

Inhibitors of pyrophosphate metabolism and signaling as anti- *Trypanosoma cruzi* agents: in vitro, in vivo and 3D-QSAR studies

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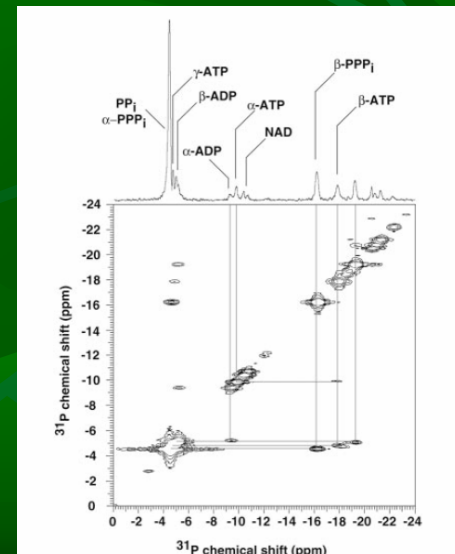
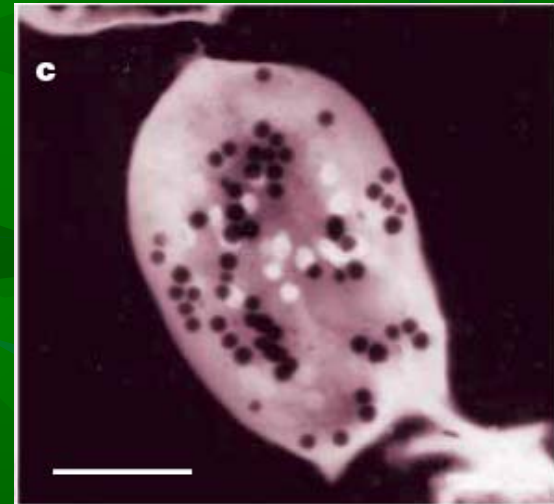
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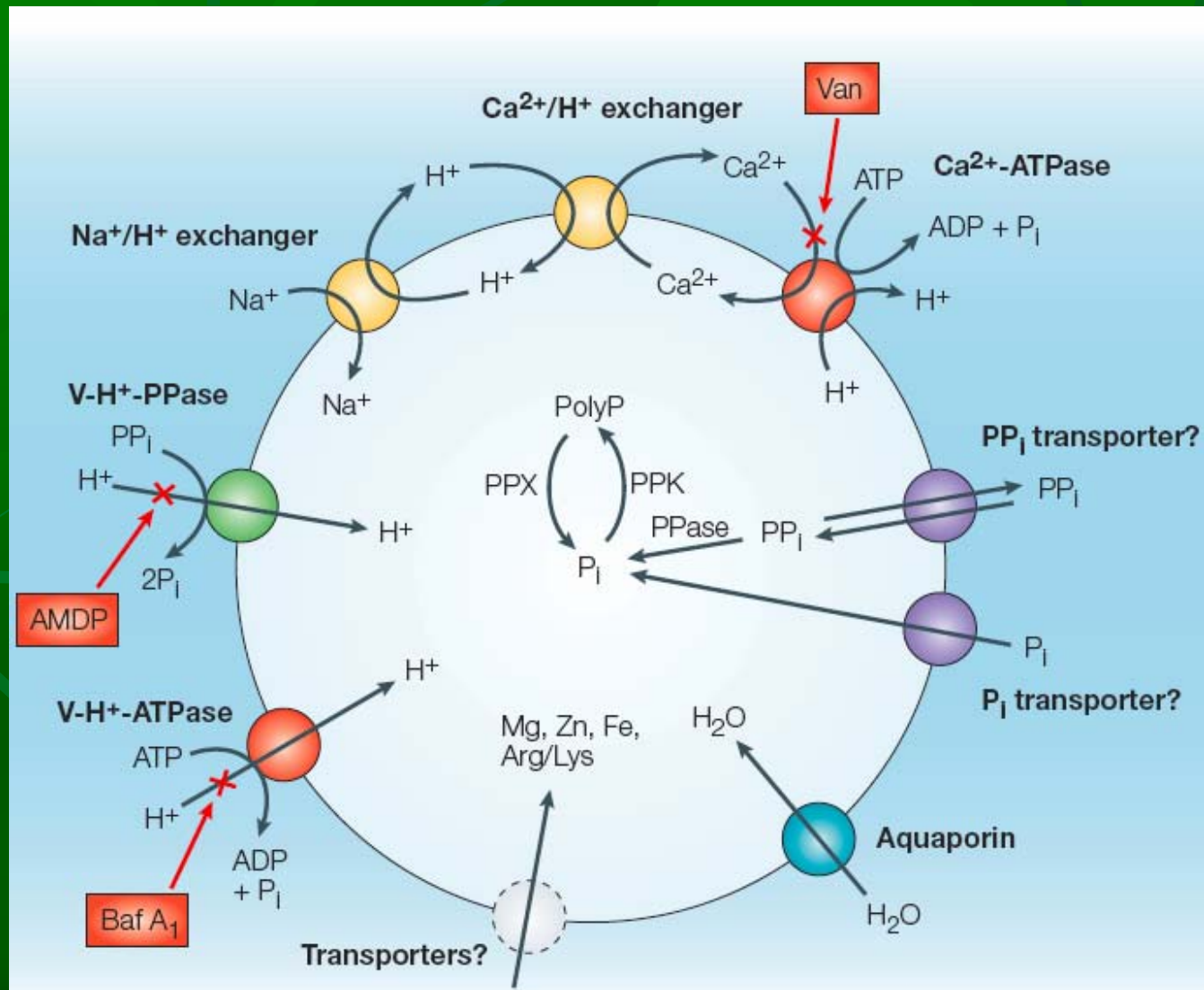
XIII Summer School in Medicinal and Pharmaceutical Chemistry, *February
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Inhibitors of pyrophosphate metabolism and signaling for the specific treatment of Chagas disease: strategy and basic findings

- *T. cruzi*, as well as other Kinetoplastida and Apicomplexa parasites, contains large amounts of inorganic pyrophosphate and other short-chain polyphosphates, which are stored as Ca^{2+} and Mg^{2+} complexes in special organelles termed acidocalcisomes, but also intervene in many metabolic processes in these cells (Docampo et al. 2005. Nature Rev. Microbiol. 3, 251; Urbina et al. 1999. JBC 274, 33609)
- Bisphosphonates, metabolically stable pyrophosphate analogs, accumulate in acidocalcisomes and interfere with pyrophosphate metabolism and signaling

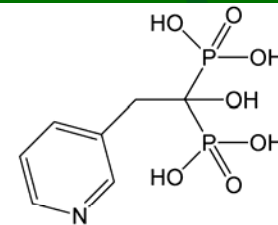


Physiological functions of acidocalcisomes (Docampo et al. 2005. Nature Rev. Microbiol. 3, 251)

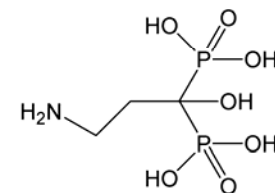


Inhibitors of pyrophosphate metabolism for the specific treatment of Chagas disease. 1. Inhibitors of farnesyl-pyrophosphate synthase (FPPS)

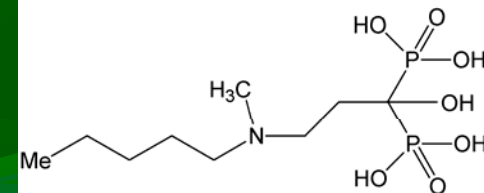
- N-alkyl- and N-aryl-bisphosphonates, inhibitors of farnesyl-pyrophosphate (FPPS) synthase and currently used in the treatment of bone resorption disorders (including osteoporosis) in humans, have also potent and selective anti-*T. cruzi* activity, in vitro and in vivo
- Their potential use as antiparasitic agents could require new pharmacological formulations (including pro-drugs) with pharmacokinetic properties appropriate for this new application
- The clinical development of these compounds is estimated in the medium term (within 10 years)



Risedronate

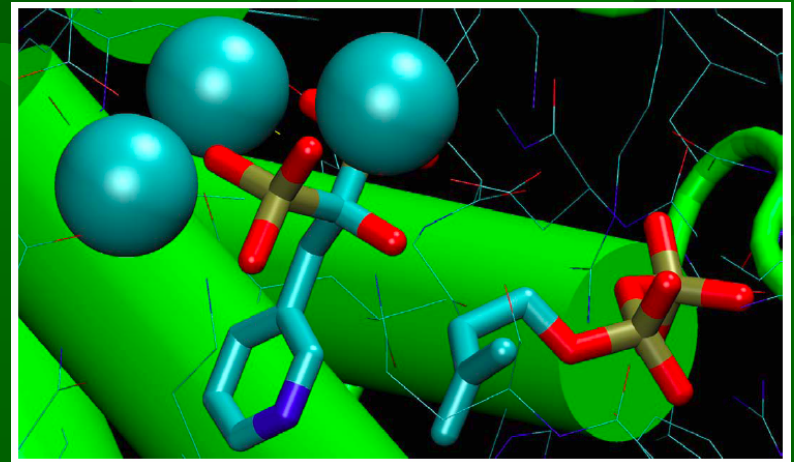
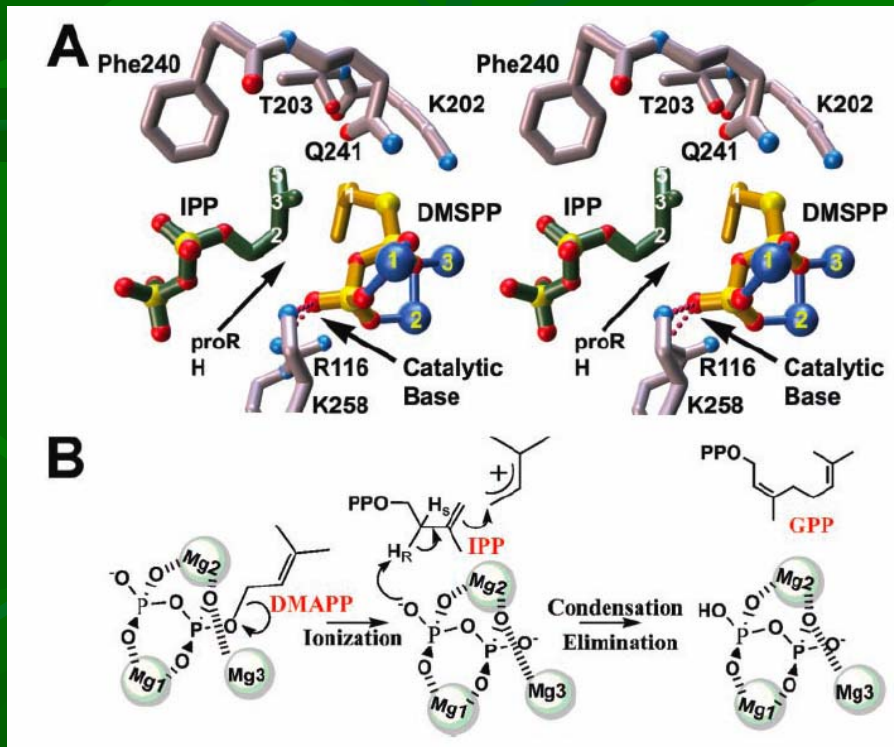


Pamidronate



Ibandronate

N-alkyl- and N-aryl-bisphosphonates inhibit FPPS by mimicking the transition state of the reaction (Martin et al. 1999. BBRC 263, 754)



E. coli FPPS; Hosfield et al. 2004. JBC 279, 8526

N-alkyl- and N-aryl-bisphosphonates are potent inhibitors of *Trypanosoma cruzi* FPPS

(Montalvetti et al. 2001, J.Biol.Chem. 276, 33930)

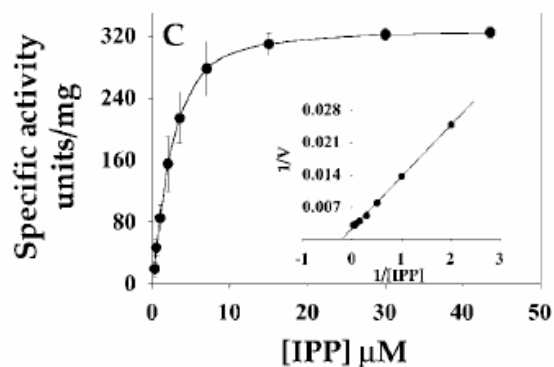
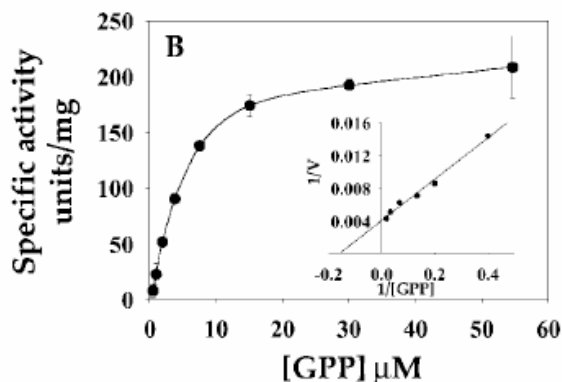


TABLE II

The effects of bisphosphonates on FPPS activity

The activity of the *T. cruzi* enzyme was assayed in the presence of bisphosphonates in mixtures containing 10 mM Hepes (pH 7.4), 5 mM MgCl₂, 2 mM dithiothreitol, 47 μM [4-¹⁴C]IPP (10 μCi/μmol), 18 μM GPP, and 10 ng of protein in a final volume of 100 μl. Reactions were incubated for 30 min at 37 °C, and the prenyl product was extracted and measured by liquid scintillation counting as described under "Experimental Procedures."

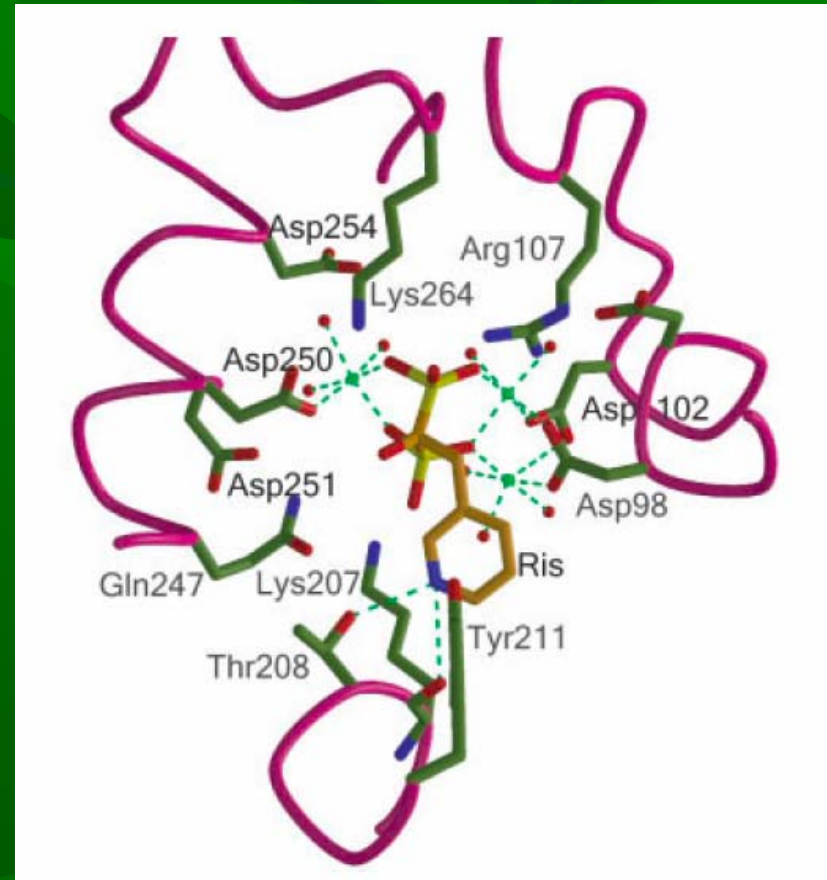
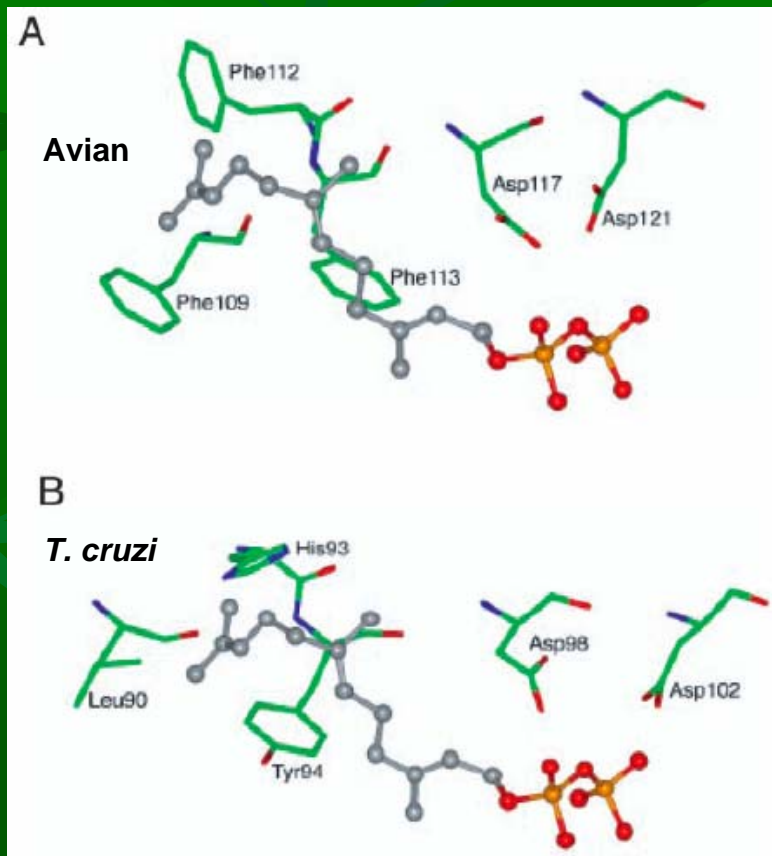
Bisphosphonate	<i>T. cruzi</i> FPPS		Human FPPS IC ₅₀
	K_i^a	IC ₅₀ ^a	
	μM	μM	μM
Etidronate	61.08 ± 8.7	57.7 ± 1.69	
Homorisedronate	8.17 ± 1.36	9.07 ± 0.7	2.93 ± 0.58 ^b
Alendronate	1.04 ± 0.18	0.77 ± 0.0	0.05 ± 0.001 ^b ; 0.46 ^c
Pamidronate	2.02 ± 0.10	1.08 ± 0.4	0.20 ± 0.01 ^b ; 0.5 ^c
Risedronate	0.032 ± 0.002	0.037 ± 0.003	0.01 ± 0.002 ^b ; 0.0039 ^c

^a Values are the means ± S.D. of three independent experiments.

^b From Ref. 22.

^c From Ref. 14.

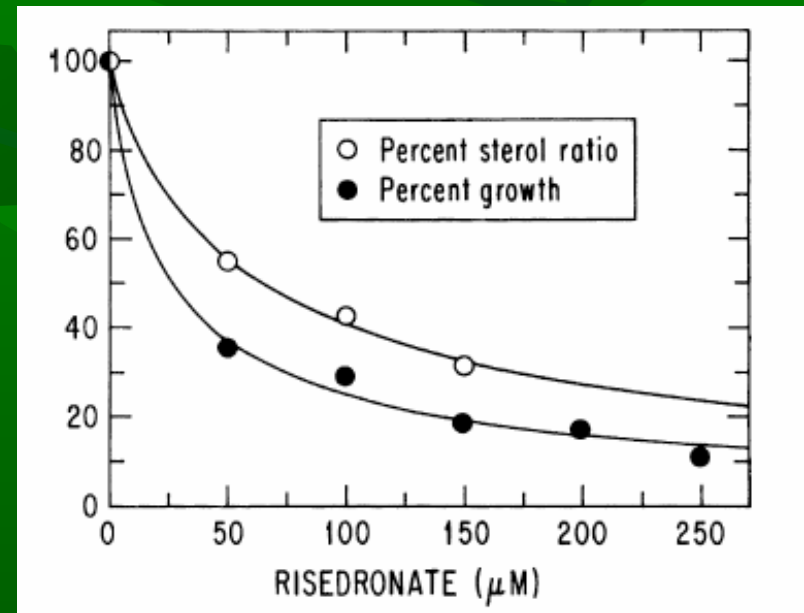
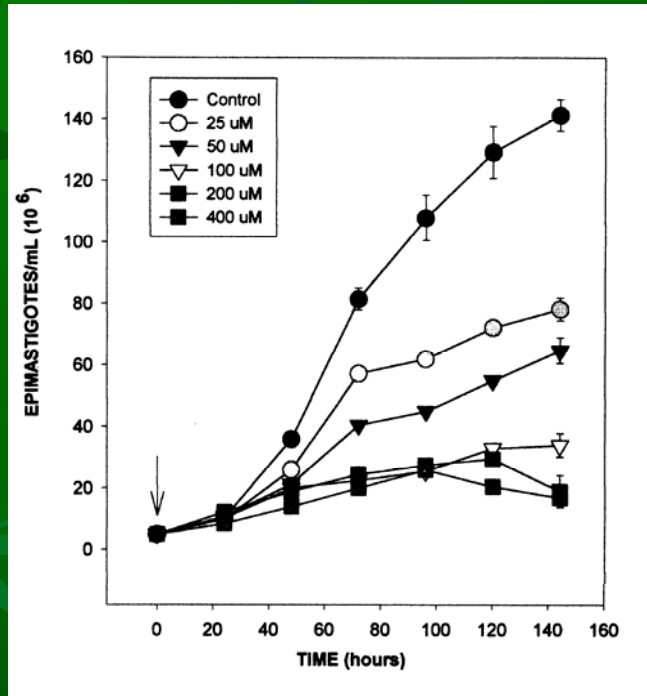
Risedronate binds at the active site *T. cruzi* FPPS as a transition state analog



Montalvetti et al. 2001, J.Biol.Chem. 276, 33930

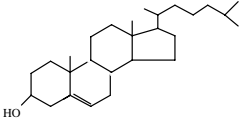
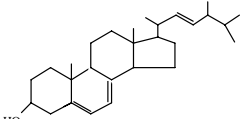
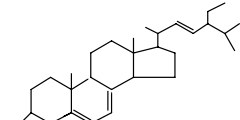
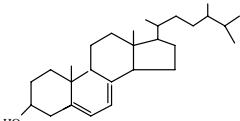
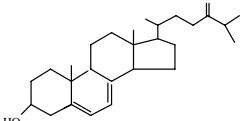
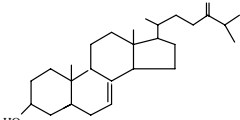
T. cruzi FPPS (Gabelli et al. 2006. Proteins 62, 80)

In vitro activity of risedronate against *Trypanosoma cruzi* extracellular epimastigotes (Garzoni et al. 2004a. IJAA 23, 273)



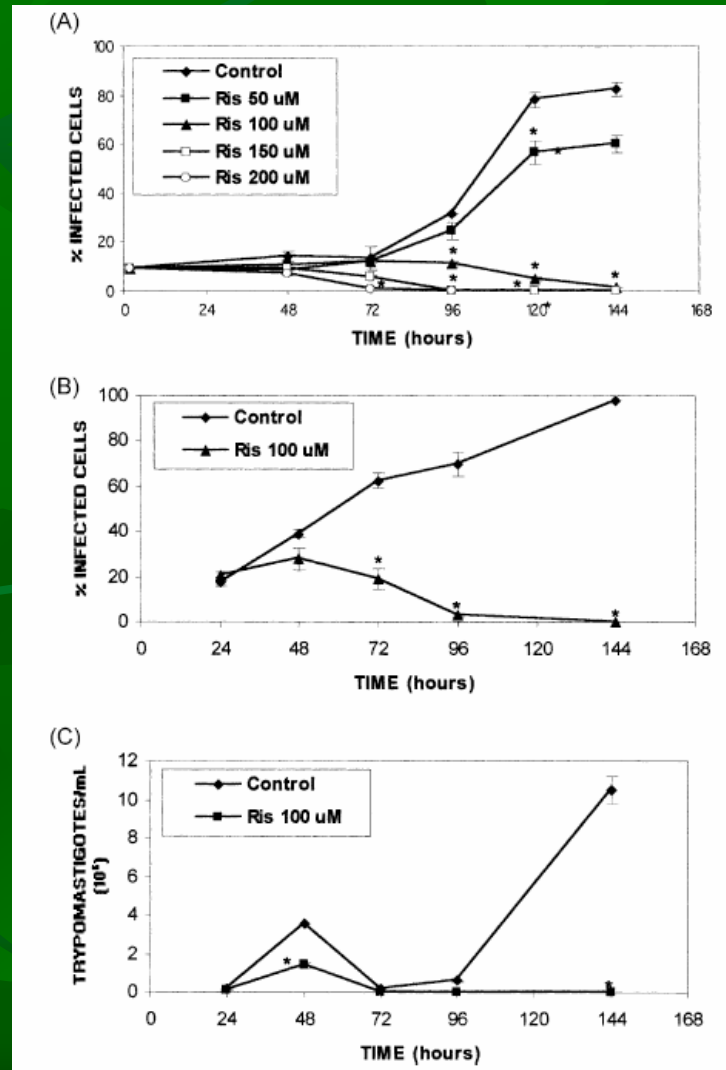
Risedronate-induced growth inhibition is associated with the depletion of *T. cruzi*-endogenous sterols (Garzoni et al. 2004a. IJAA 23a, 273)

TABLE 1. Effects of risedronate on the free sterol composition of *Trypanosoma cruzi* epimastigotes (EP stock) ^a

NAME	STRUCTURE	RIS 0 μ M	RIS 50 μ M	RIS 100 μ M	RIS 150 μ M
<i>EXOGENOUS:</i>					
CHOLESTEROL		33.9	48.2	54.8	62.1
<i>ENDOGENOUS:</i>					
24-METHYL-5 α ,22-C HOLESTA-TRIEN-3 β -OL (ERGOSTEROL)		22.5	20.8	20.2	17.3
24-ETHYL-5 α ,22-C HOLESTA-TRIEN-3 β -OL		16.0	22.8	25.0	20.6
ERGOSTA-5,7-DIEN-3 β -OL		9.6	3.2	n.d.	n.d.
ERGOSTA-5,7,24(24')-DIEN-3 β -OL		13.9	5.0	n.d.	n.d.
ERGOSTA-7,24(24')-DIEN-3 β -OL		4.1	n.d.	n.d.	n.d.
ENDOGENOUS/EXOGENOUS STEROLS		1.95	1.07	0.82	0.61

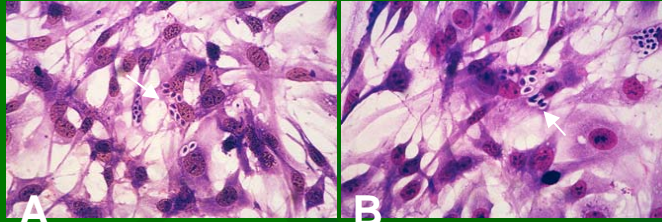
^aSterols were extracted from *T. cruzi* epimastigotes cultured in LIT medium and drugs added at a cell density of $5 \cdot 10^6$ epimastigotes/ml; they were separated from polar lipids by silicic acid column chromatography and analyzed by quantitative capillary gas-liquid chromatography and mass spectrometry. Results are expressed as mass per cent.

In vitro activity of risedronate against *Trypanosoma cruzi* intracellular amastigotes (Garzoni et al. 2004a. IJAA 23, 273)

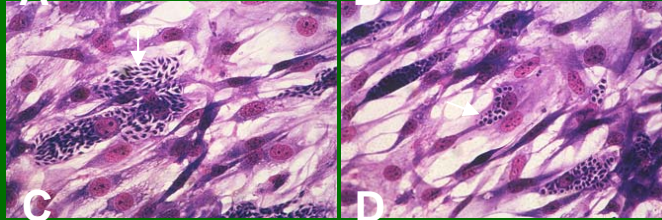


In vitro activity of risedronate against *Trypanosoma cruzi* intracellular amastigotes (Garzoni et al. 2004a. IJAA 23, 273)

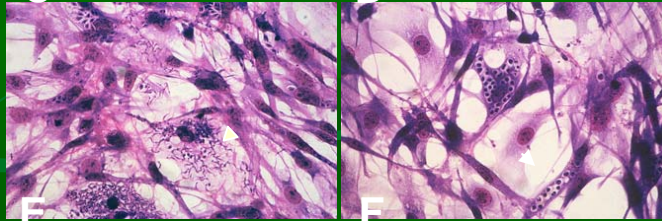
48 h



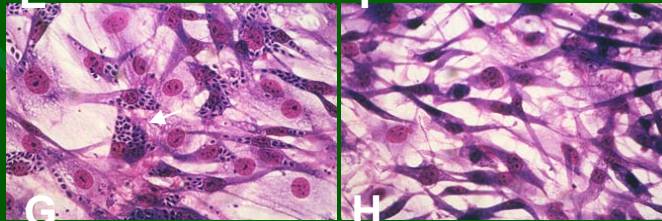
72 h



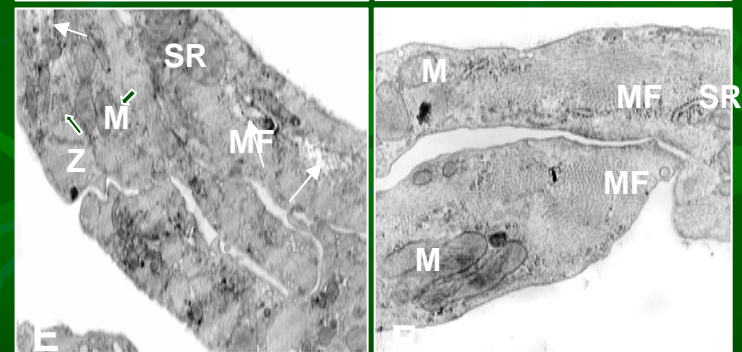
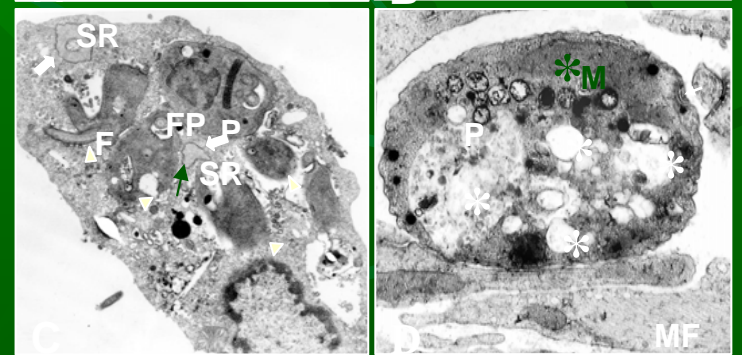
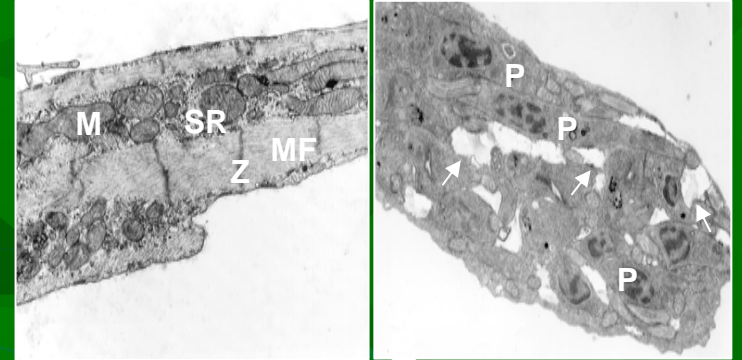
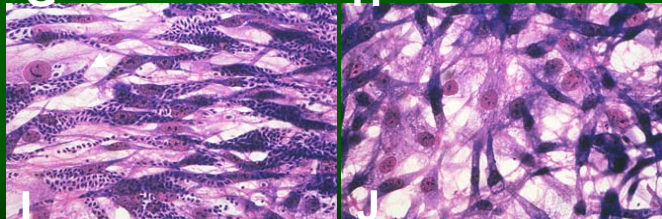
96 h



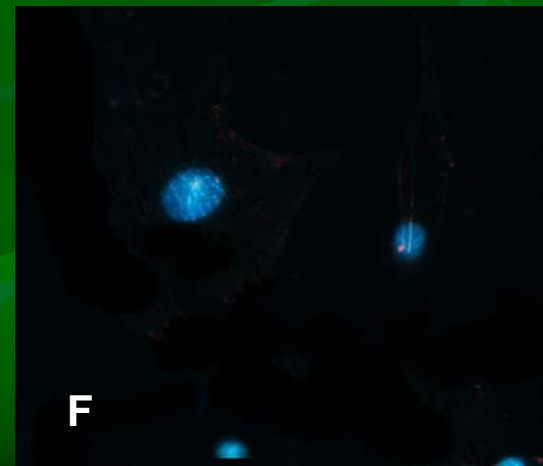
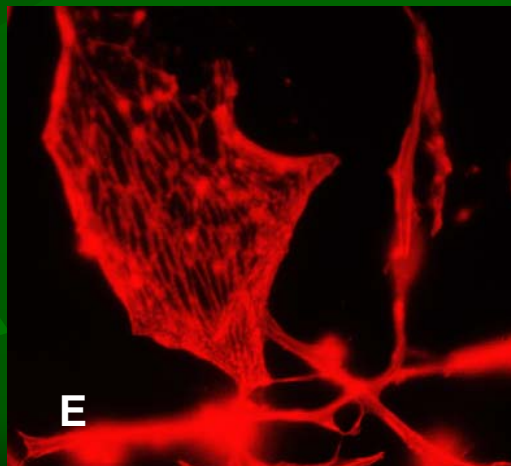
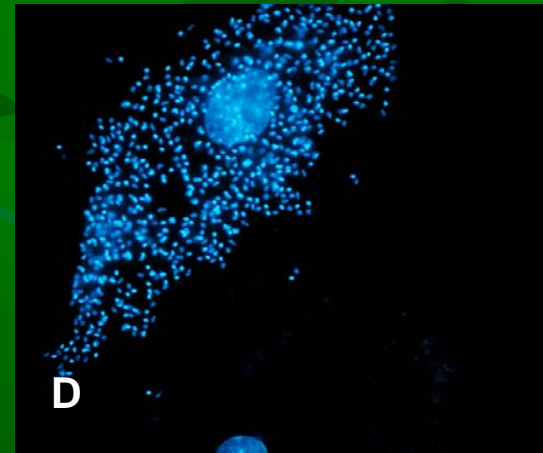
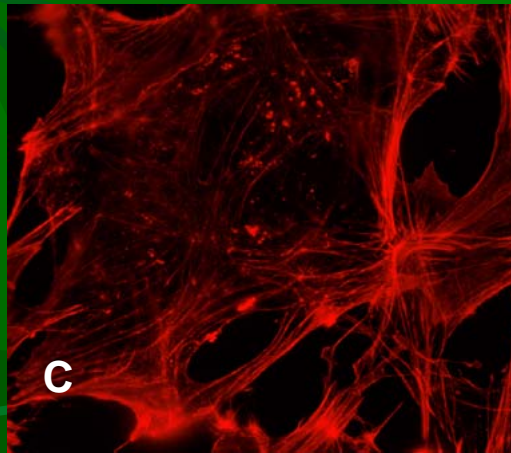
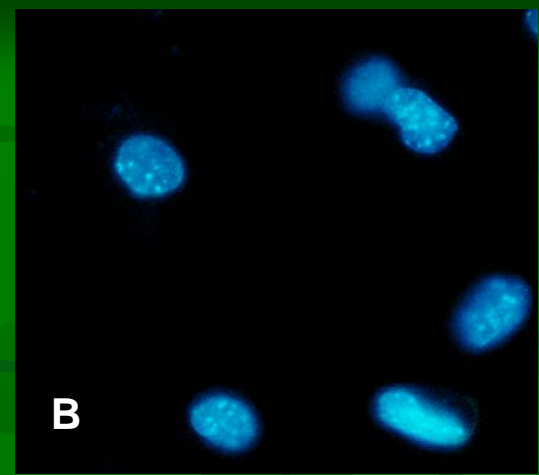
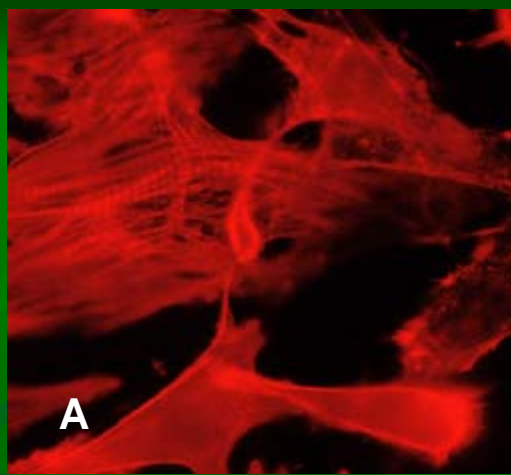
120 h



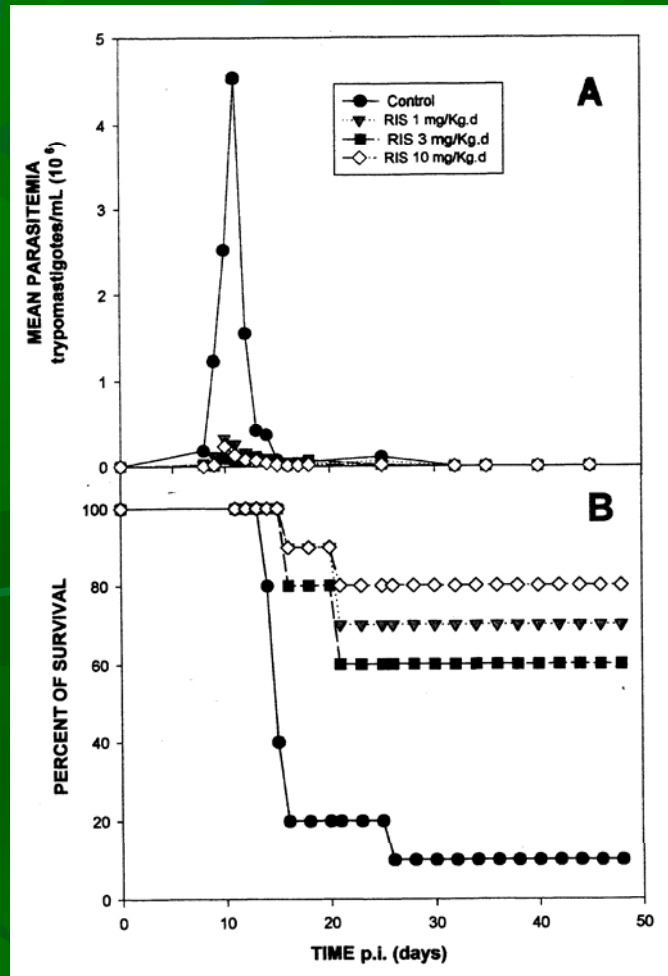
144 h



Recovery of
T. cruzi-infected
cardiomyocytes
after treatment
with risedronate
(Garzoni et al.
2004a. IJAA 23,
273)



Activity of risedronate in a murine model of acute Chagas disease: parasitemia and survival (Garzoni et al. 2004b. IJAA 23, 286)



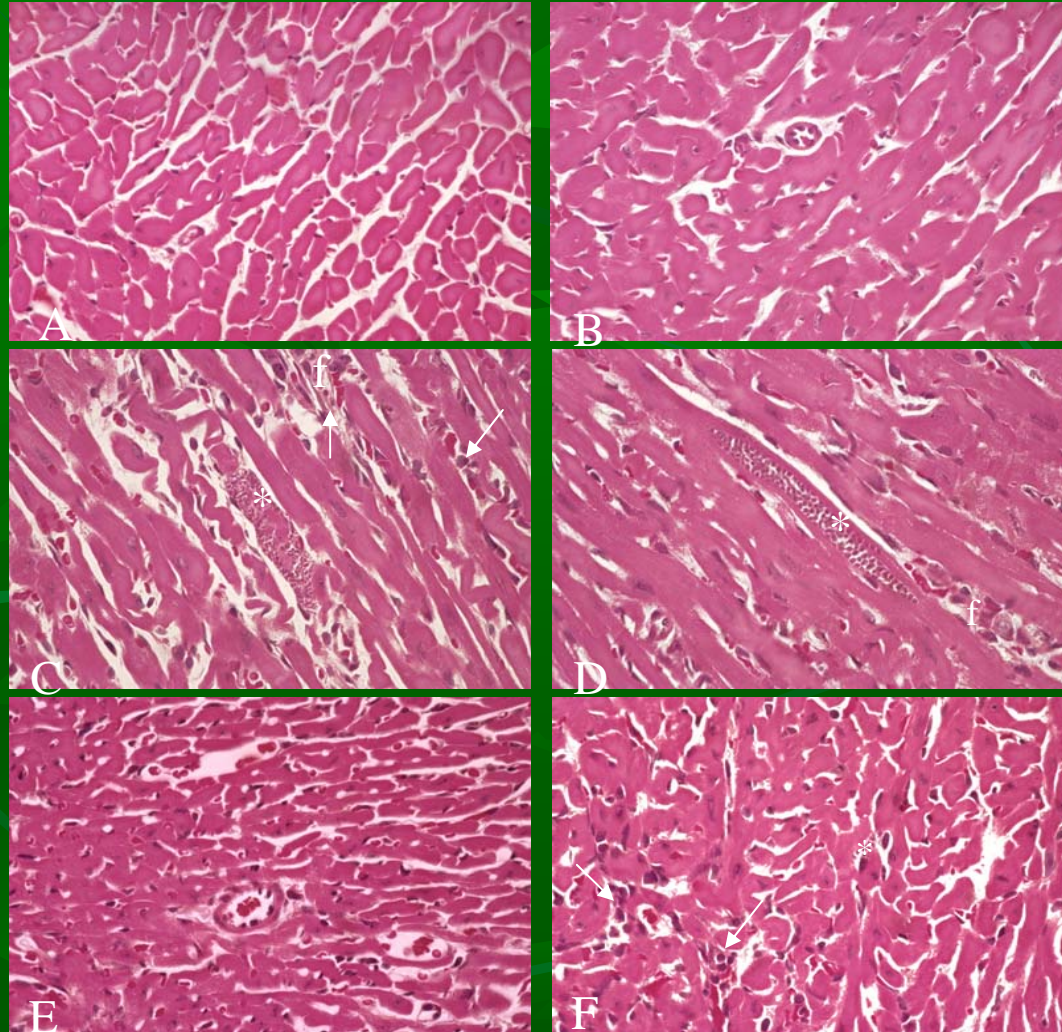
Activity of risedronate in a murine model of acute Chagas disease: parasitemia and survival (Garzoni et al. 2004b. IJAA 23, 286)

Table 1
Effects of risedronate on parasitaemia and survival in a murine model of acute Chagas' disease^a

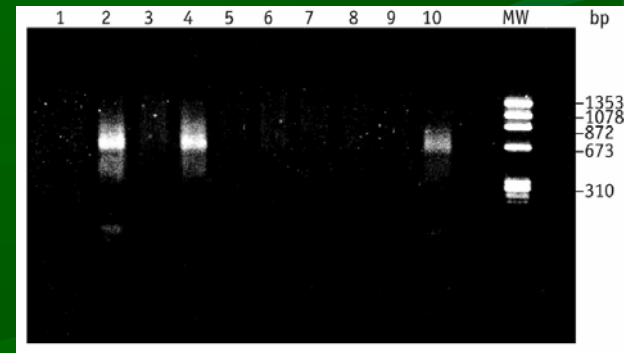
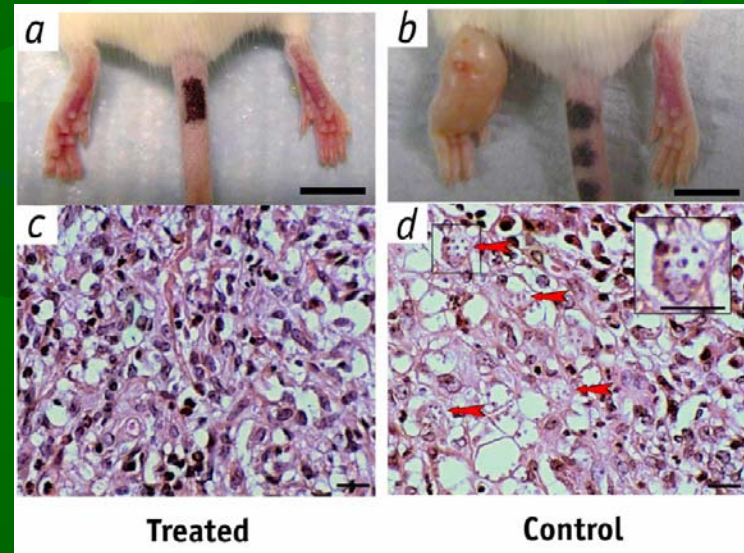
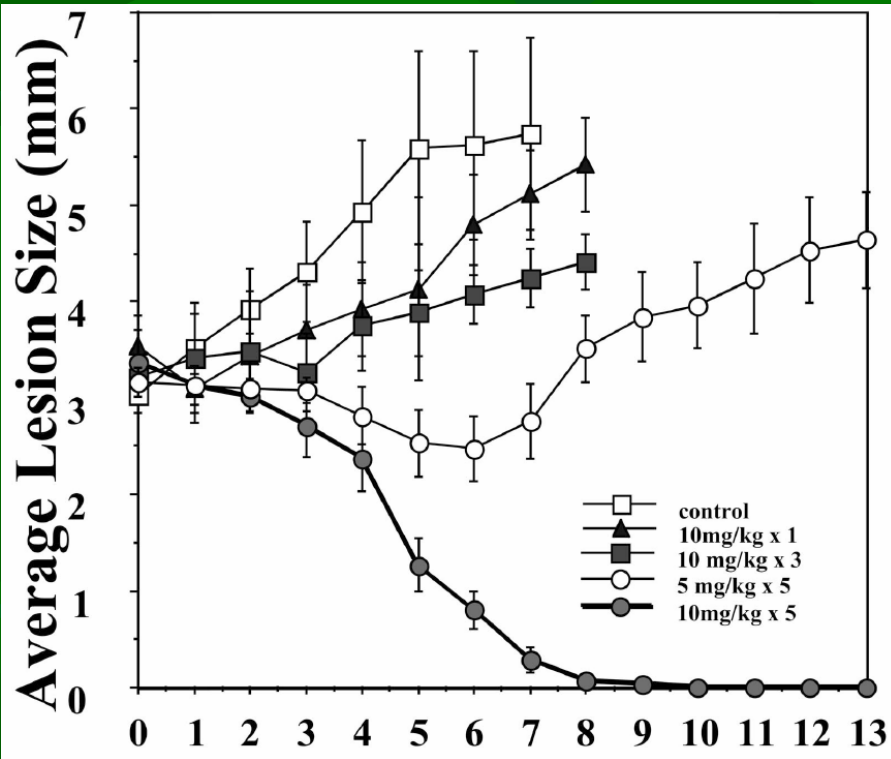
Experiment	Treatment	Mean peak parasitaemia (trypomastigotes/ml $\times 10^4$)	Survival
1	Control	458.2 \pm 316.3	2/10 ^c
	1 mg/kg per day Ris \times 7 days	45.2 \pm 33.8 ^b	7/10 ^{c,d}
	3 mg/kg per day Ris \times 7 days	19.9 \pm 15.0 ^b	6/10 ^{c,e}
	10 mg/kg per day Ris \times 7 days	24.0 \pm 14.1 ^b	8/10 ^{c,f}
2	Control	478.4 \pm 209.5	3/10 ^g
	10 mg/kg per day Ris \times 7 days	16.4 \pm 15.0 ^b	9/10 ^{g,h}
3	Control	253.2 \pm 171.2	4/10 ⁱ
	10 mg/kg per day Ris \times 14 days	18.0 \pm 11.4 ^b	8/10 ^{i,j}

^a Female Swiss albino mice (20–25 g) were infected with 10^3 trypomastigotes/ml and i.v. treatment was started 24 h post-infection. Animals were followed for 40–60 days. Other details are described in [Section 2](#).

**Activity of risedronate in a murine model of acute
Chagas disease: heart parasitism (Garzoni et al. 2004b.
IJAA 23, 286)**

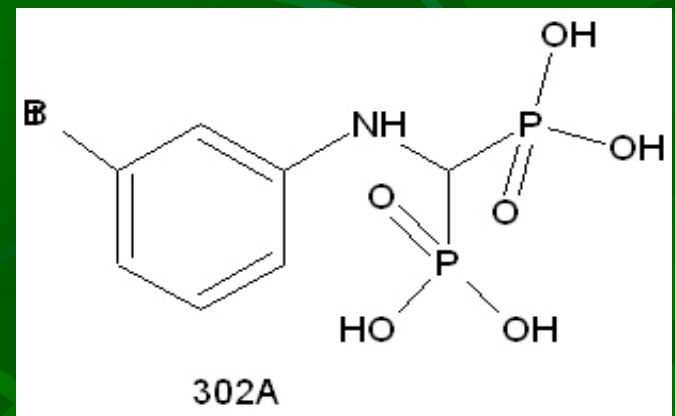
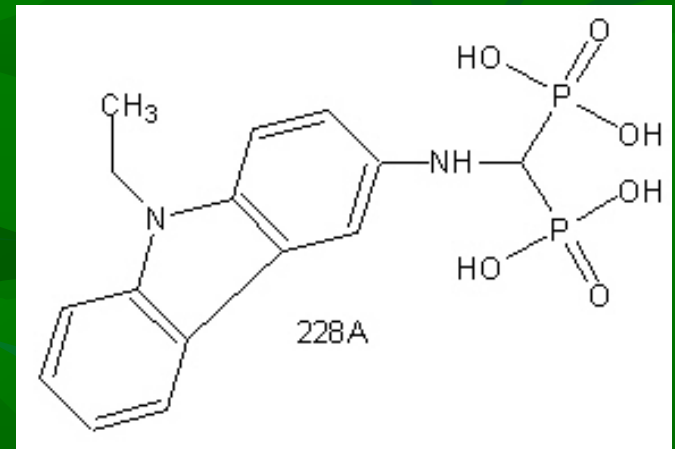


Activity of pamidronate in a murine model of cutaneous Leishmaniasis caused by *L. amazonensis* (Rodriguez et al. 2002. J. Infect. Dis. 186, 138)

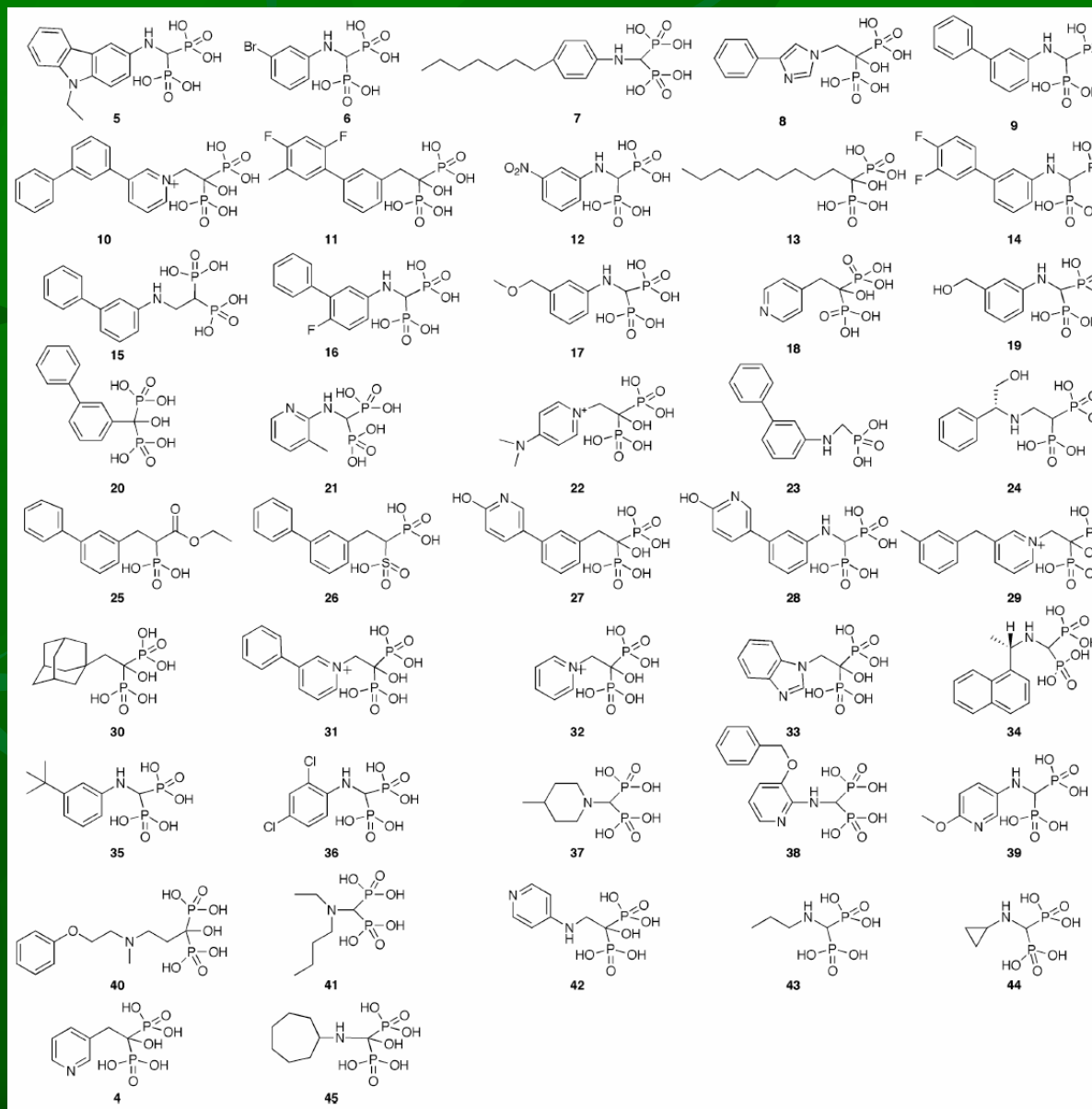


Inhibitors of pyrophosphate signaling for the specific treatment of Chagas disease. 2. Inhibitors of pyrophosphate- modulated hexokinase

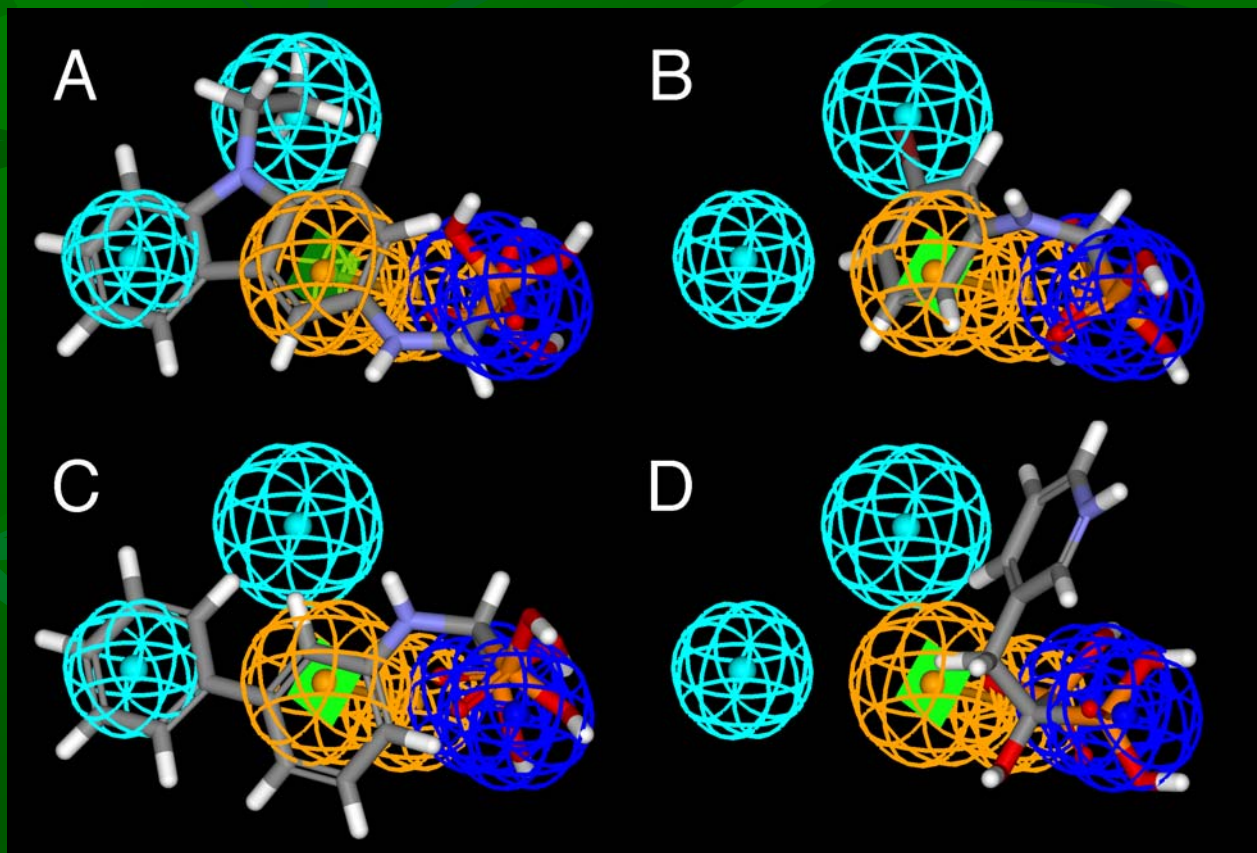
- *T. cruzi* hexokinase (TcHK) is unusual as it is not regulated by D-glucose-6-phosphate, but is inhibited non-competitively by PPi (Caceres et al. 2003. MBP 126, 251)
- A novel series of aromatic amino-methylene bisphosphonates has been identified, which are 100- to 1000-fold more potent than PPi as inhibitors of TcHK (Hudok et al. 2006. JMC 49, 215)
- These compounds block glycolysis and growth of both proliferative stages of the parasite, while not affecting mammalian cells



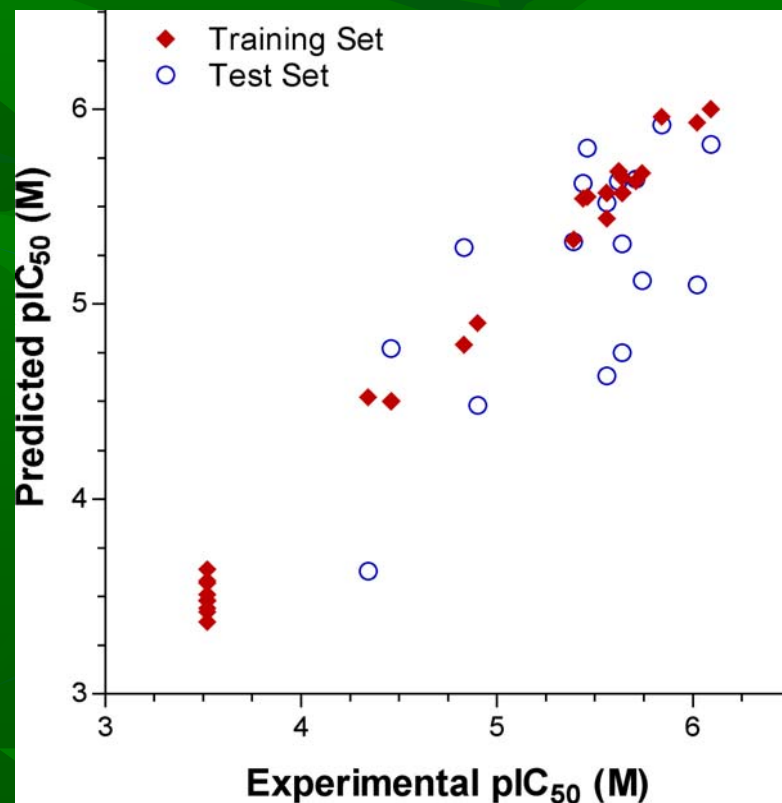
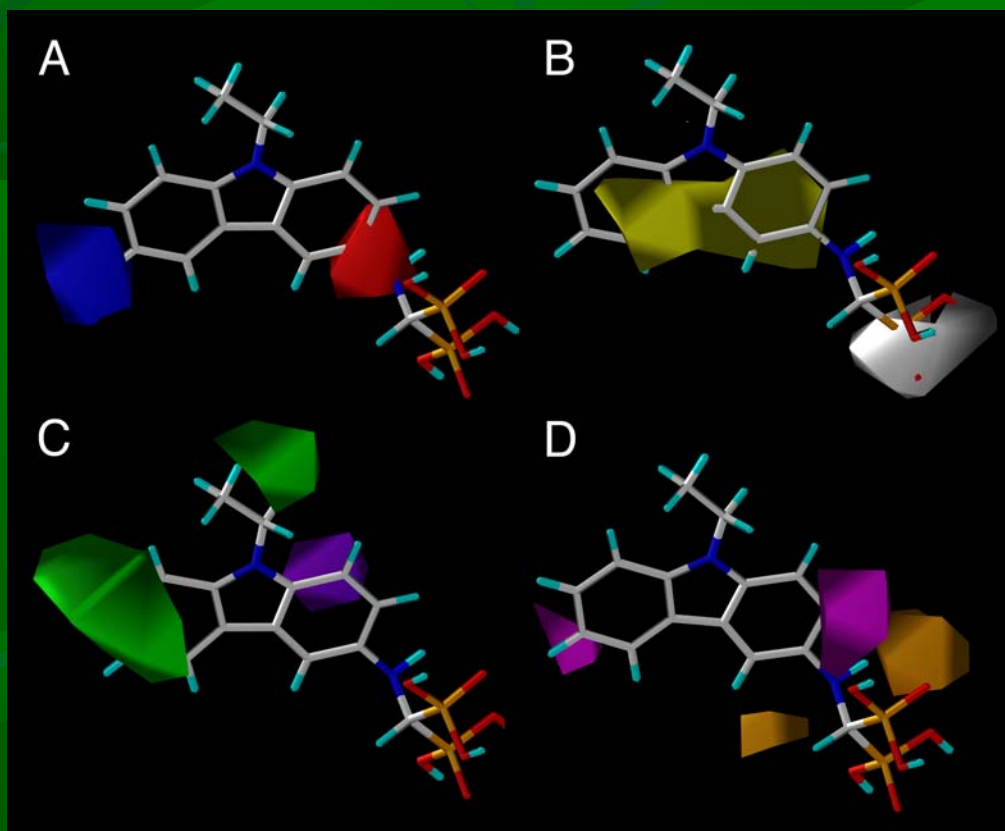
Aromatic amino-methylene bisphosphonate hexokinase inhibitors (Hudok et al. 2006. JMC 49, 215)



3D-QSAR studies of *T. cruzi* hexokinase inhibitors: Catalyst pharmacophore (Hudok et al. 2006. JMC 49, 215)



3D-QSAR studies of *T. cruzi* hexokinase inhibitors: Comparative molecular similarity indices analysis (CoMSIA; Hudok et al. 2006. JMC 49, 215)

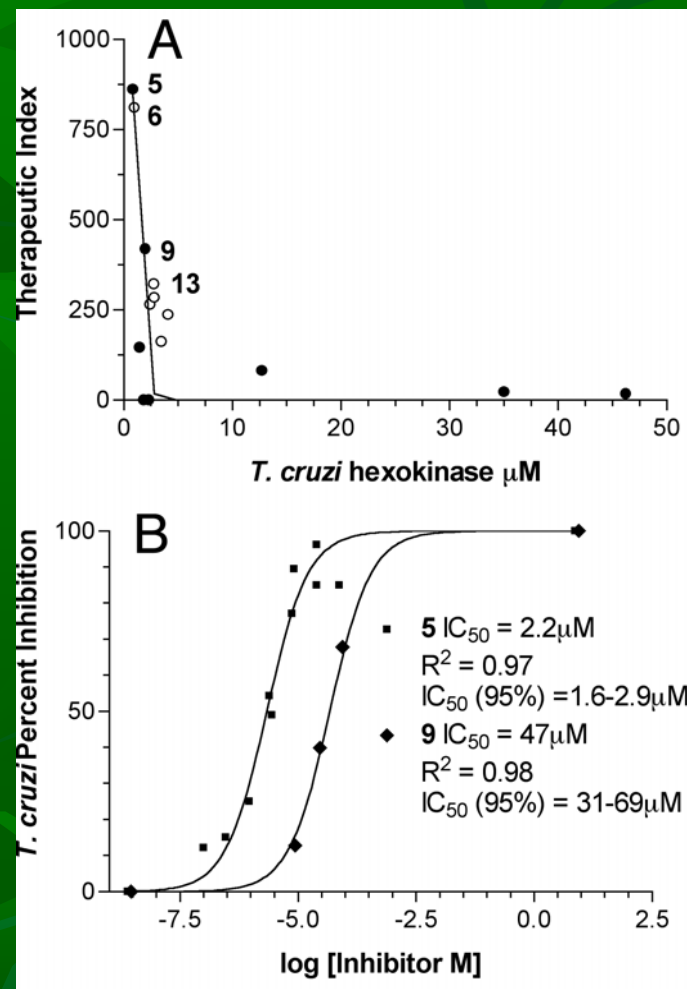


Effects of aromatic amino-ethyl bisphosphonates on *T. cruzi* hexokinase, amastigote and mammalian cells growth (Hudok et al. 2006. JMC 49, 215)

Table 2. *T. cruzi* Hexokinase IC₅₀, Human Nasopharyngeal Carcinoma (KB) LD₅₀, and *D. discoideum* IC₅₀

compd	TcHK IC ₅₀ (μM)	KB LD ₅₀ (μM)	therapeutic index ^a	DD IC ₅₀ (μM)
5	0.81	698	862	2052
6	0.95	≥ 771	≥ 812	112
7	1.44	211	147	10
8	1.81	<i>b</i>	<i>b</i>	122
9	1.97	827	420	211
10	2.29	<i>b</i>	<i>b</i>	2
12	2.39	≥ 635	≥ 266	78
13	2.77	≥ 892	≥ 322	10
14	2.78	≥ 791	≥ 285	77
15	3.46	≥ 560	≥ 162	62
17	4.06	≥ 964	≥ 237	253
18	12.7	1041	82	<i>b</i>
19	14.5	≥ 980	≥ 67	351
20	35	797	23	103
21	46.2	840	18	3
4	≥ 300	822	≤ 2.7	3
45	≥ 300	163	≤ 0.5	2
44	≥ 300	242	≤ 0.8	30
43	≥ 300	457	≤ 1.5	4
42	≥ 300	384	≤ 1.3	3
40	≥ 300	311	≤ 1.0	2
41	≥ 300	≥ 1055	<i>c</i>	4
39	≥ 300	302	≤ 1.0	52
38	≥ 300	≥ 765	<i>c</i>	1
37	≥ 300	446	≤ 1.5	2
29	≥ 300	728	≤ 2.4	889
35	≥ 300	<i>b</i>	<i>b</i>	75
34	≥ 300	<i>b</i>	<i>b</i>	7
33	≥ 300	<i>b</i>	<i>b</i>	2
32	≥ 300	≥ 1060	<i>c</i>	3
31	≥ 300	12	≤ 0.04	3
29	≥ 300	<i>b</i>	<i>b</i>	8
22	≥ 300	<i>b</i>	<i>b</i>	30

^a Therapeutic index = KB LD₅₀/TcHK IC₅₀. ^b Value not determined.
^c Indeterminate value.



Effects of aromatic amino-ethyl bisphosphonates inhibit *T. cruzi* hexokinase but not FPPS (Hudok et al. 2006. JMC 49, 215)

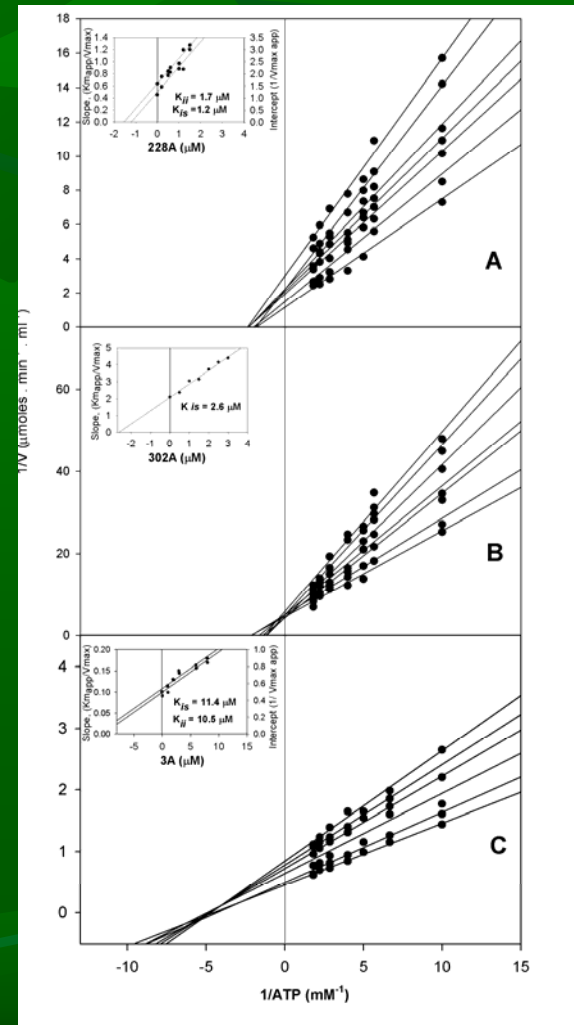
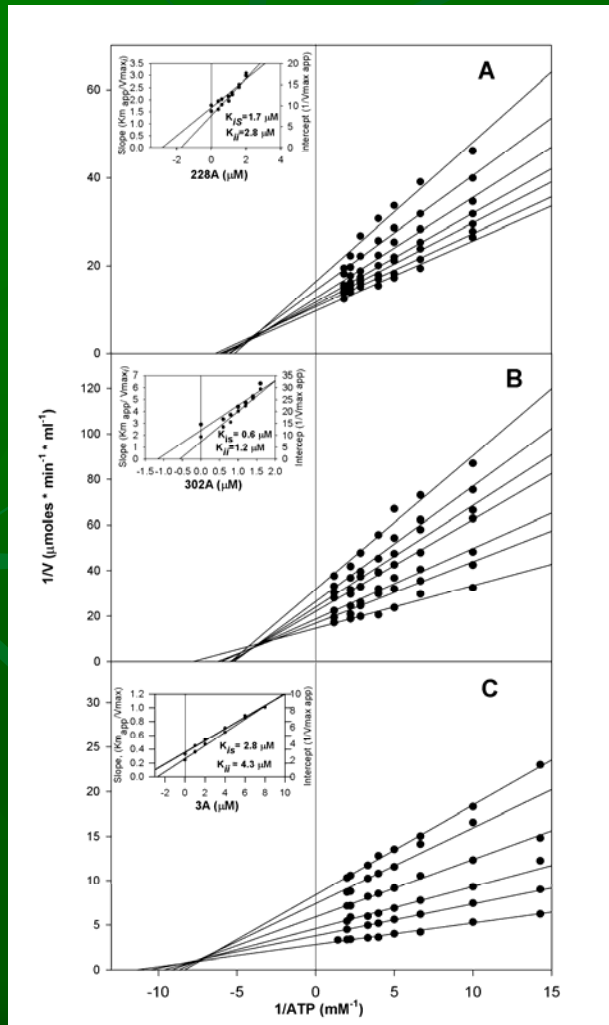
Table 3. Differential pIC₅₀^a Values for *T. cruzi* Hexokinase and *L. major* FPPS Inhibition

compd	TcHK activity		<i>L. major</i> FPPS activity		Δ pIC ₅₀ ^a
	IC ₅₀ (μ M)	pIC ₅₀	IC ₅₀ (μ M)	pIC ₅₀	
5	0.81	6.09	≥ 100	4.00	≥ 2.09
7	1.45	5.84	≥ 100	4.00	≥ 1.84
9	1.95	5.71	11.46	4.94	0.77
11	2.29	5.64	12.28	4.91	0.73
13	2.75	5.56	2.37	5.63	-0.07
16	3.63	5.44	28.6	4.54	0.90
18	12.60	4.90	1.34	5.87	-0.97
21	45.74	4.34	0.112	6.95	-2.61
45	≥ 300	3.52	0.228	6.64	≤ -3.12
26	≥ 300	3.52	11	4.96	≤ -1.44
40	≥ 300	3.52	0.42	6.38	≤ -2.85
4	≥ 300	3.52	0.17	6.77	≤ -3.25

^a Δ pIC₅₀ = pIC₅₀(TcHK) - pIC₅₀(FPPS).

Aromatic amino-ethyl bisphosphonates are non-competitive inhibitors of *T. cruzi* hexokinase (Sanz-Rodriguez et al, in press)

Soluble TcHK



Glycosome-bound
TcHK

Aromatic amino-ethyl bisphosphonates are non-competitive inhibitors of *T. cruzi* hexokinase (Sanz-Rodriguez et al, in press)

Kinetics of inhibition of pure *T. cruzi* hexokinase by bisphosphonates

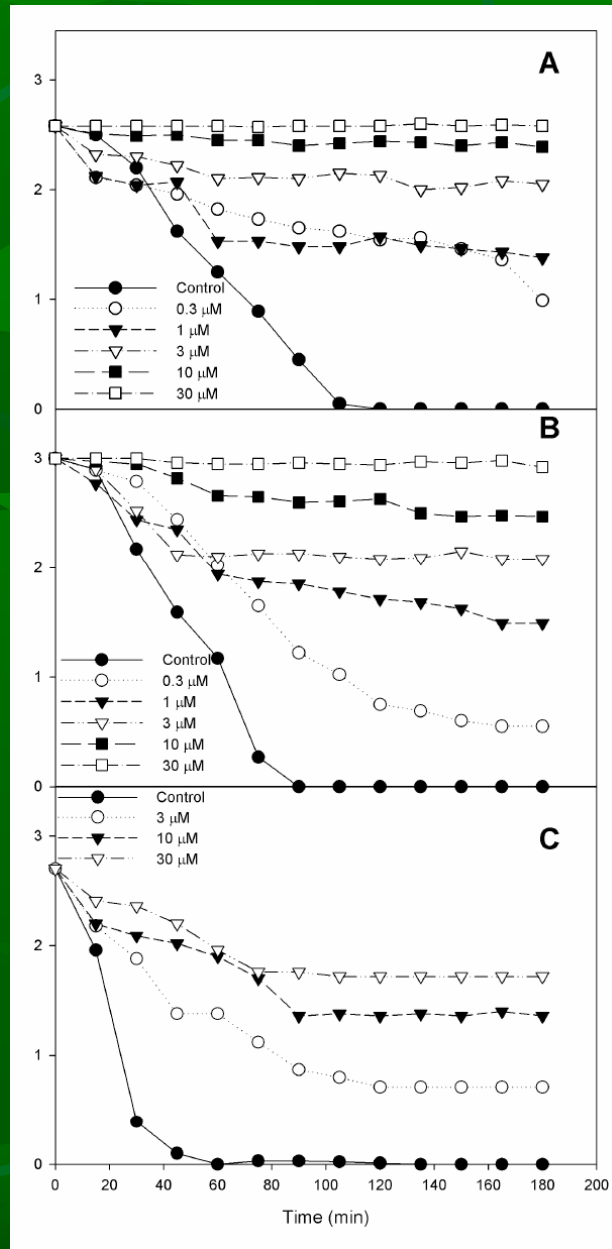
Inhibitor/Substrate	K _{ii} ¹	K _{is} ¹	Mechanism
228/ATP ²	2.8	1.7	mixed
228/D-glucose ³	-	0.5	competitive
302/ATP ²	1.2	0.6	mixed
302/D-glucose ³	-	0.4	competitive
3/ATP ²	4.3	2.8	mixed
3/D-glucose ³	6.0	3.3	non-competitive

¹Inhibition constants given in μM

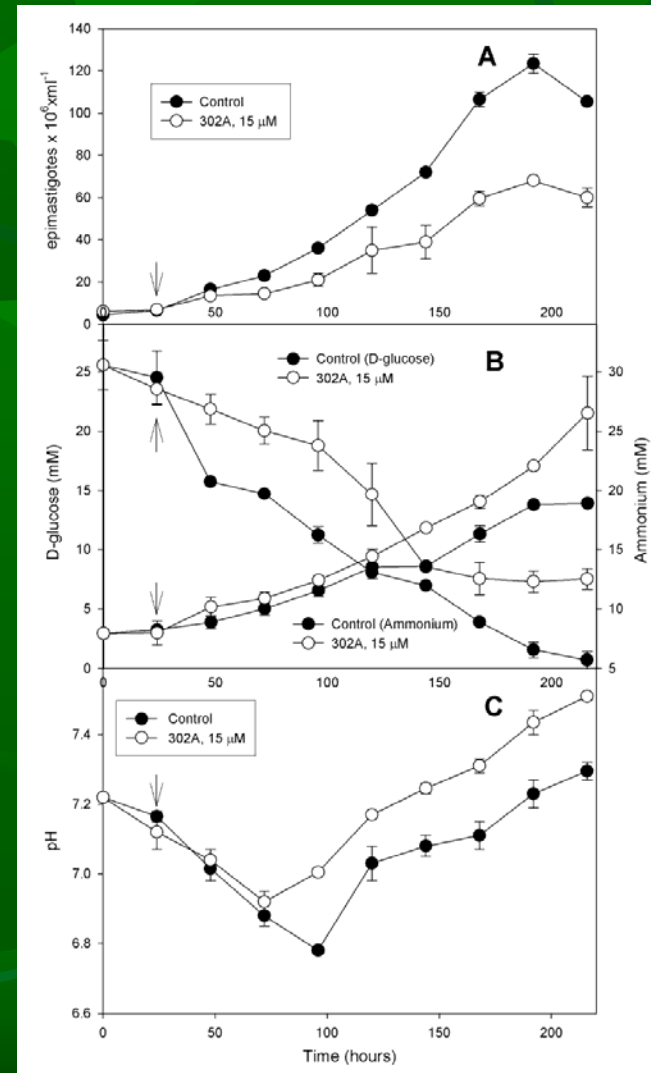
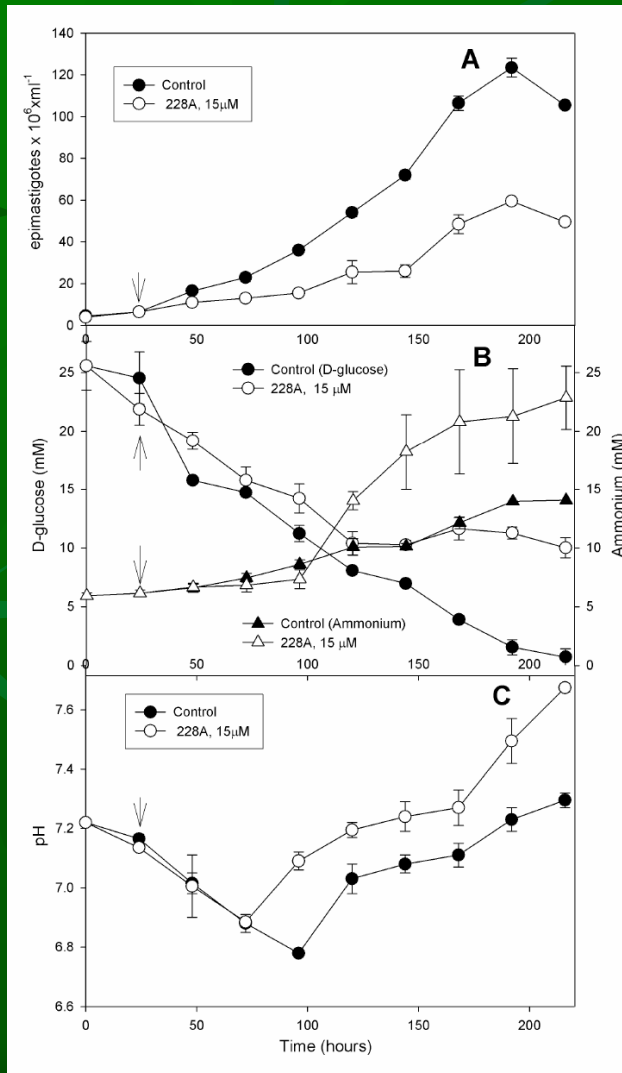
²In the presence of 2 mM D-glucose and 3 mM MgCl_2

³In the presence of 1 mM ATP and 3 mM MgCl_2

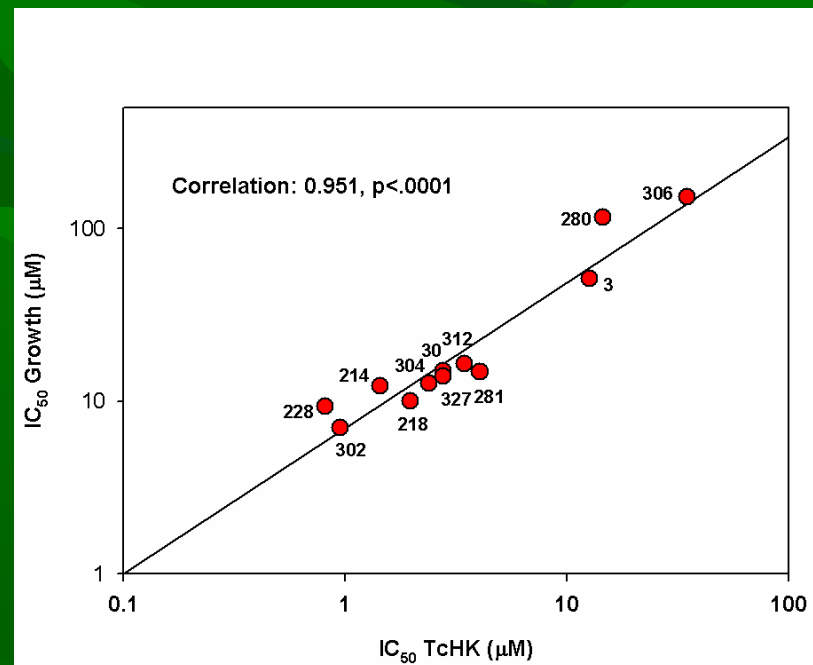
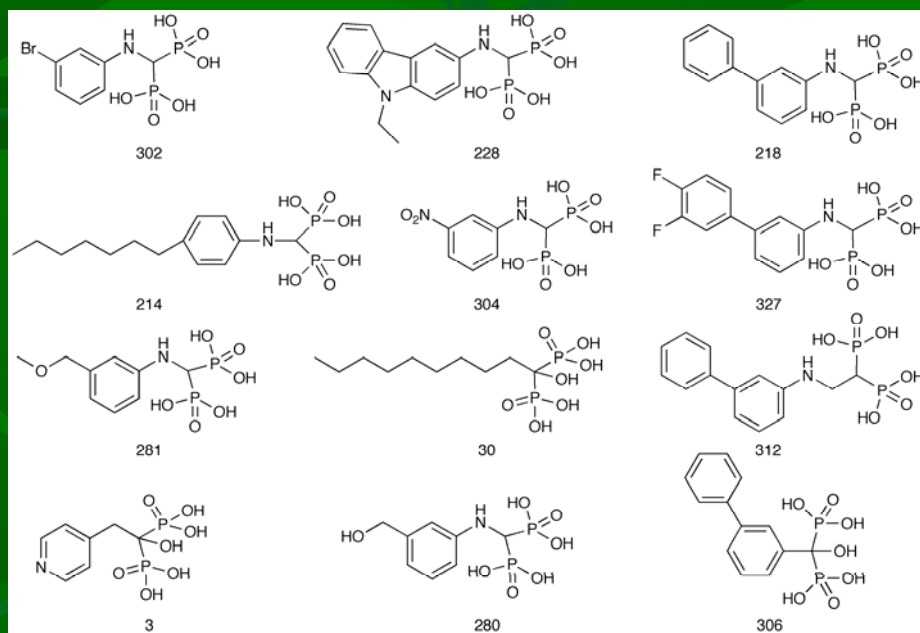
**Aromatic amino-ethyl
bisphosphonates
block glucose
consumption of
digitonin-treated *T.
cruzi* epimastigotes
(Sanz-Rodriguez et al,
in press)**



Growth inhibition of *T. cruzi* epimastigotes by aromatic amino-ethyl bisphosphonates (Sanz-Rodriguez et al, in press)



Correlation of TcHK and epimastigote growth inhibition by aromatic amino-ethyl bisphosphonates (Sanz-Rodriguez et al, in press)



Conclusions

- *T. cruzi*, as well as other Kinetoplastida and Apicomplexa parasites, contains large amounts of inorganic pyrophosphate and other short-chain polyphosphates, which are stored as Ca^{2+} and Mg^{2+} complexes in special organelles termed acidocalcisomes, but also intervene in many metabolic processes in these cells
- Bisphosphonates, metabolically stable pyrophosphate analogs, accumulate in acidocalcisomes and interfere with pyrophosphate metabolism and signaling
- N-alkyl and N-aryl-bisphosphonates, inhibitors of FPPS currently used in the treatment of bone resorption disorders, are also potent anti-*T. cruzi* agents, in vitro and in vivo
- A novel group of aromatic amino-methylene bisphosphonates are potent inhibitors of *T. cruzi* hexokinase and selectively block parasite's glycolysis and proliferation
- The potential use of bisphosphonates as antiparasitic agents may require new pharmacological formulations and its clinical development is estimated in the medium term (within 10 years)

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