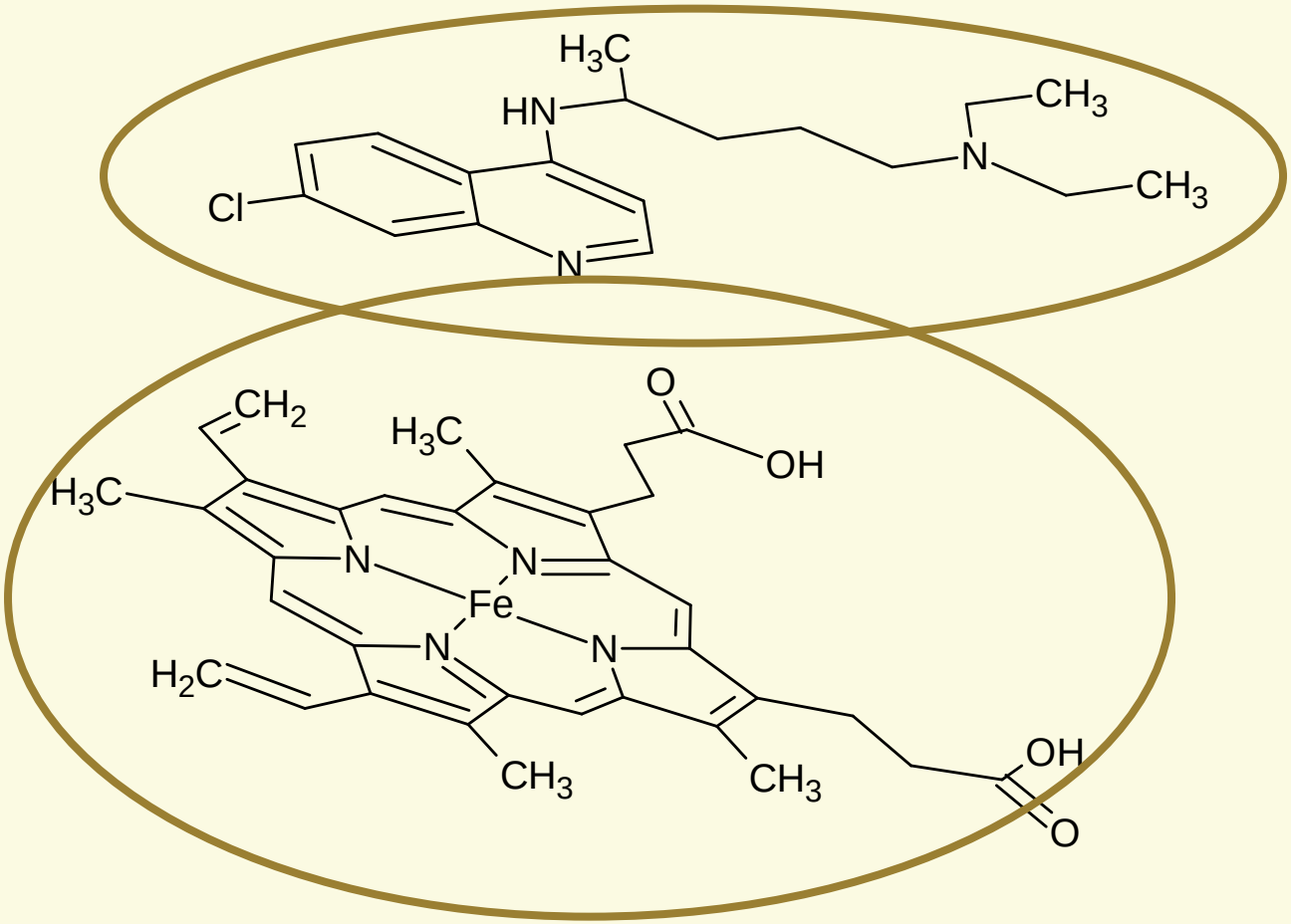
β –Hematin



Biochem. **365**, 343, (2002)

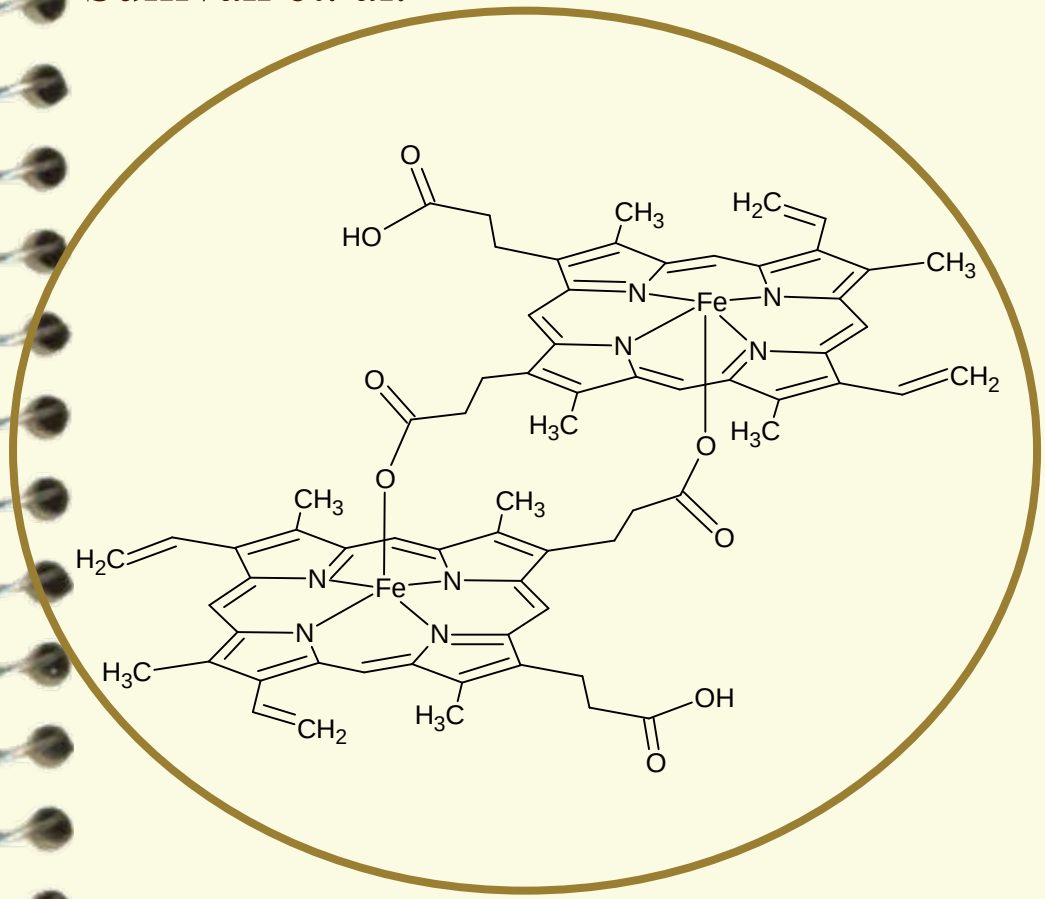
Hipótesis de la inhibición de la formación de la hemozoína

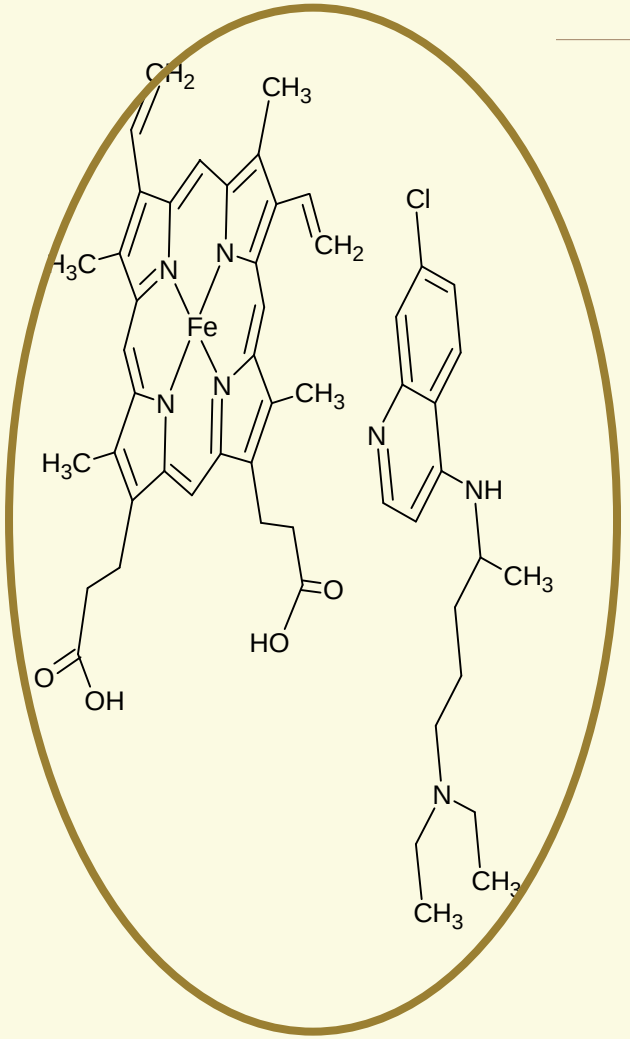
Egan T.



Egan T.J; Ross David; Adams Paul; *Quinoline anti-malarial drugs inhibit spontaneous formation of β-haematin (malaria pigment)* FEBS Letters 352 (1994) 54-57

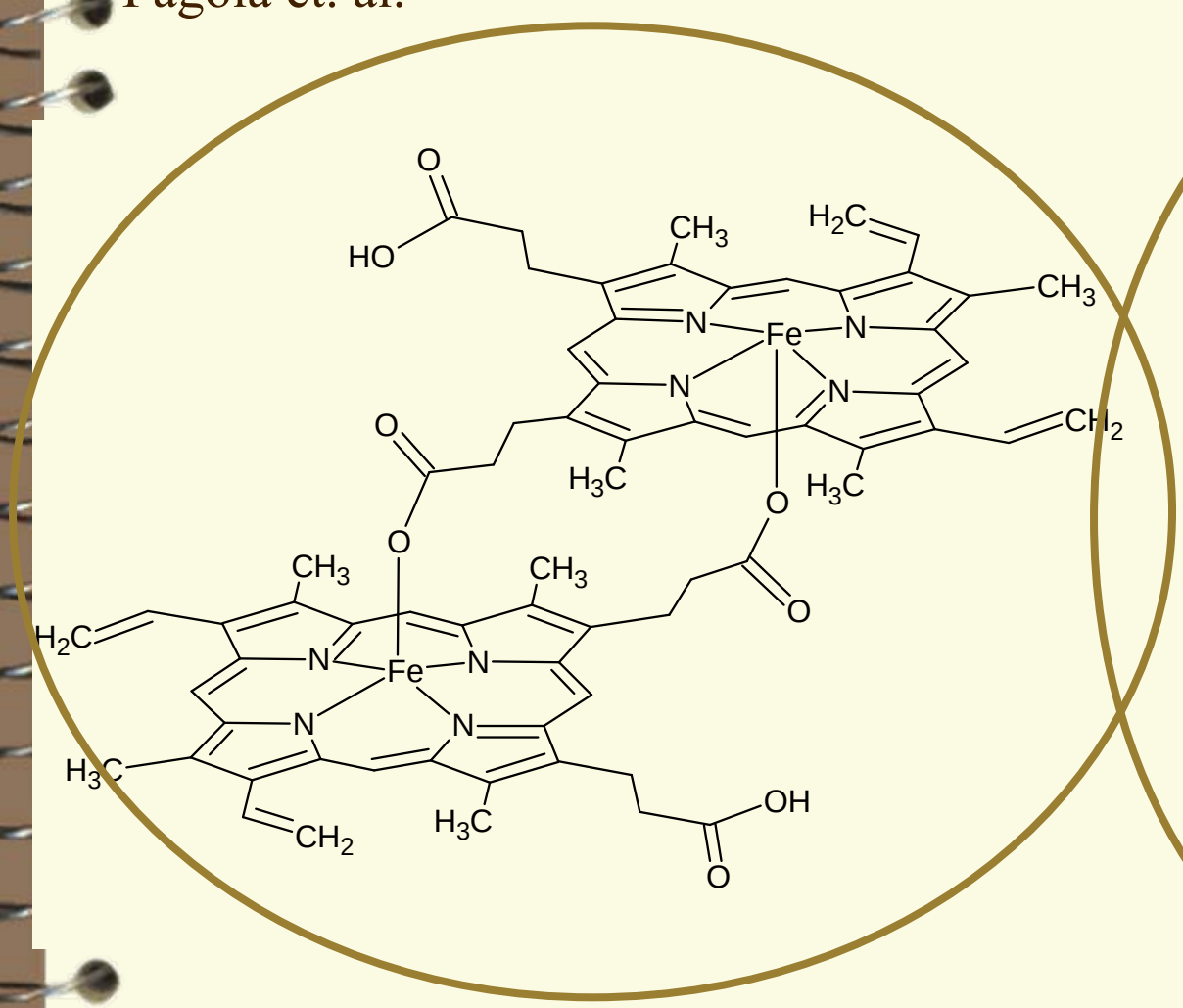
Sullivan et. al.

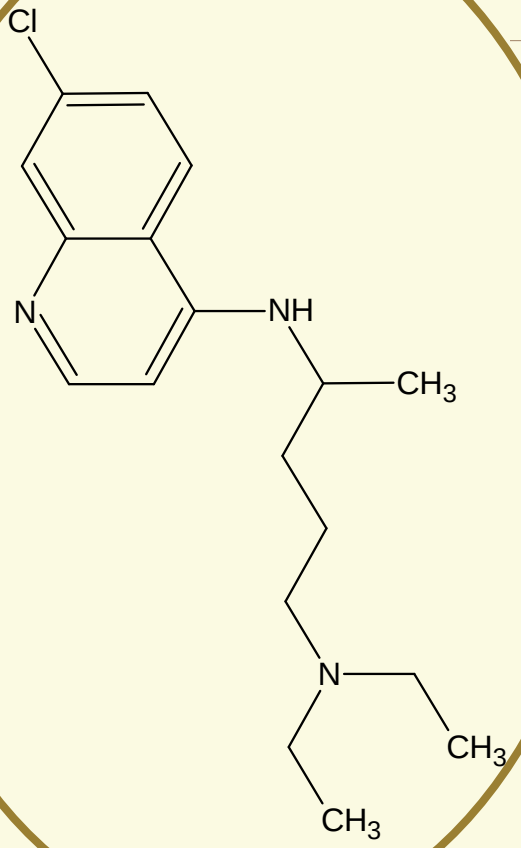




Sullivan, D. J.; Gluzman, I. Y.; Russell, D. G.; *Proc. Natl. Acad. Sci. USA*, **1996,** *93*, 11865

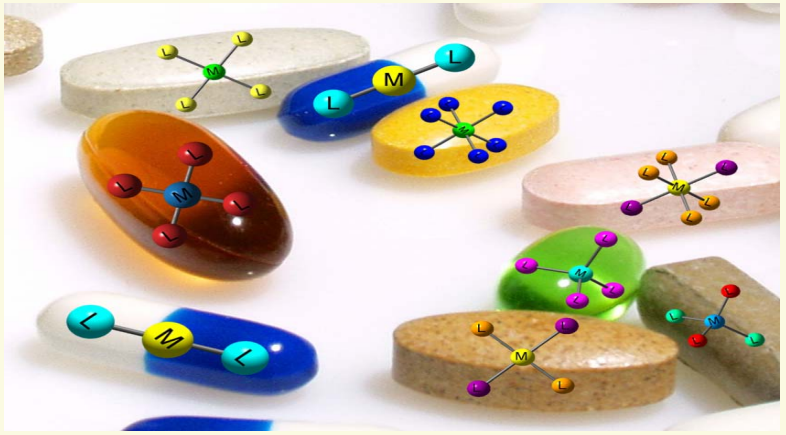
Pagola et. al.





Pagola, S; Stephens, P. W; Bohle, D. S; Nature **2000**, *404*, 307

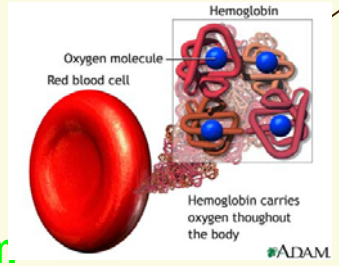
At the Laboratory



Interference of the drug with the detoxification of heme

Food Vacuole

PEPTIDS



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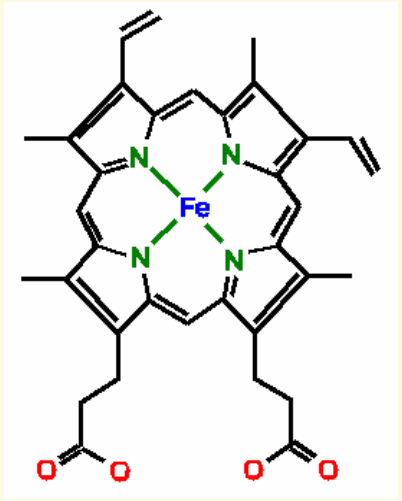
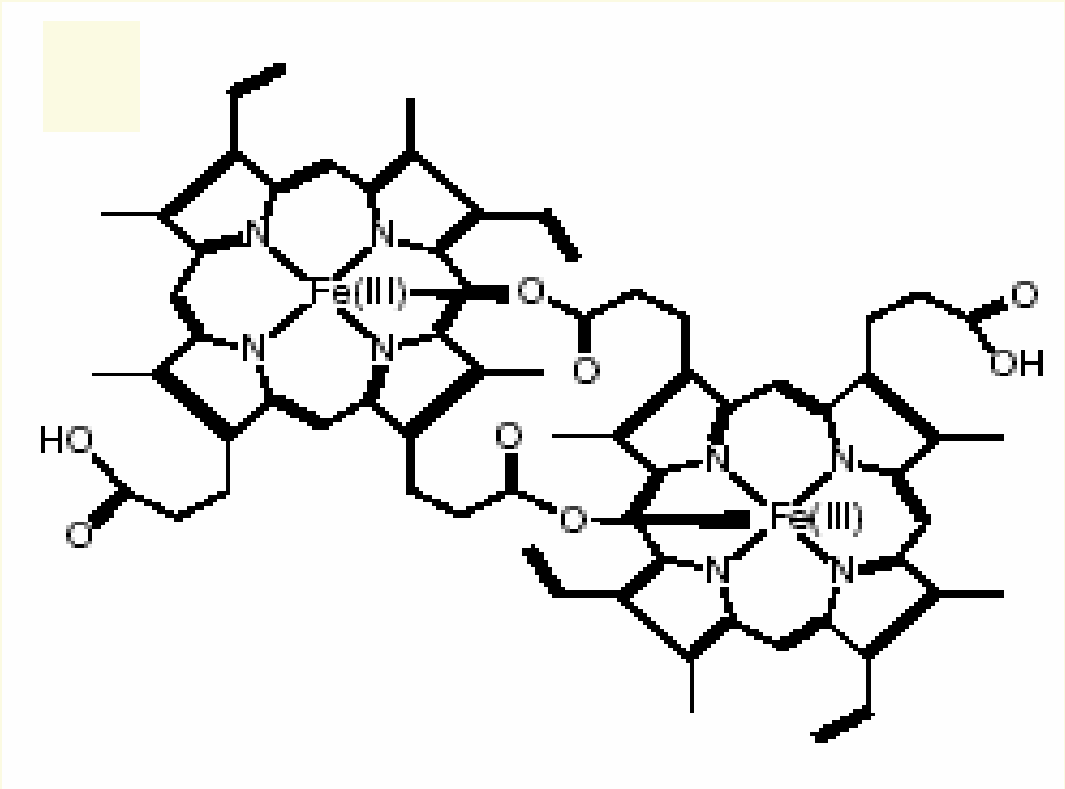


Oxidation

HEMATIN
H_20/OH^-Fe(III)PPIX

Hemozoín

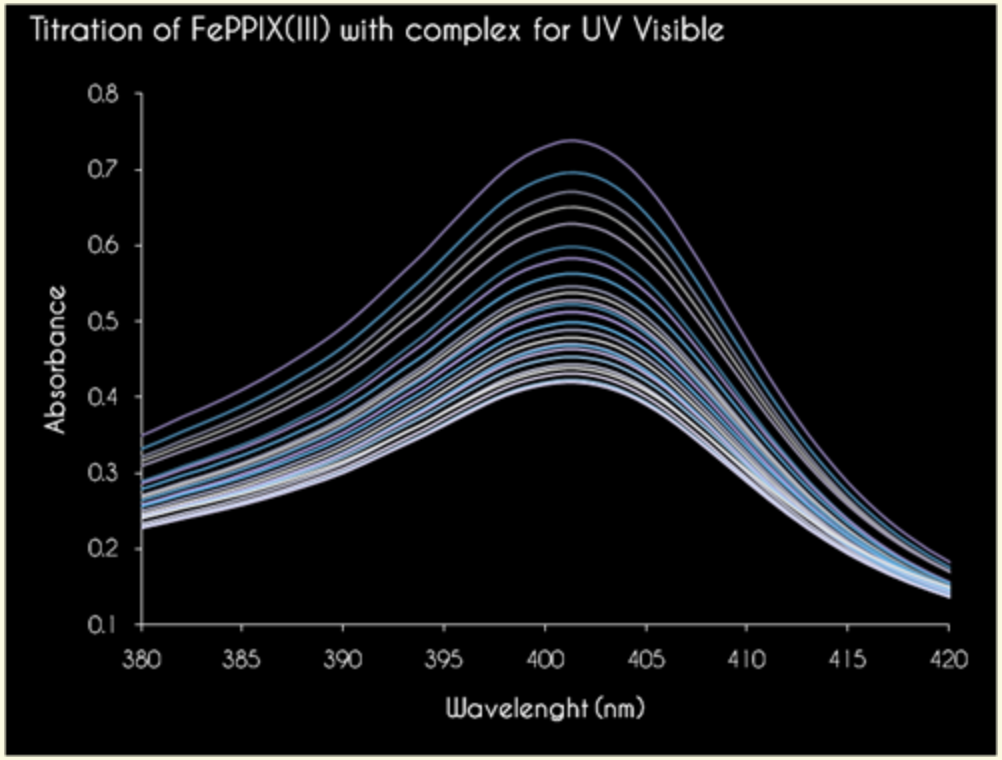
Inhibition of the formation of β –Hematin by Infrared



FEBS Letters, **352**, 54, (1994)

Techniques used for the evaluation of the Ferriprotoporphiryn target

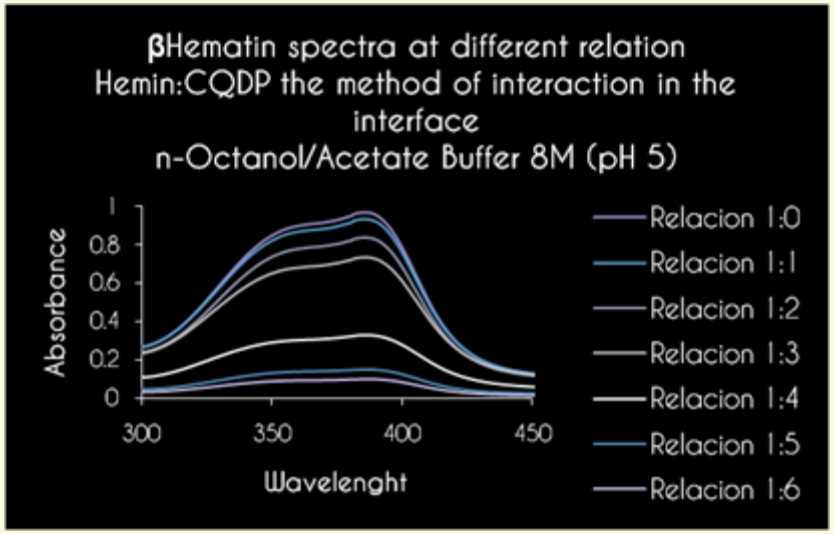
•Titration of FePPIX(III) with Complex by UV Visible

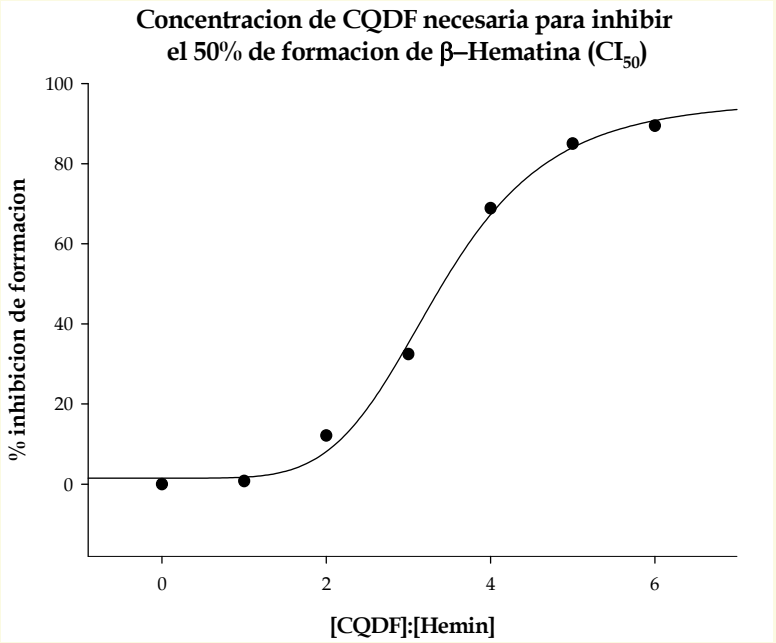


Egan T. J. et. al. J. Inorg. Biochem. (1997), 68, 137-145

Techniques used for the evaluation of the Ferriprotoporphiryn target

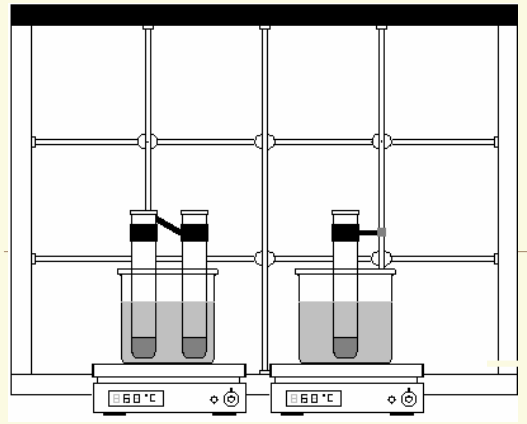
Inhibition of the formation of β-Hematin near the interface water/n-octanol mixture



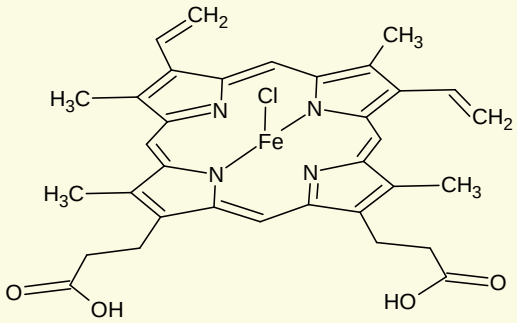


Roberto A. Sanchez-Delgado, et. al. J Biol Inorg Chem (2008), *online version*

Qualitative Analysis by IR

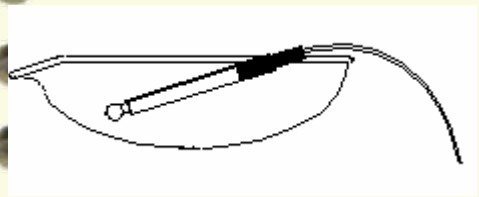


15 mg hemin in
3 ml. de NaOH al 0,1 M

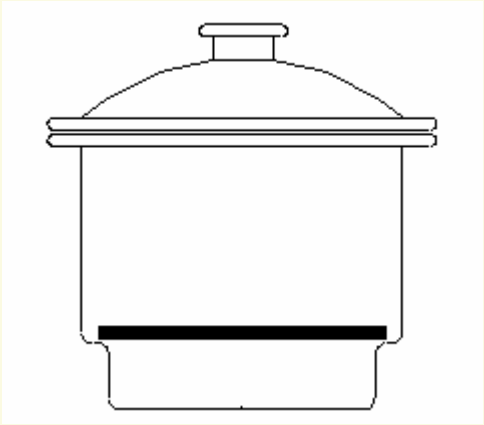


hemin

0,30 ml HCL at 1,0 M and
1,74 ml of acetate buffer (pH=5) al 12,9 M
t= 1h

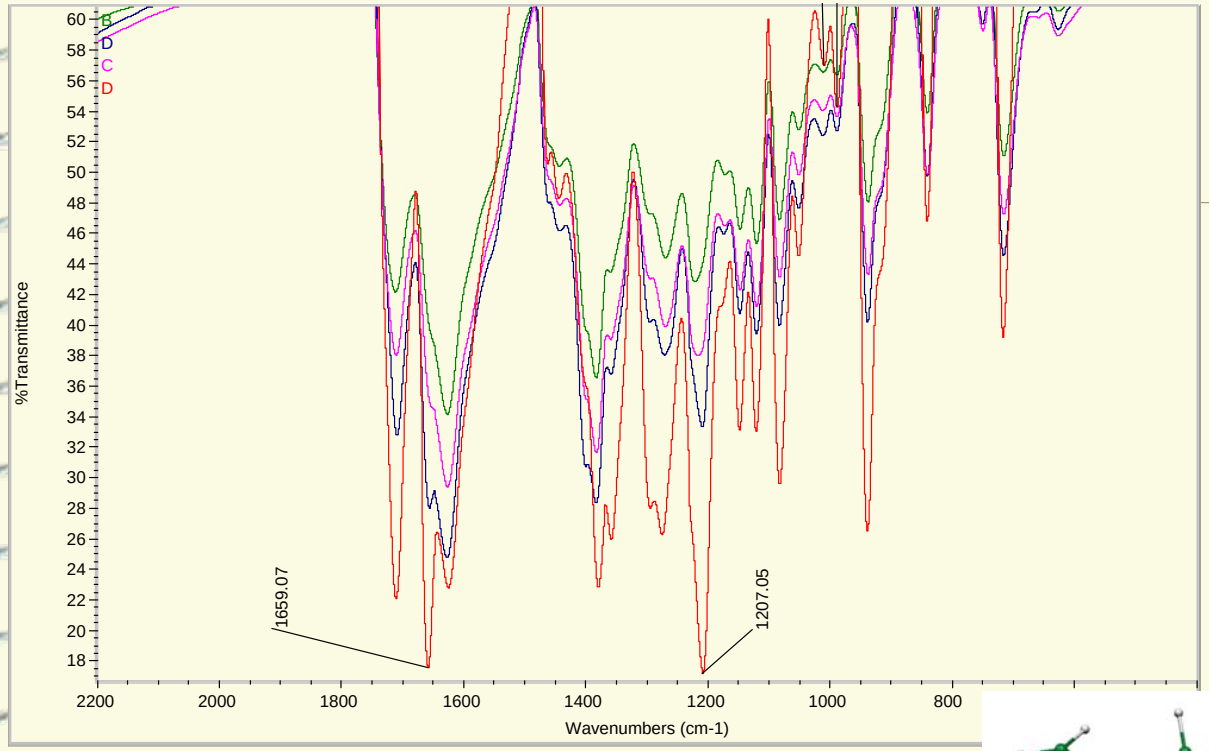


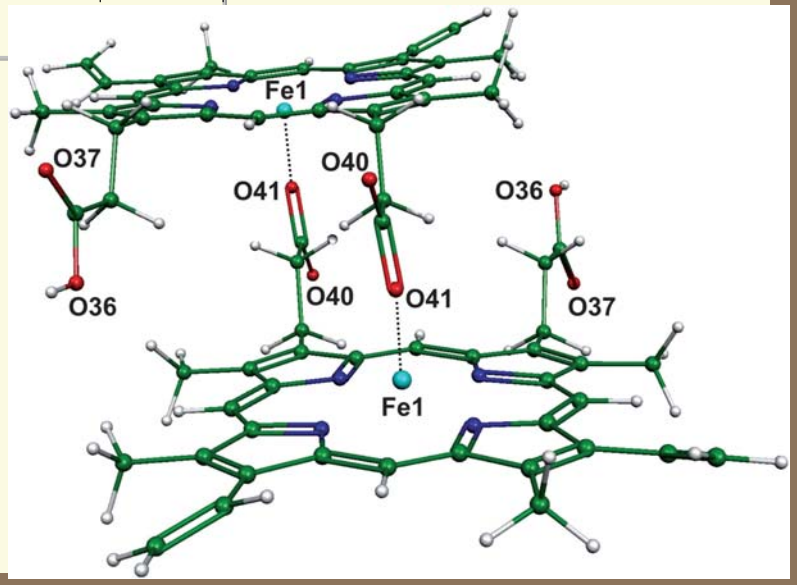
Filtrar
Cool in ice



Dry in P2O5
48 h

Infrared

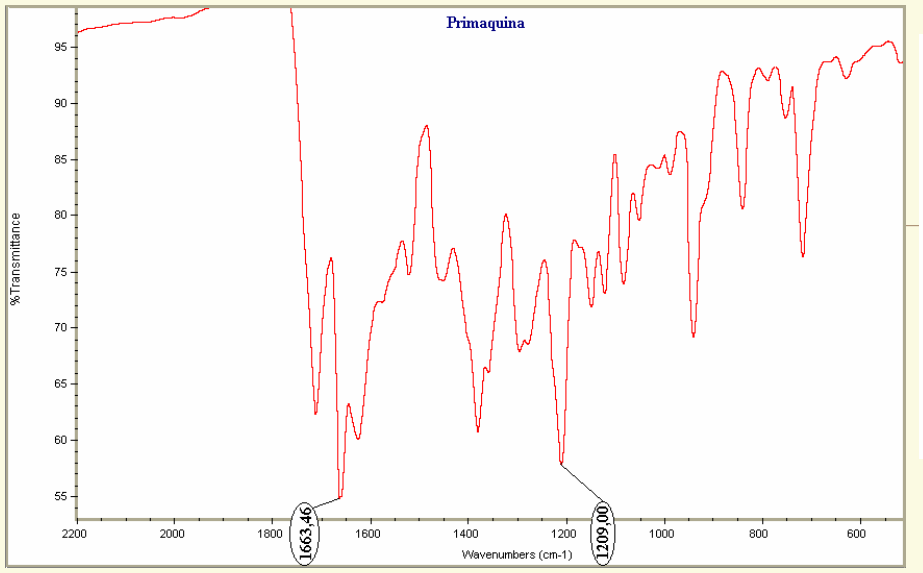

B
D
C
D
%Transmittance
1659.07
1207.05
Wavenumbers (cm-1)

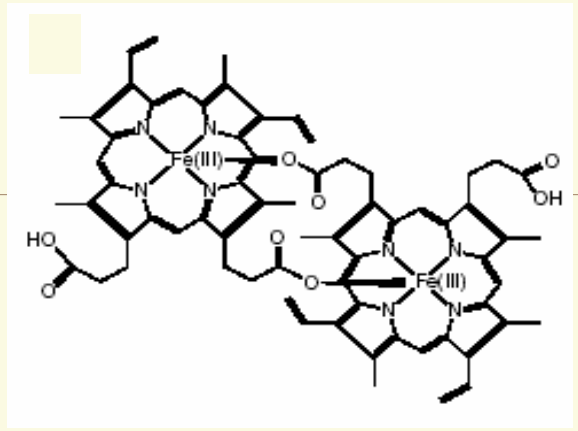

Fe1
O37
O40
O41
O36
O36
O40
O41
O37
Fe1

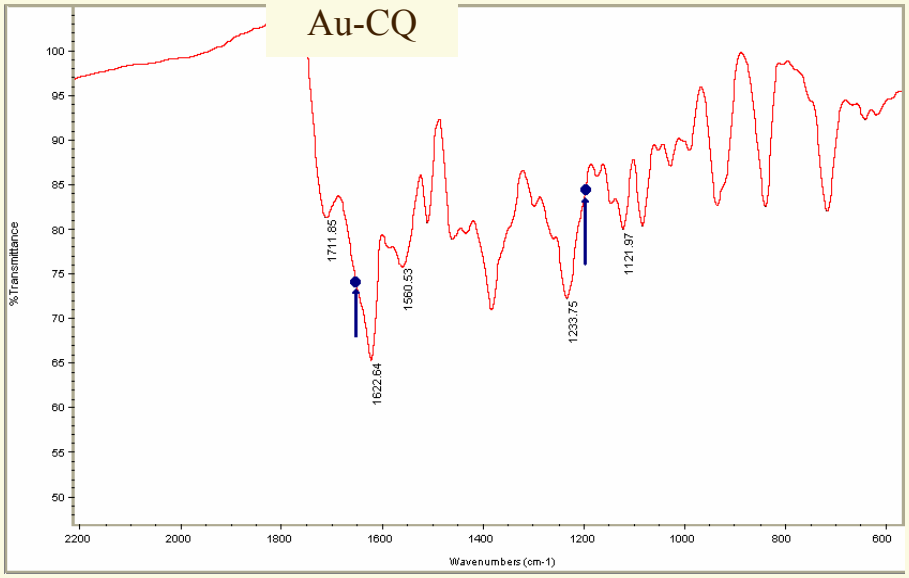
Infrared spectroscopy to monitor the β-Hematin characteristics bands which appear in 1660 y 1210 cm-1 due to the binds iron-propianate of the dimmer

Drug	Intra-erythrocytic Antimalarical Activity	Capacity to bind heme group	Inhibition of the formation of β-Hematin
Cloroquine	+	+	+
Quinine	+	+	+
Amodiaquine	+	+	+
8-hidroxiquinoline	-	Not determine	-

FEBS Letters, **352**, 54, (1994)

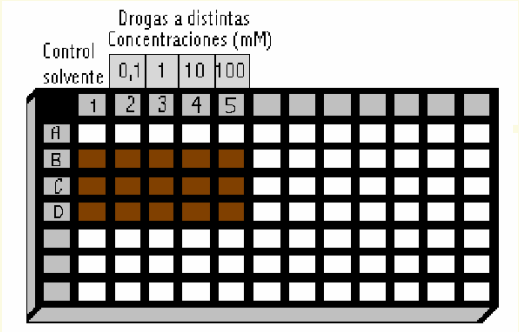




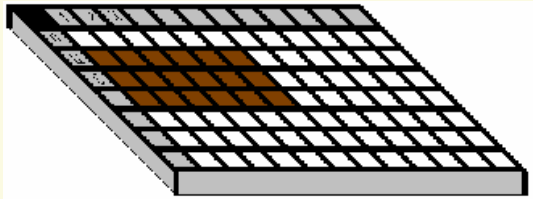


IR spectrum after incubated with $[Au(CQ)(PPh_3)]PF_6$. Arrow indicate the position of the absent β-Hematin bands .

The IC50 of β-Hematin formation in buffer assay in 96-well micro plates.



Incubation during 48 h. a 37 °C



Centrifuge

Remove of sobrenadate

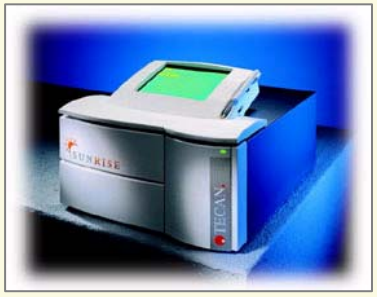
Each well contain:

- ✓ 50 µL hemin solution
- ✓ 50 µL of complex

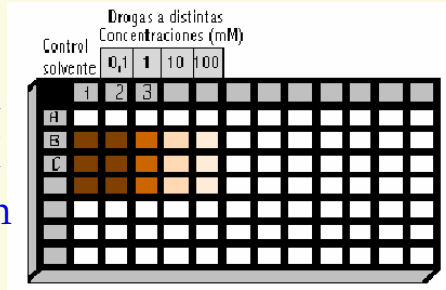
At different concentrations

- ✓ 100 µL of acetic acid /sodium acetate buffer pH 4.4

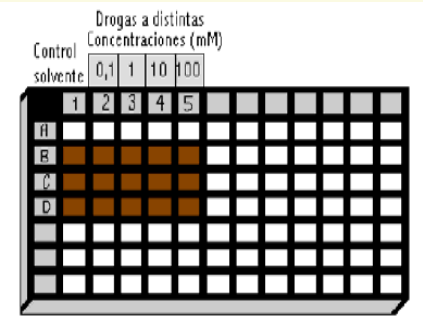
405 nm (hemina)



Dilute in NaOH solution

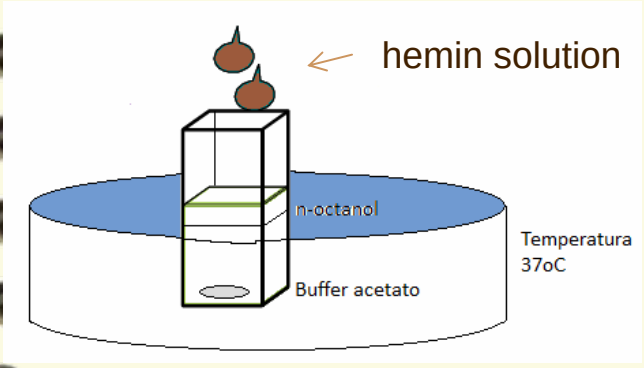


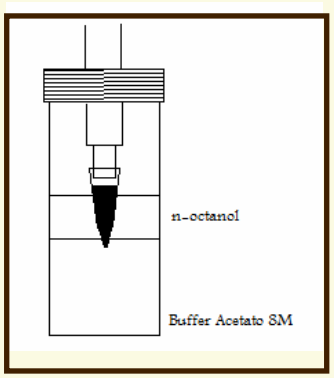
Wash with 200 µL of DMSO



[i] J. N. Domínguez, C. León, J. Rodrigues, N. Gamboa de Domínguez, P. J. Rosenthal, II Fármaco, 60 (2005) 307-311

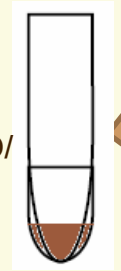
The IC50 of β-Hematin formation in interface



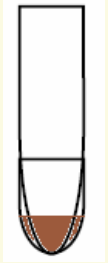


2 h

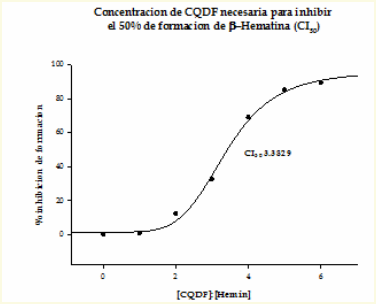
Se disuelve en NaOH

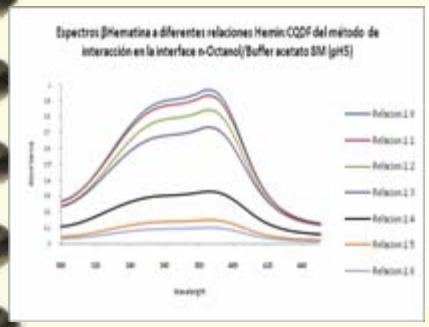


Washing with DMSO/ EtOH

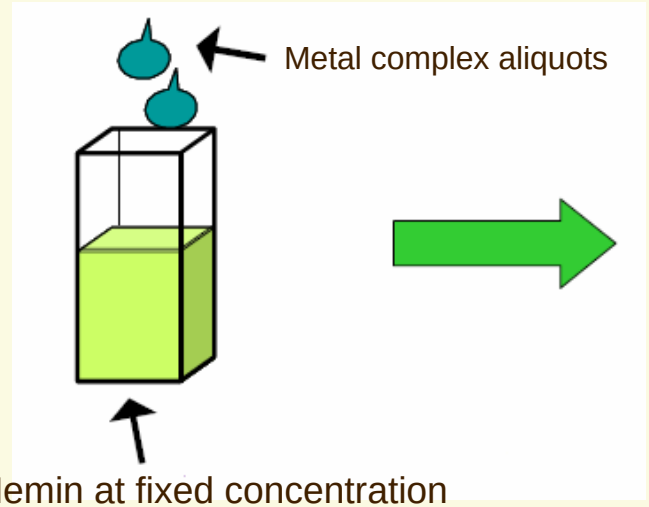


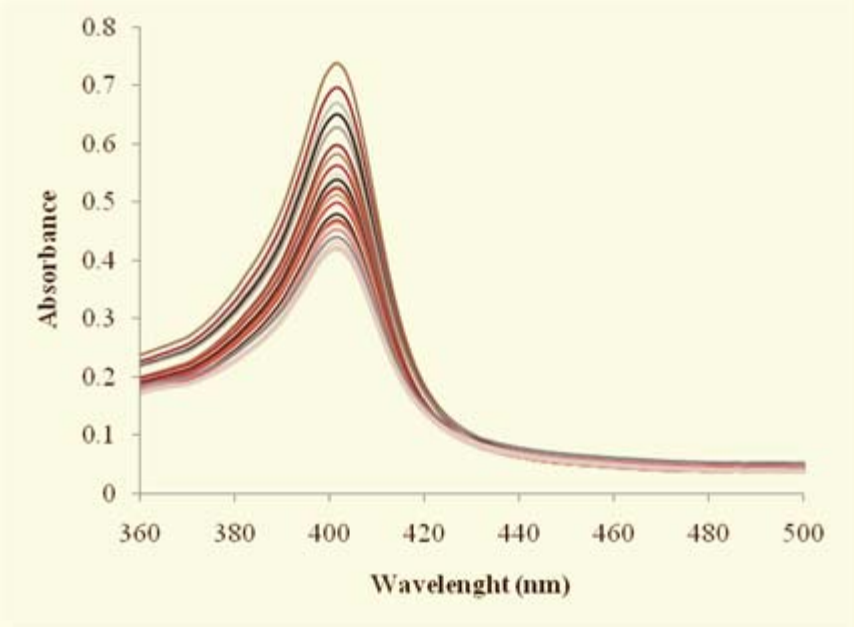
Abs at 385nm





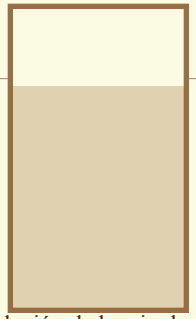
The association constant of [Au(CQ)(PPh3)]PF6 with Fe(III)PPIX





The association constant of [Au(CQ)(PPh3)]PF6 with Fe(III)PPIX

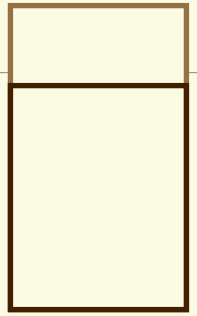
Muestra



Solución de hemin de concentración 4µM, Buffer Trizma (pH7) 40% de DMSO

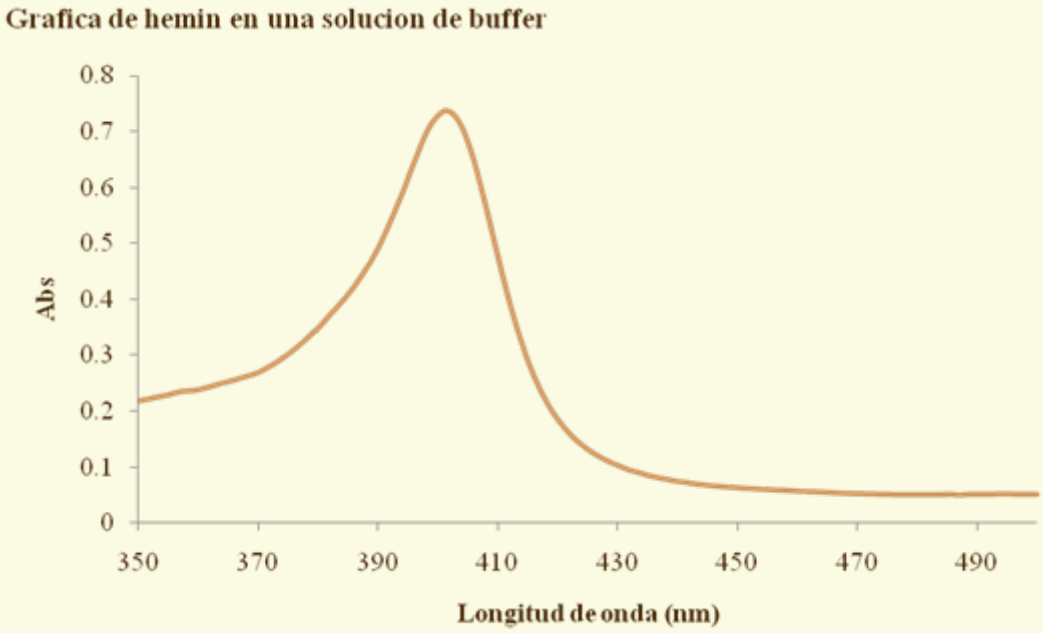
Temperature: 25 °C

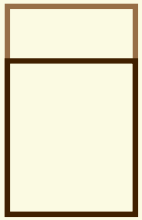
Blanco



Buffer Trizma (pH7) 40% de DMSO

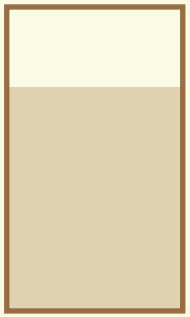
Absorbance readings at 402 nm



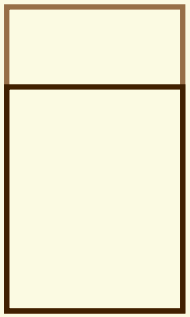


10 µL

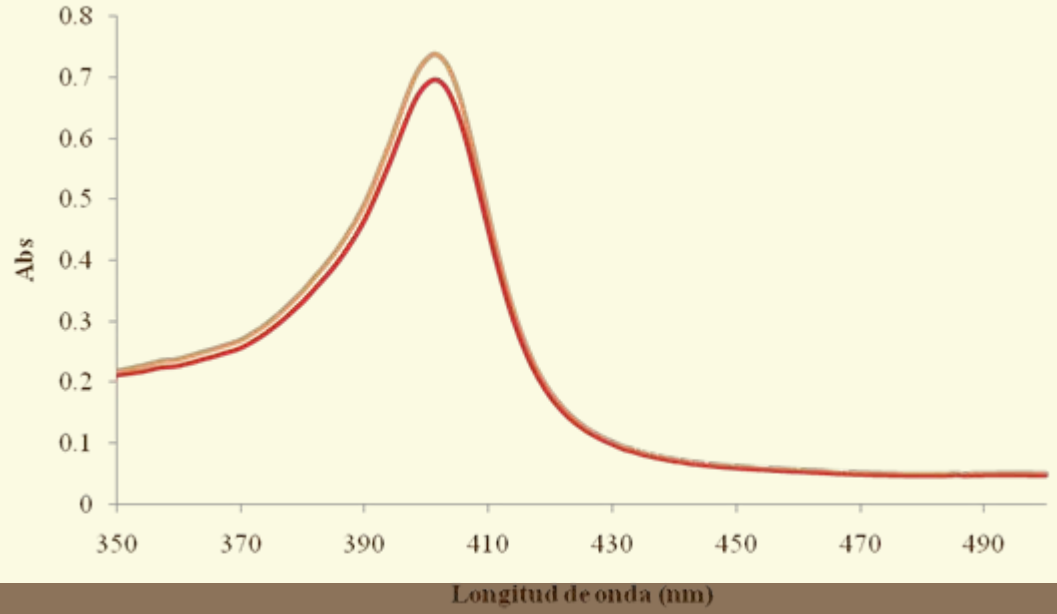
Muestra



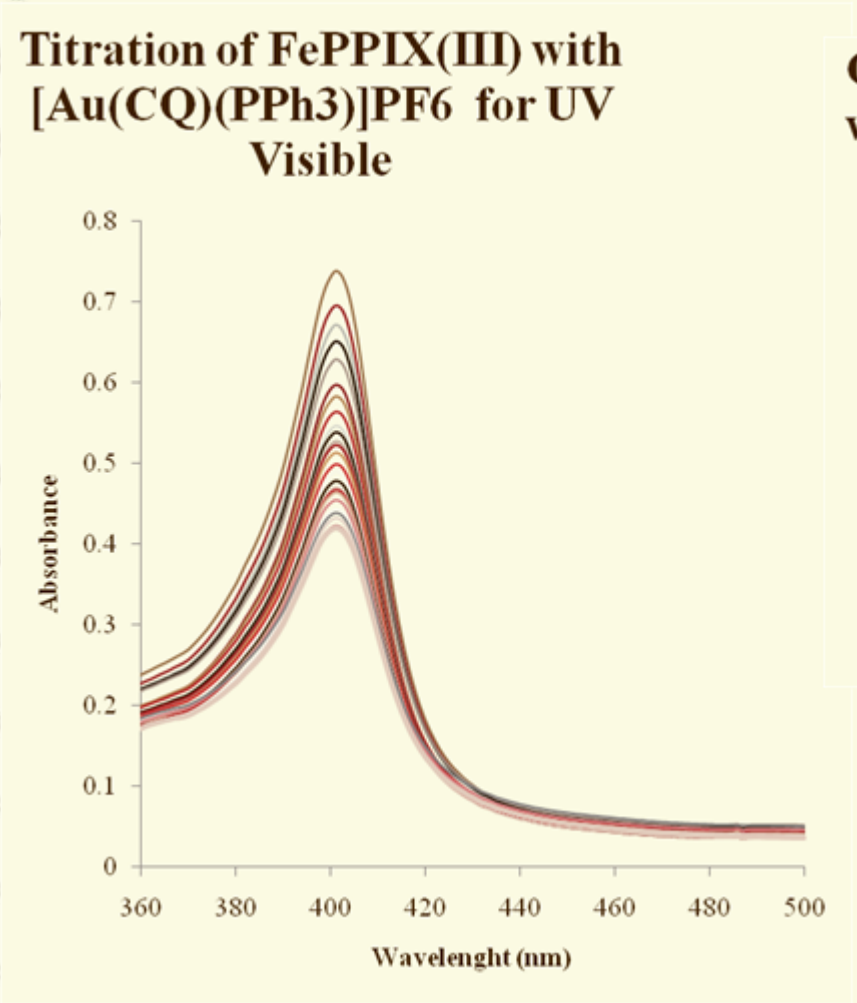
Blanco

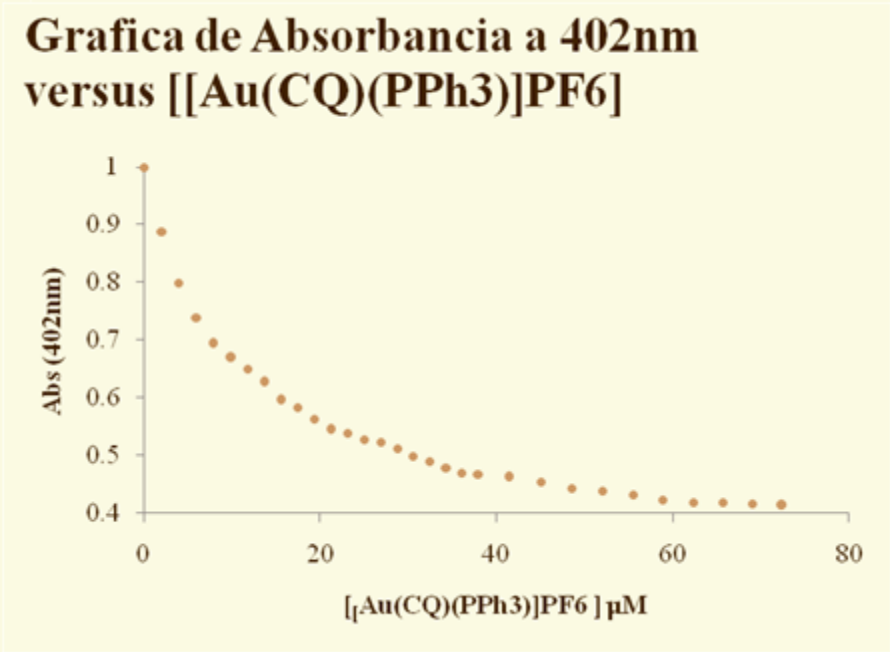


Solución de [Au(CQ)(PPh3)]PF6 a una concentración de 1 mM en buffer al 40% DMSO



The association constant of [Au(CQ)(PPh3)]PF6 with Fe(III)PPIX





$$A = \frac{A_0 + A_\infty K[Q]_{free}}{1 + K[Q]_{free}}$$

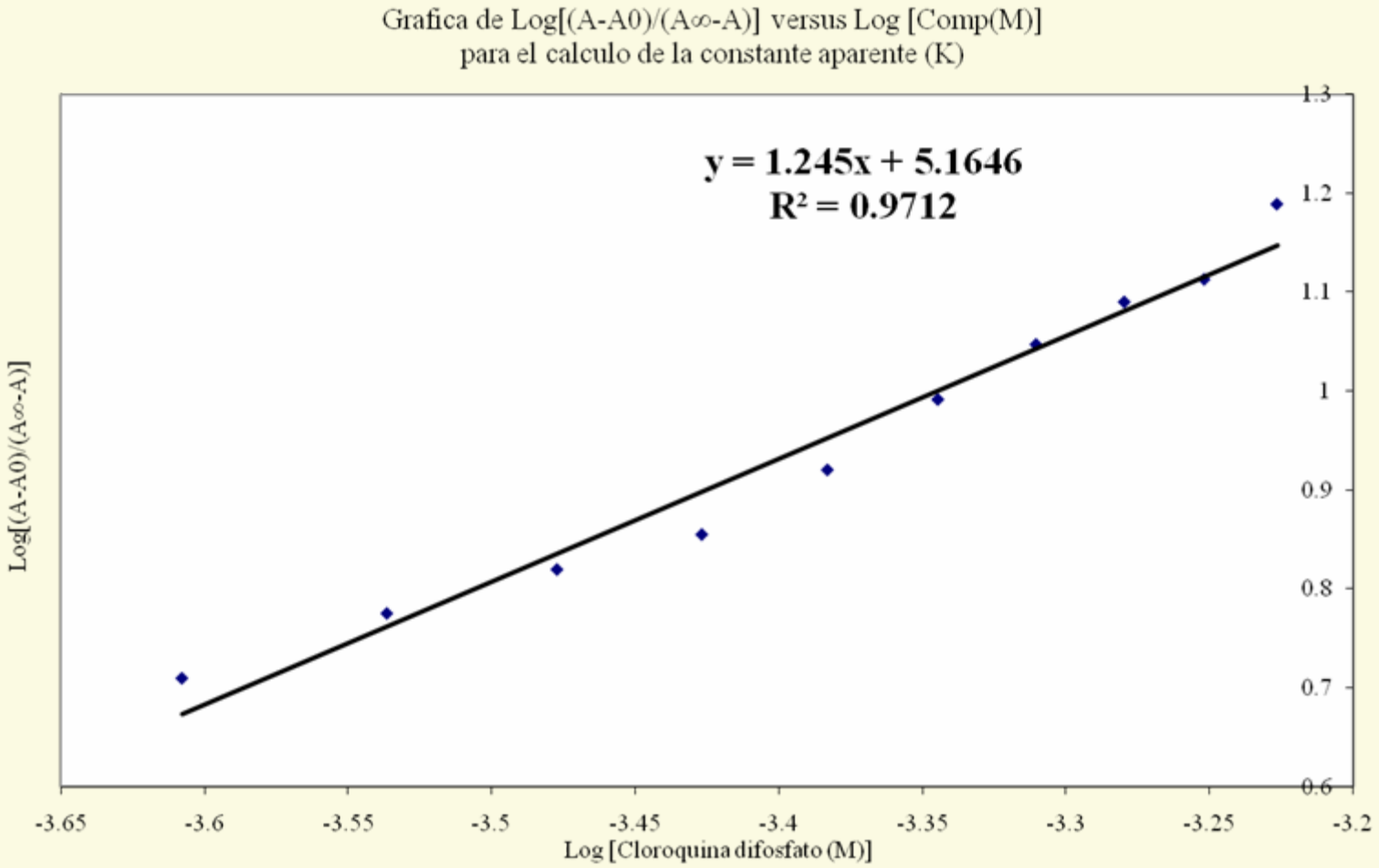
A_0 is the absorbance of hemin before addition of the complex, A∞ is the absorbance for the drug–hemin adduct at saturation, A is the absorbance at each point of the titration, and K is the association constant

Manual

$$A = \frac{A_0 + A_\infty K[Q]_{free}}{1 + K[Q]_{free}}$$

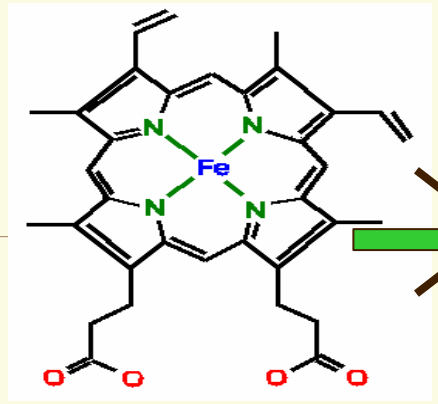
SigmaPlot 10.0

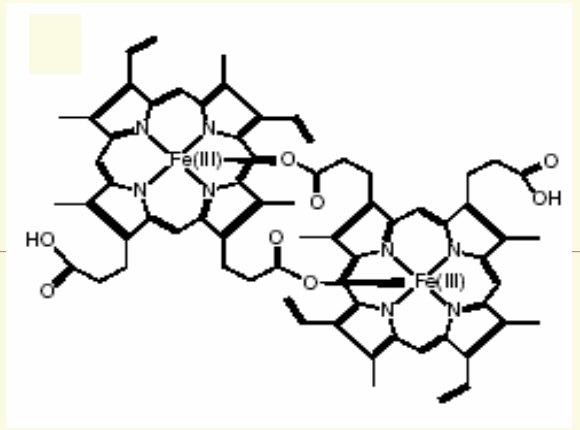
$$y = \frac{a + bx}{1 + cx}$$



Effects of Au-CQ and Ru-CQ complexes on inhibition of β-hematin formation

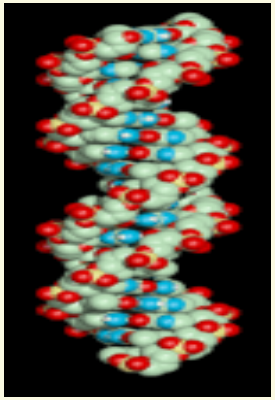
Complex	Inhibition of β-hematin Formation	IC50 (mM)[a] in buffer	IC50 (mM)[b] in interface	Log K[c]	Effect on the *in vitro* growth strains of *P. falciparum* (IC50 nm)[d]	
					FCB1	FCB2
[Au(CQ)(PPh3)]PF6	+	**3,42 ± 1,89**	**0.64 ± 0.02**	**7,69**	**5.1**	**23**
CQDP	+	0,35 ± 0,09 (1)	3.38 ± 0.13 (1)	5,84	47	110
[RuCl2(CQ)]2	- - -	**1.0**	**1.2**	**5.06**	**10.5**	**46.5**

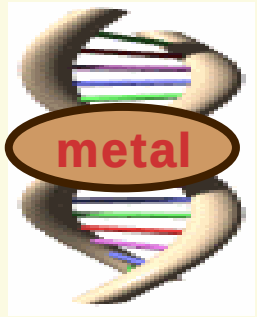




inhibition of β-hematin formation

$[Au(CQ)PPh_3]^+PF_6$





Intercalation
With the ADN