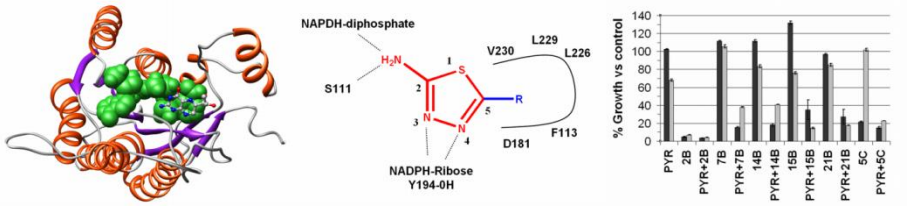
UNIVERSITÀ DEGLI STUDI
DI MODENA E REGGIO EMILIA

de Duve Institute

The discovery of thiadiaza derivatives as pteridine reductase inhibitors against Trypanosomatidae infections.

Maria Paola Costi
University of Modena and Reggio Emilia
Italy



New Indigo Workshop: Antiparasitic and Antitumour drugs | 8 and 9 of September 2011, Porto, Portugal

Classical Antifolates do not work properly against Leishmania parasites.

EXPERIMENTAL PARASITOLOGY **87,** 157–169 (1997)

Biochemical and Genetic Tests for Inhibitors of Leishmania Pteridine Pathways

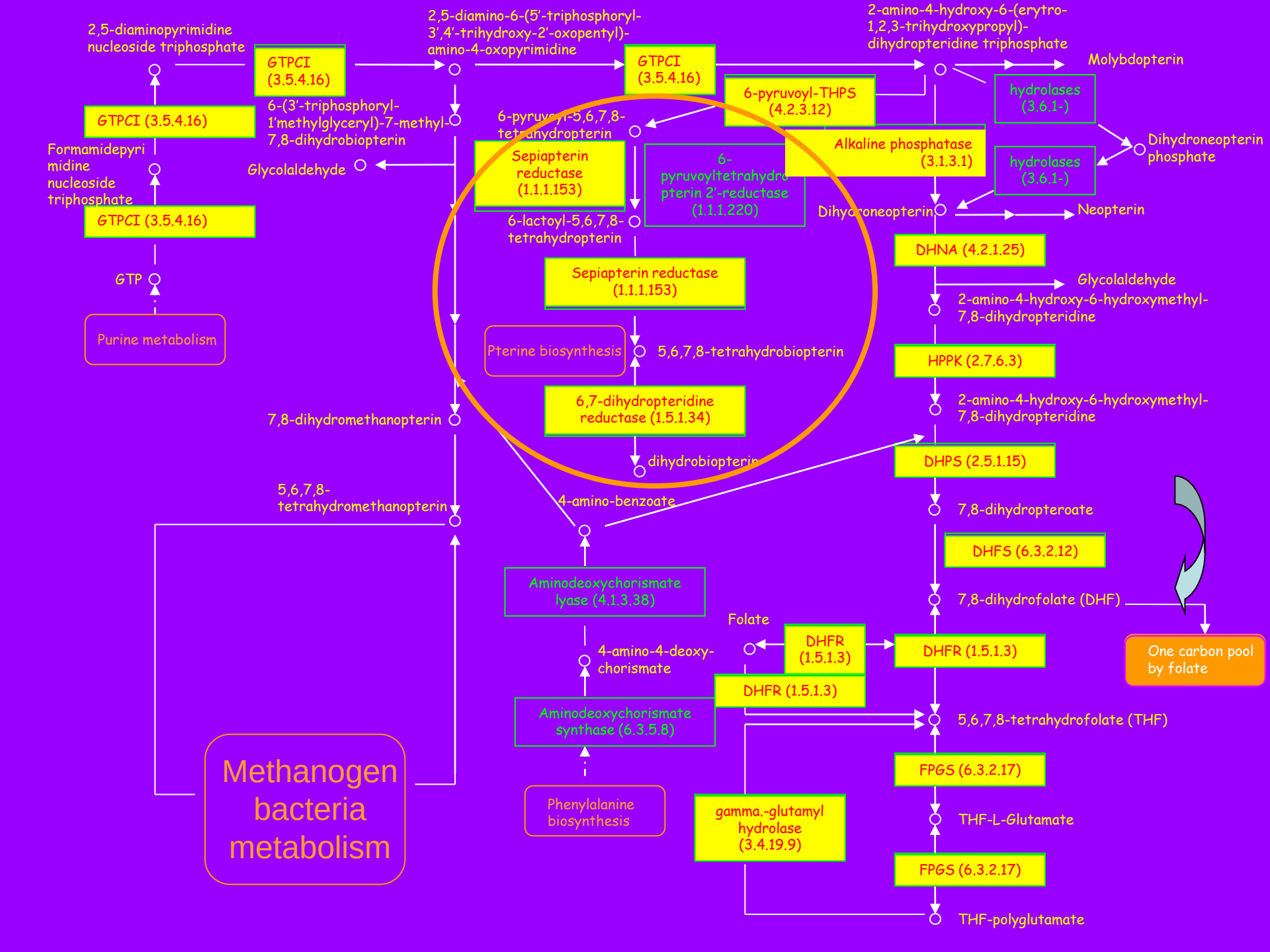
L. W. Hardy,* W. Matthews,* B. Nare,†,1 and S. M. Beverley†,2

**Department of Pharmacology and Molecular Toxicology and Program in Molecular Medicine, Biotech 2, University of Massachusetts Medical Center, Worcester, Massachusetts 01605, U.S.A.; and †Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, U.S.A.*

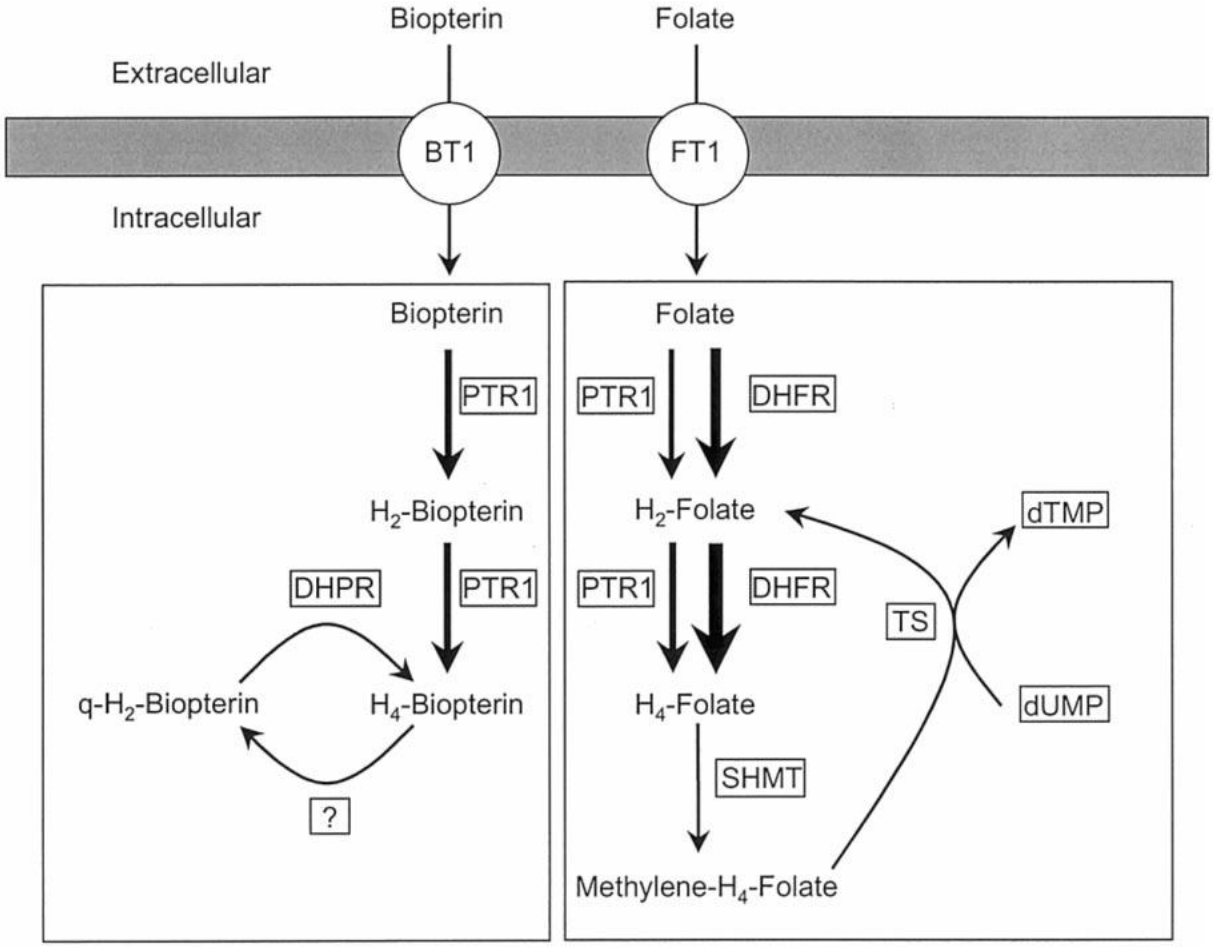
Leishmania exhibit many unusual features in pteridine metabolic pathways which are essential for growth, suggesting that these should be excellent targets for chemotherapeutic attack (summarized in Nare *et al.* 1997a). However, unlike some other protozoal infectious diseases where antifolates such as pyrimethamine, sulfa drugs, and trimethoprim have enjoyed success (Ferone 1984; Grossman and Remington 1979; McDougald 1982), effective antifolate chemotherapy has not been achieved in infections by *Leishmania,* despite this parasite's extreme divergence from the host.

Either or both of the *PTR1* and *DHFR-TS* genes are often found to be amplified in methotrexate (MTX) resistant lines. Investigations of the genes and enzymes identified by studies of antifolate resistance have provided important beginnings for efforts to develop effective antifolate chemotherapy and to understand why previously tested inhibitors did not work.

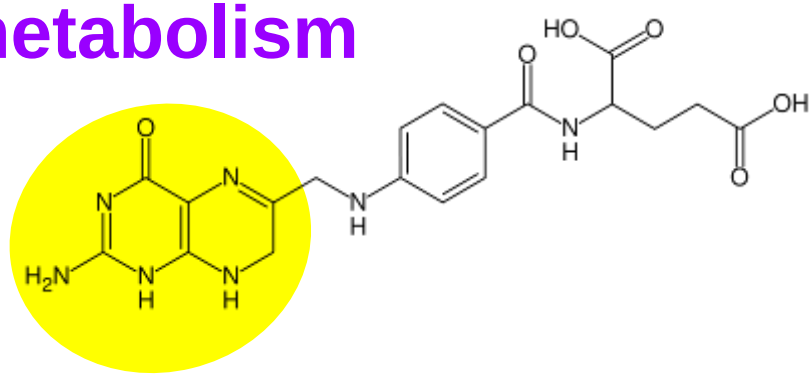
Folate pathway:
which proteins can be considered as validated targets?

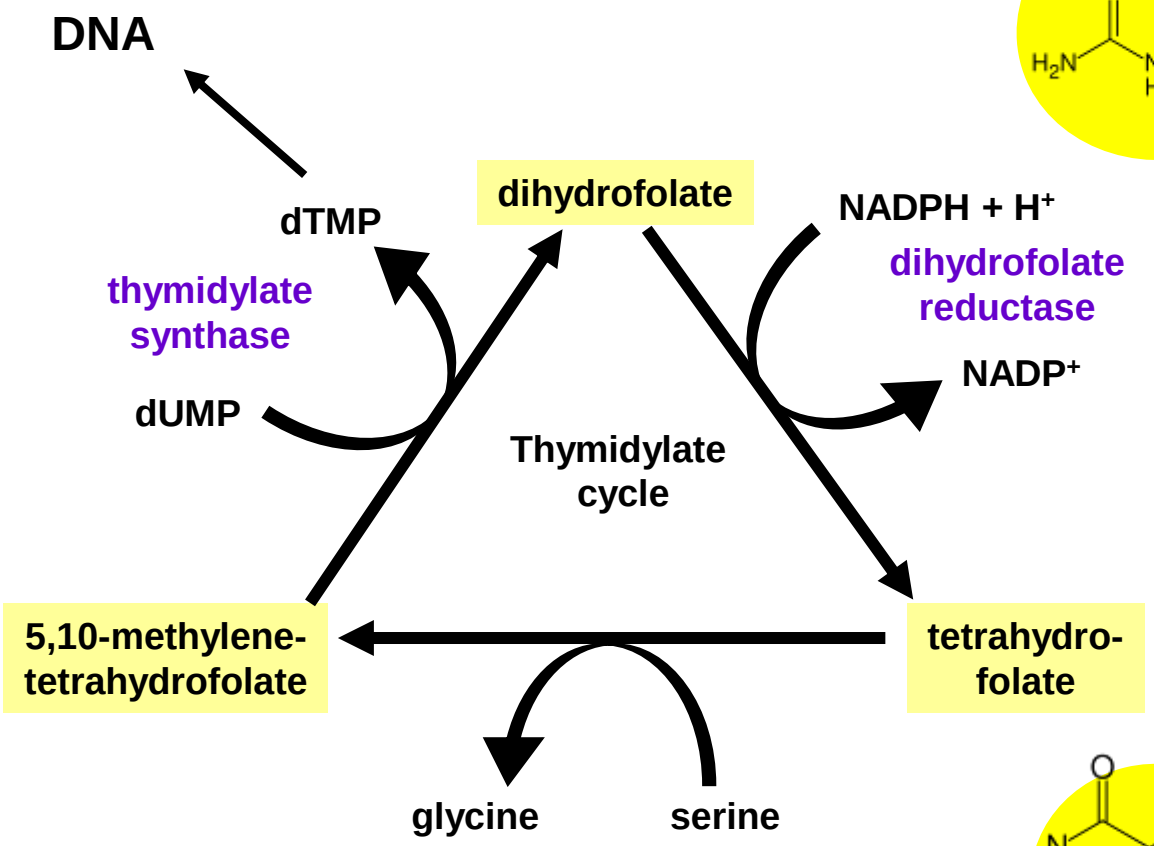

2,5-diaminopyrimidine nucleoside triphosphate
GTPCI (3.5.4.16)
2,5-diamino-6-(5'-triphosphoryl-3',4'-trihydroxy-2'-oxopentyl)-amino-4-oxopyrimidine
GTPCI (3.5.4.16)
2-amino-4-hydroxy-6-(erytro-1,2,3-trihydroxypropyl)-dihydropteridine triphosphate
Molybdopterin
hydrolases (3.6.1-)
Dihydroneopterin phosphate
GTPCI (3.5.4.16)
6-(3'-triphosphoryl-1'methylglyceryl)-7-methyl-7,8-dihydrobiopterin
6-pyruvoyl-THPS (4.2.3.12)
6-pyruvoyl-5,6,7,8-tetrahydropterin
Alkaline phosphatase (3.1.3.1)
hydrolases (3.6.1-)
Formamidepyrimidine nucleoside triphosphate
Glycolaldehyde
Sepiapterin reductase (1.1.1.153)
6-pyruvoyltetrahydropterin 2'-reductase (1.1.1.220)
Dihydroneopterin
Neopterin
GTPCI (3.5.4.16)
6-lactoyl-5,6,7,8-tetrahydropterin
DHNA (4.2.1.25)
GTP
Sepiapterin reductase (1.1.1.153)
Glycolaldehyde
2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine
Purine metabolism
Pterine biosynthesis
5,6,7,8-tetrahydrobiopterin
HPPK (2.7.6.3)
6,7-dihydropteridine reductase (1.5.1.34)
2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine
7,8-dihydromethanopterin
dihydrobiopterin
DHPS (2.5.1.15)
5,6,7,8-tetrahydromethanopterin
4-amino-benzoate
7,8-dihydropteroate
DHFS (6.3.2.12)
Aminodeoxychorismate lyase (4.1.3.38)
7,8-dihydrofolate (DHF)
Folate
DHFR (1.5.1.3)
DHFR (1.5.1.3)
One carbon pool by folate
4-amino-4-deoxy-chorismate
DHFR (1.5.1.3)
Aminodeoxychorismate synthase (6.3.5.8)
5,6,7,8-tetrahydrofolate (THF)
FPGS (6.3.2.17)
Phenylalanine biosynthesis
gamma.-glutamyl hydrolase (3.4.19.9)
THF-L-Glutamate
FPGS (6.3.2.17)
THF-polyglutamate
Methanogen bacteria metabolism

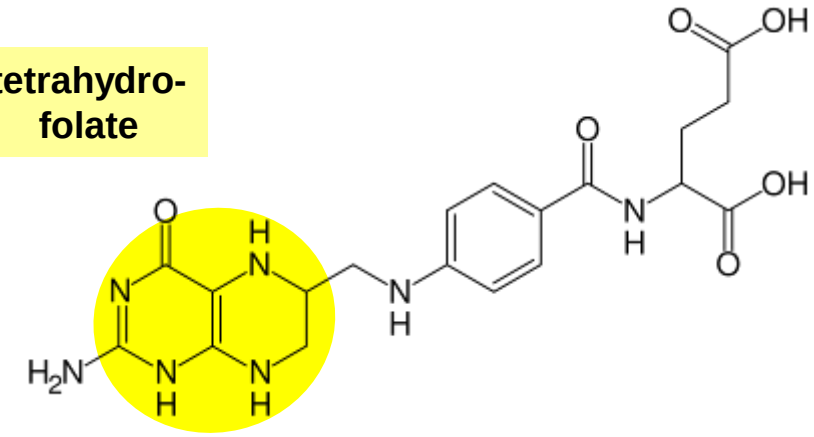
Folate pathway in Trypanosomatidae



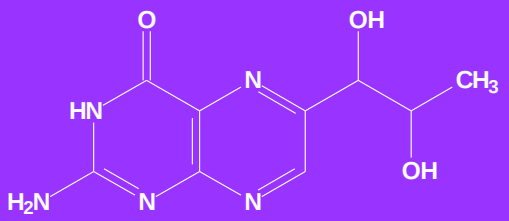
Folate pathway: one carbon metabolism



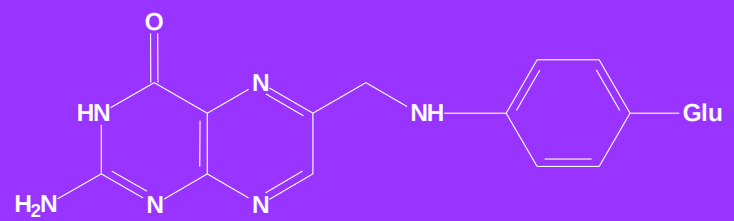




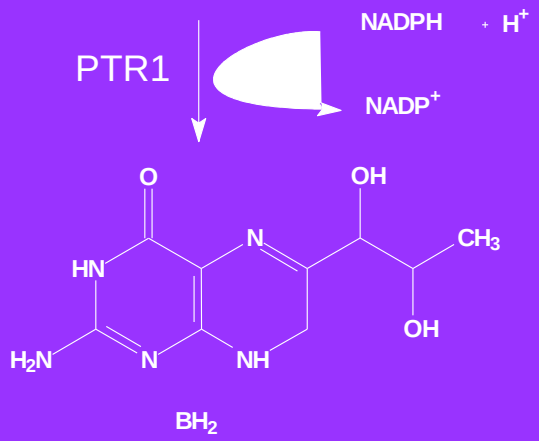
PTR1 mechanism



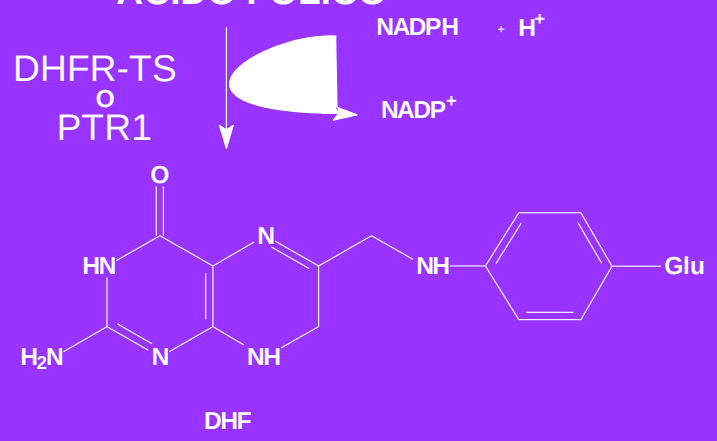
BIOPTERINA



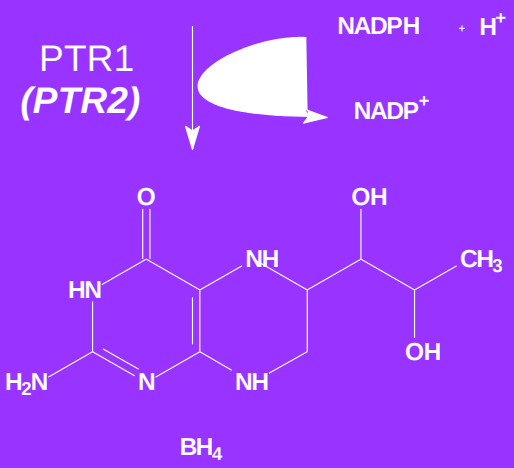
ACIDO FOLICO



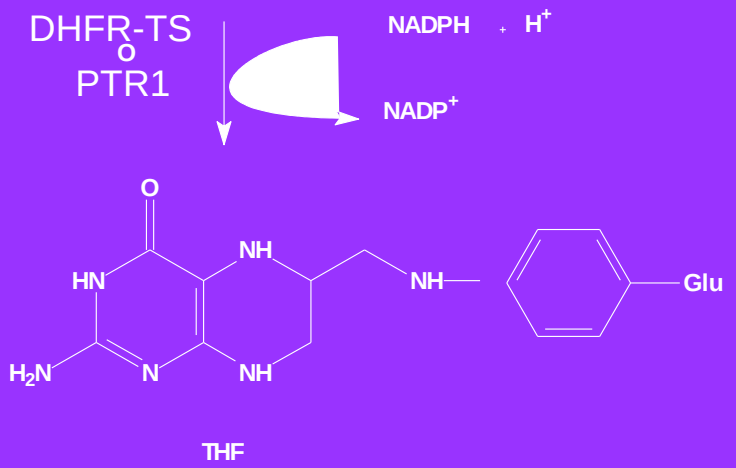
BH_2



DHF

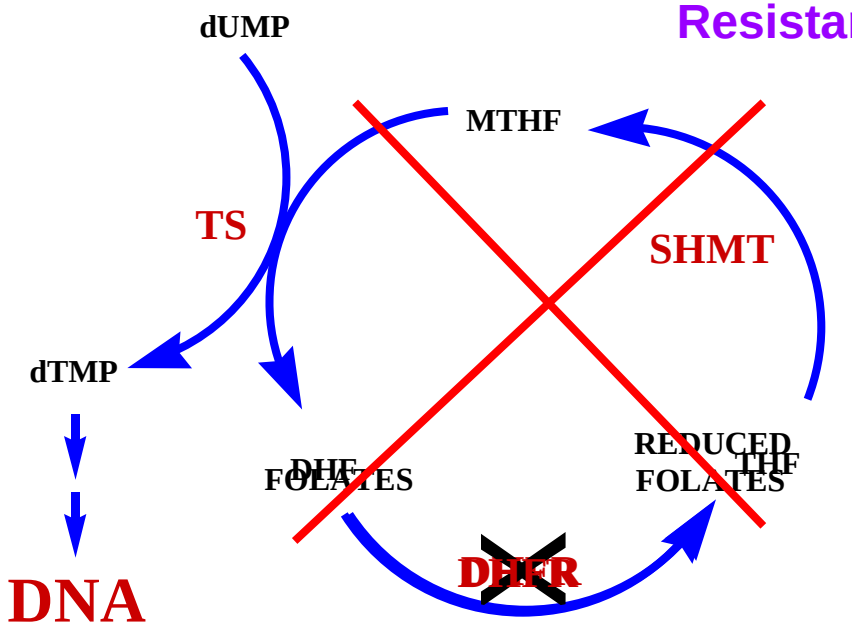


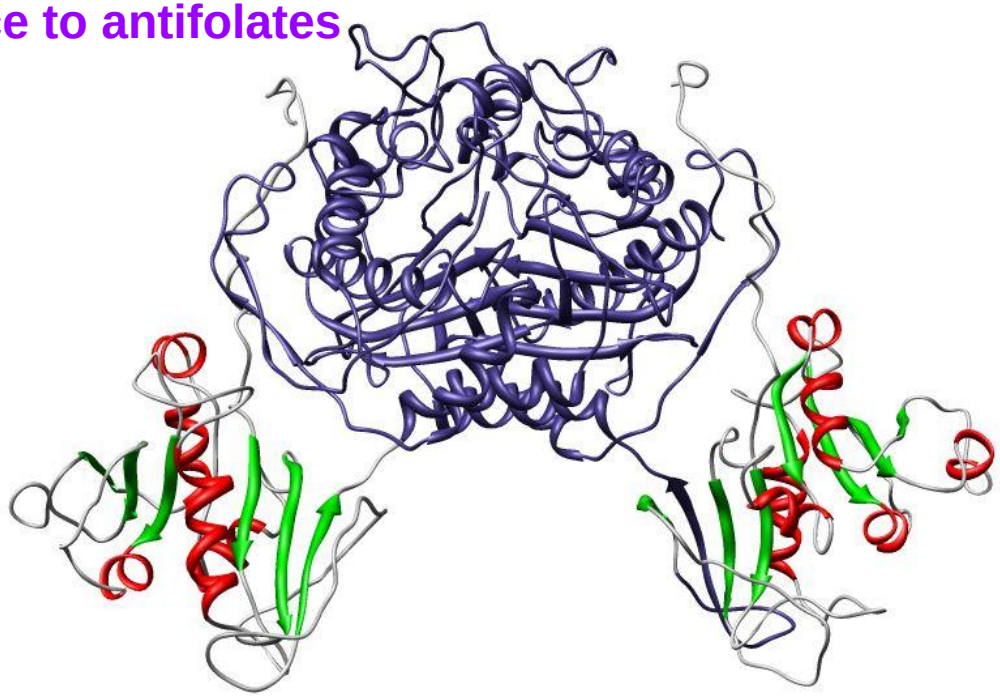
BH_4

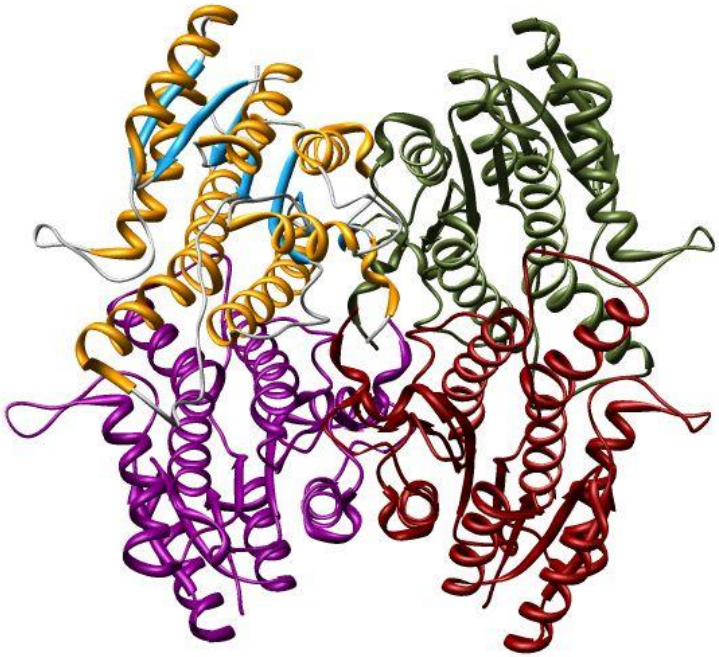


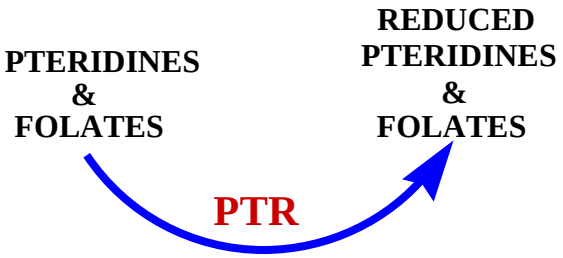
THF

Resistance to antifolates

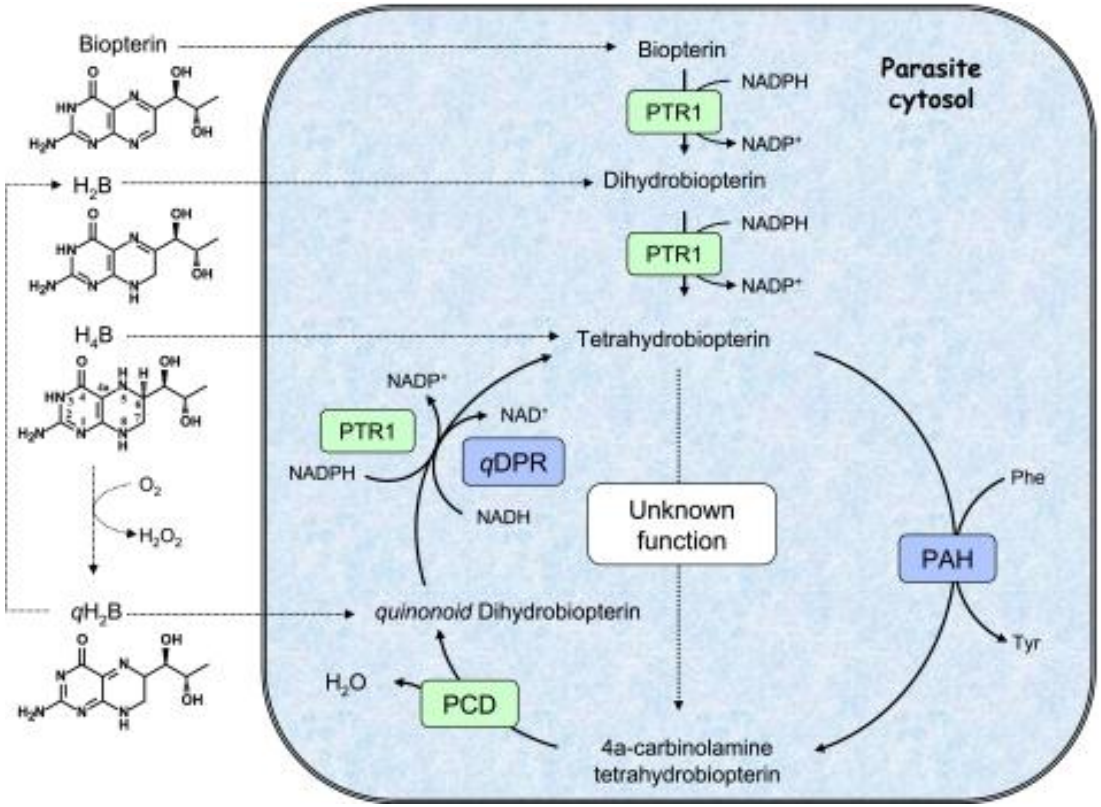








Additional enzymes in the BTs metabolism



Enzymes and metabolic pathways in *T. brucei* and *L. major*. Enzymes present in both parasites are shown in *green* and enzymes only present in *L. major* are shown in *blue*. The *dotted lines* indicate pterins that can be taken up from the medium.

Dissecting the metabolic roles of pteridine reductase 1 in Trypanosoma brucei and Leishmania major.

J Biol Chem. 2011 Mar 25;286(12):10429-38. Epub 2011 Jan 14.Ong HB, Sienkiewicz N, Wyllie S, Fairlamb AH.

Strategies for the pursuit of drugs to treat neglected tropical diseases.

- (A) **label extension**, extending the indications of existing drugs for other conditions to tropical diseases;
- (B) **piggy-back discovery**, in which the discovery of new drugs is focused on one or a few classes of well-studied and validated targets; and
- (C) **de novo drug discovery**.

These strategies collectively seek to exploit two possible sets of drug targets: those that have been validated in other organisms and diseases, and those that have not – perhaps because they are unique to neglected-disease pathogens – but that nevertheless have potential as novel sites of action.

PLoS Negl Trop Dis. 2010 Aug 24;4(8):e804.
Identification of attractive drug targets in neglected-disease pathogens using an in silico approach.
Crowther GJ, Shanmugam D, Carmona SJ, Doyle MA, Hertz-Fowler C, Berriman M, Nwaka S, Ralph SA, Roos DS, Van Voorhis WC, Agüero F.

Table 2

Preliminary genome-wide prioritization of *Leishmania major* targets.

Ranking	Gene_name	Gene product	Weight
1	LmjF29.0820	cysteine peptidase C (CPC),CPC cysteine peptidase, Clan CA, family C1, Cathepsin B-like	416
2	LmjF05.0350	trypanothione reductase	386
2	LmjF06.0860	dihydrofolate reductase-thymidylate synthase	386
2	LmjF23.0050	cyclophilin, putative,peptidyl-prolyl cis-trans isomerase, putative	386
2	LmjF25.0910	cyclophilin a	386
2	LmjF06.0120	cyclophilin	386
2	LmjF18.0270	protein kinase, putative,glycogen synthase kinase, putative	386
8	LmjF36.1960	phosphomannomutase, putative	366
8	LmjF23.0270	pteridine reductase 1	366
10	LmjF30.2970	glyceraldehyde 3-phosphate dehydrogenase, glycosomal	351
10	LmjF12.0220	hydroxyacylglutathione hydrolase, putative,glyoxalase II, putative	351
10	LmjF24.0850	triosephosphate isomerase	351
13	LmjF27.1870	trypanothione synthetase, putative	341
13	LmjF06.0560	deoxyuridine triphosphatase, putative,dUTP diphosphatase	341
15	LmjF21.0250	hexokinase, putative	336
15	LmjF25.1320	serine/threonine protein phosphatase, putative	336
15 Family	LmjF19.0550	methionine aminopeptidase, putative,metallo-peptidase, Clan MG, M24	336
15	LmjF34.1260	mitochondrial DNA polymerase I protein A, putative	336
15	LmjF30.0880	adenosine kinase, putative	336
15	LmjF33.1630	cyclophilin, putative	336

Aims

The aim is to identify new lead compounds tackling the folate pathway, active against Leishmania parasites (and Trypanosomes), non toxic against human cells.

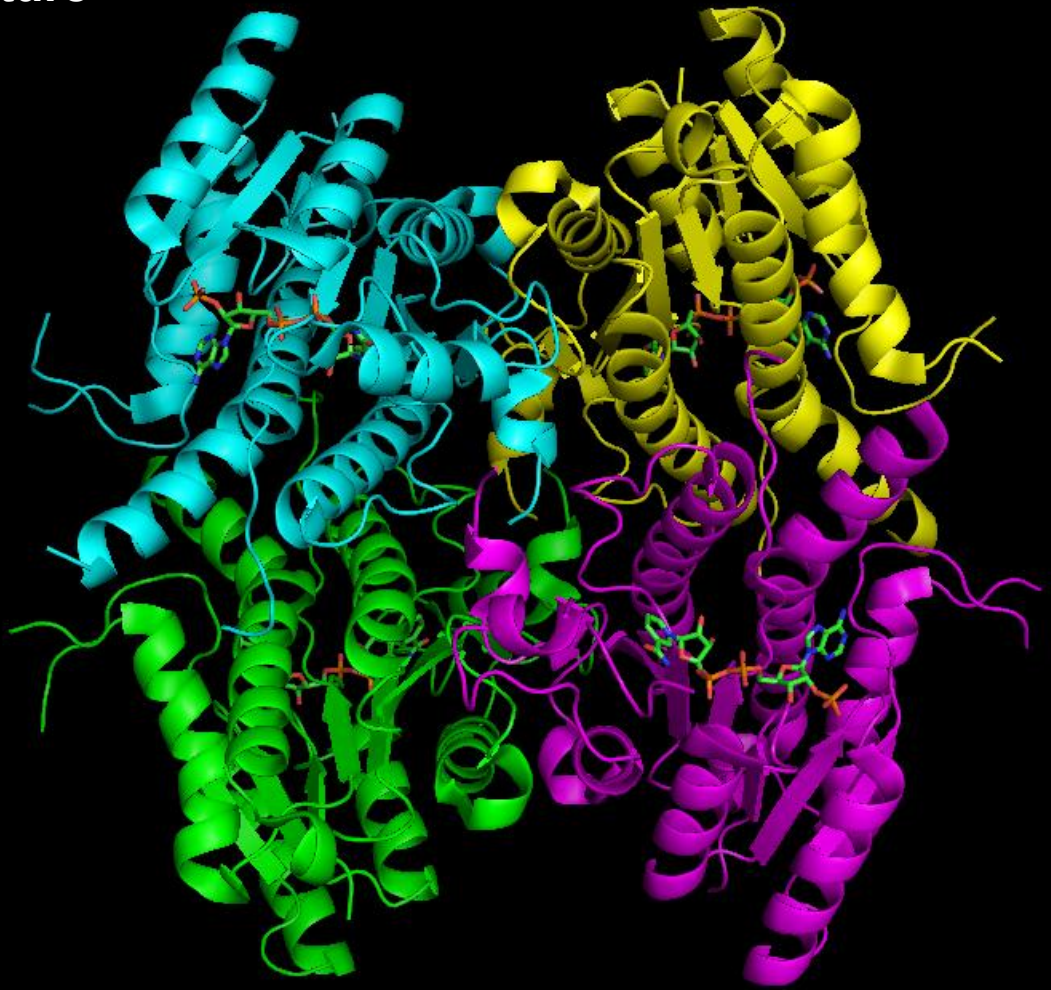
Expected biological properties of the new compounds are

SPECIFICITY
enzymes absent in human cells

SELECTIVITY
Enzymes present in human cells but quite different structure

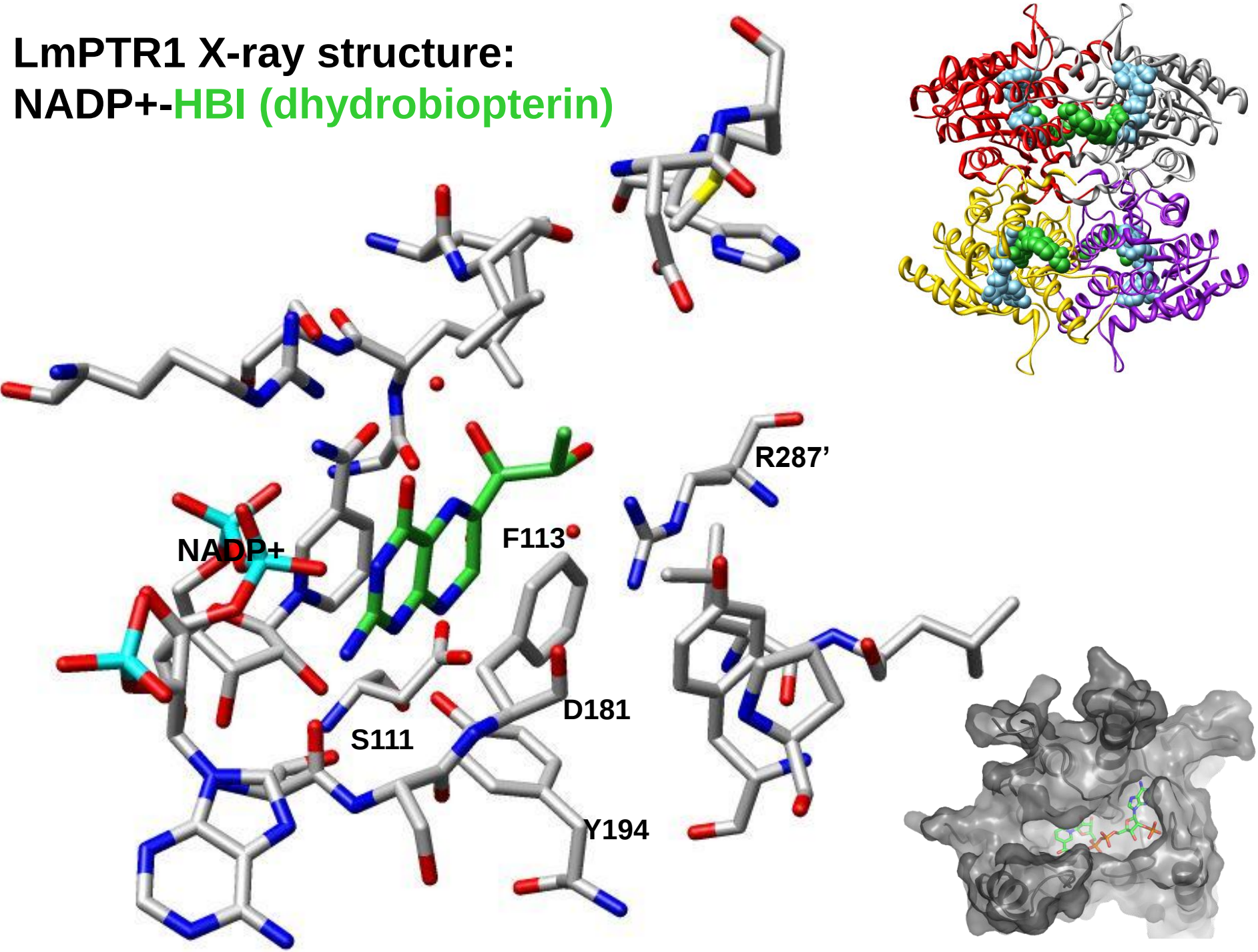
Targeting essential proteins or suitable for combination therapy

PTR1 structure



Gourley DG, Hunter WN et al. nature structural biology • volume 8 number 6 • june 2001

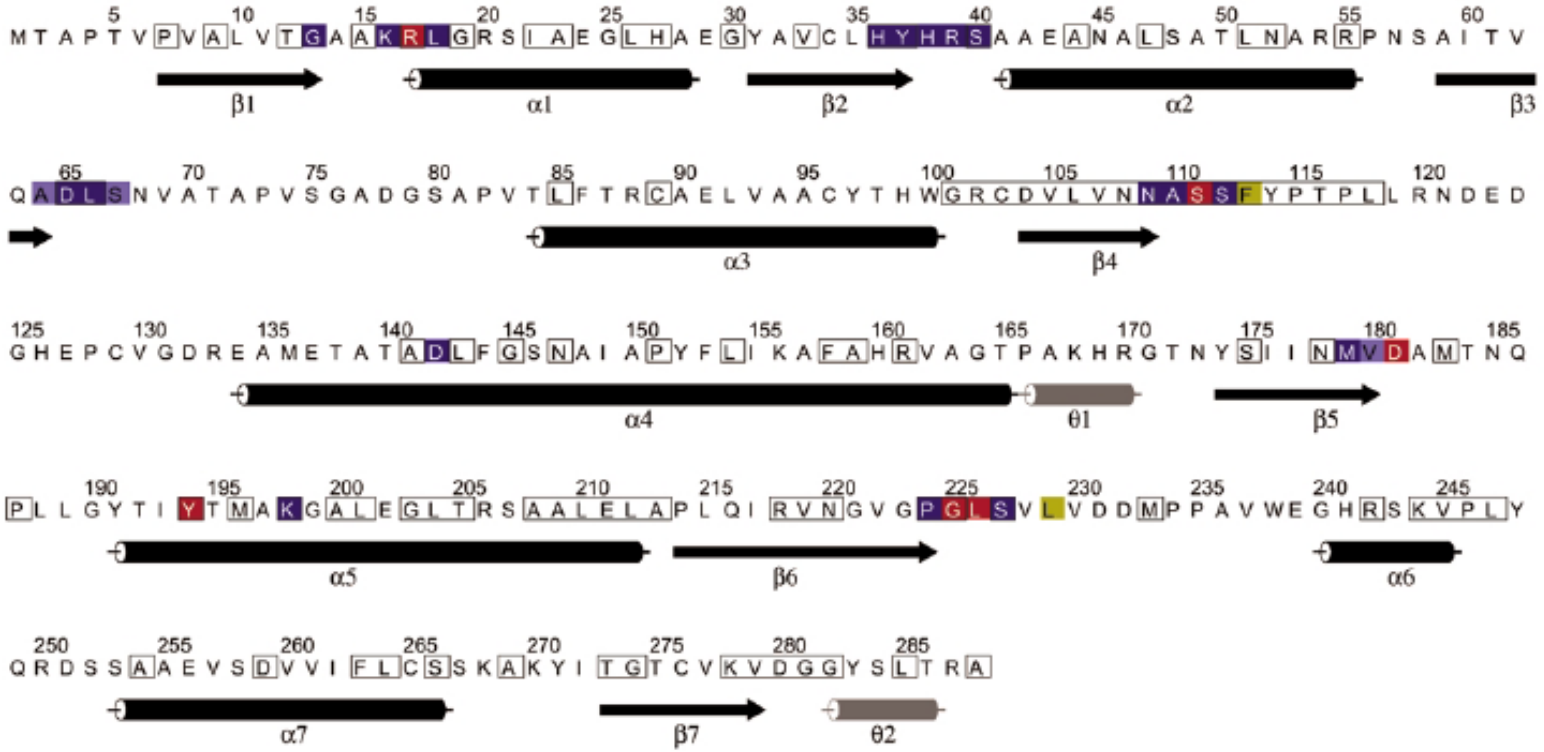
LmPTR1 X-ray structure:
NADP+-HBI (dhydrobiopterin)



Primary structure of PTR1s

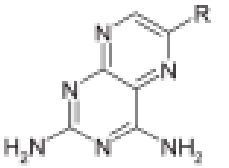
Colored box: less than 4° from the substrates

Boxes: residues conserved in all species

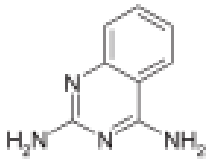


Ptr1 inhibition

Currently, no drugs are known to target this enzyme validated inhibitors exist and known drugs such as methotrexate and pyrimetamine



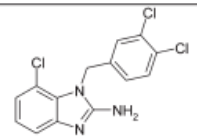
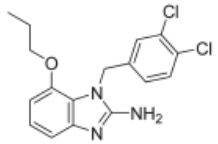
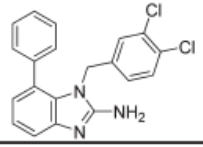
R= H: 2,4-diaminopteridine
R= CH3: 6-methylpteridine-2,4-diamine



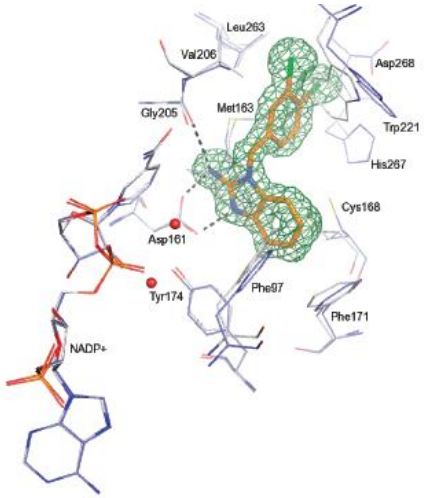
2,4-diaminoquinazoline

2,4-diaminopyrimidine

Table 4. Hit Expansion of the Aminobenzimidazole Series[a]

#	Structure	K_i^{app} [μM]	Hill slope
10		0.51	1.0
11		0.047	0.7
12		0.007	0.7

[a] K_i^{app} and Hill slopes are averaged over at least two independent measurements.



R.Brenk et al, J. Med. Chem. 2009, 52, 4454–4465

Conclusions by Beverly's work

Several significant conclusions resulted:

(1) potent inhibition of PTR1 alone is insufficient for growth inhibition, in Leishmania

(2) depletion of intracellular PTR1 levels sensitizes the parasites to growth inhibition

Proof of concept through library screening strategy

We have identified a folate-like analog class

low nanomolar PTR1 inhibition constant (Ki) and

- *high resistance index against PTR1 overexpressing Leihmania major parasites*
- *low efficacy alone against the amastigote form of Leishmania major almost synergize the activity of Pyirimetamine.*
- *Lower toxicity against human cells.*

This compound class is under development.

Cavazzuti A, Cosi MP et.al. PNAS, 2008

889R

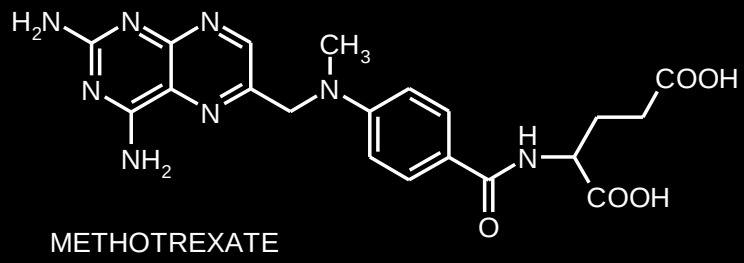
Ki 7 μM *Tc*PTR1

Ki 100 nM *Lm*PTR1

Ki 4 μM *Lm*DHFR

Ki 10 μM *h*DHFR

NI at 190 μM vs hTS, LmTS-DHFR



METHOTREXATE

Ki 110 nM *Tc*PTR1

Ki 180 nM *Lm*PTR1

Ki 130 pM *Lm*DHFR

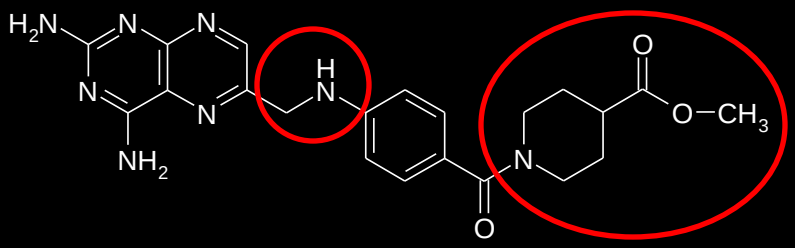
Ki 3.4 pM *h*DHFR

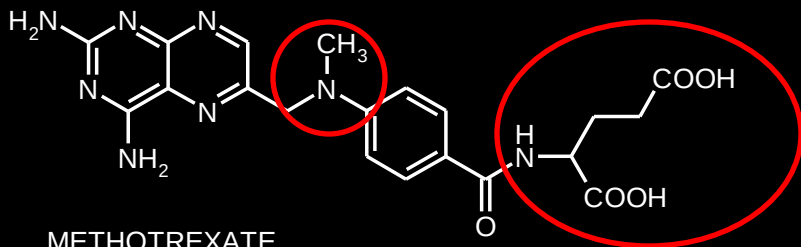
Ki 600 nM *Lm*TS

Compound 889R is very active against LmPTR1 and 100 times less active against the human enzyme (hDHFR). It is active against amastigote form of Leishmania major in combination with pyrimetamine (PYR).

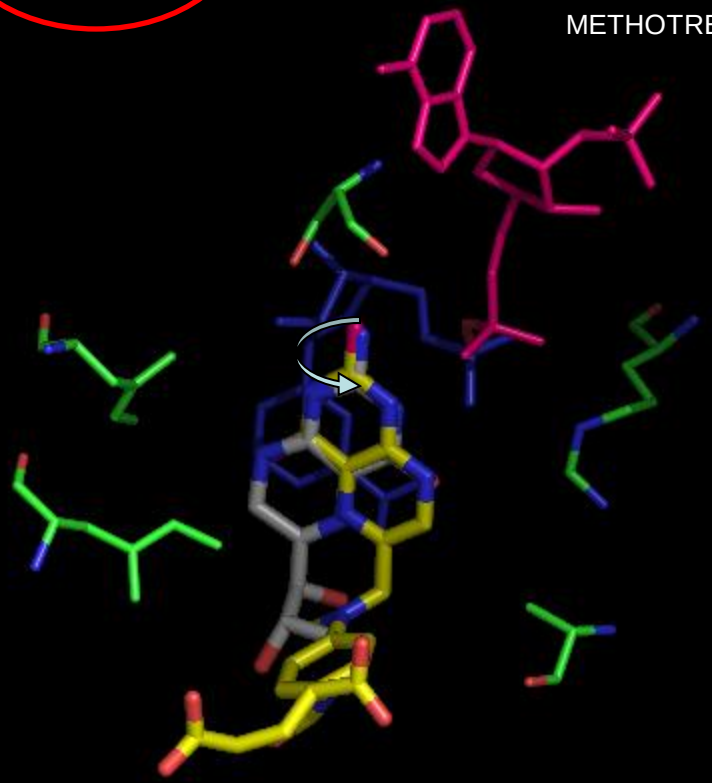
PYR is a DHFR inhibitor.

889R





METHOTREXATE



The binding mode is crucial for specificity

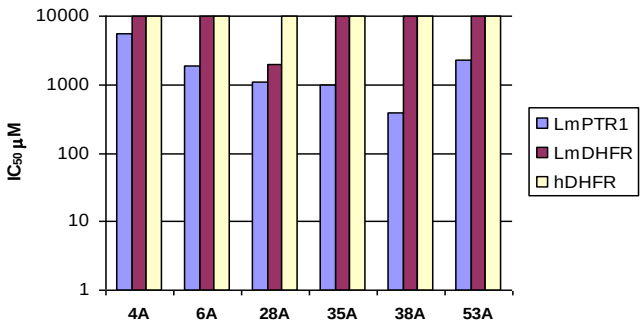
Medicinal chemistry program

- Hit identification: Virtual screening
  +
  Enzymatic assays
  +
  Scaffold identification and validation

- Hit to Lead: Design, synthesis and biological evaluation of thiadiazole compound library
  +
  Molecular Modelling
  (to explain exp data,
  to suggest new derivatives (GRID))
  +
  X-ray structure

S.Ferrari, Costi MP et al JMedChem, 2010

			LmPTR1 IC50
virtual screening	**ACD database**	~ 350000	
	structure-based virtual screening: LUDI	21394	
	filtering 1: % contacts, number of H bonds, calculated score	724	
	filtering 2: visual comparison to LmPTR1 and hDHFR, number and type of interactions	93	
in vitro tests	**in vitro testing**: LmPTR1, LmDHFR, hDHFR	53	
	active against LmPTR1	**6**	0.39- 5.6 mM
		lead 1	5.6 mM

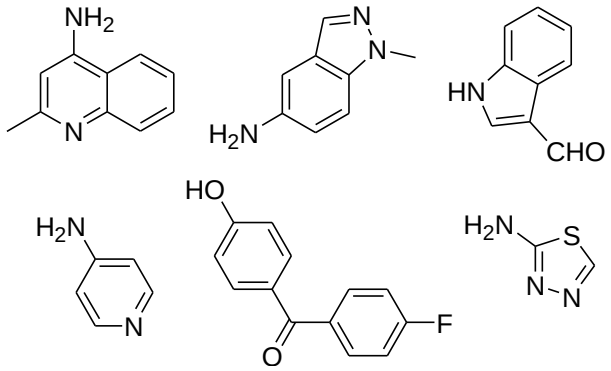


53 COMPOUNDS

WERE from the Virtual Screening Library

AND

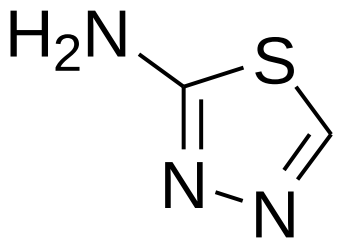
TESTED AGAINST enzyme bio-library



6 COMPOUNDS

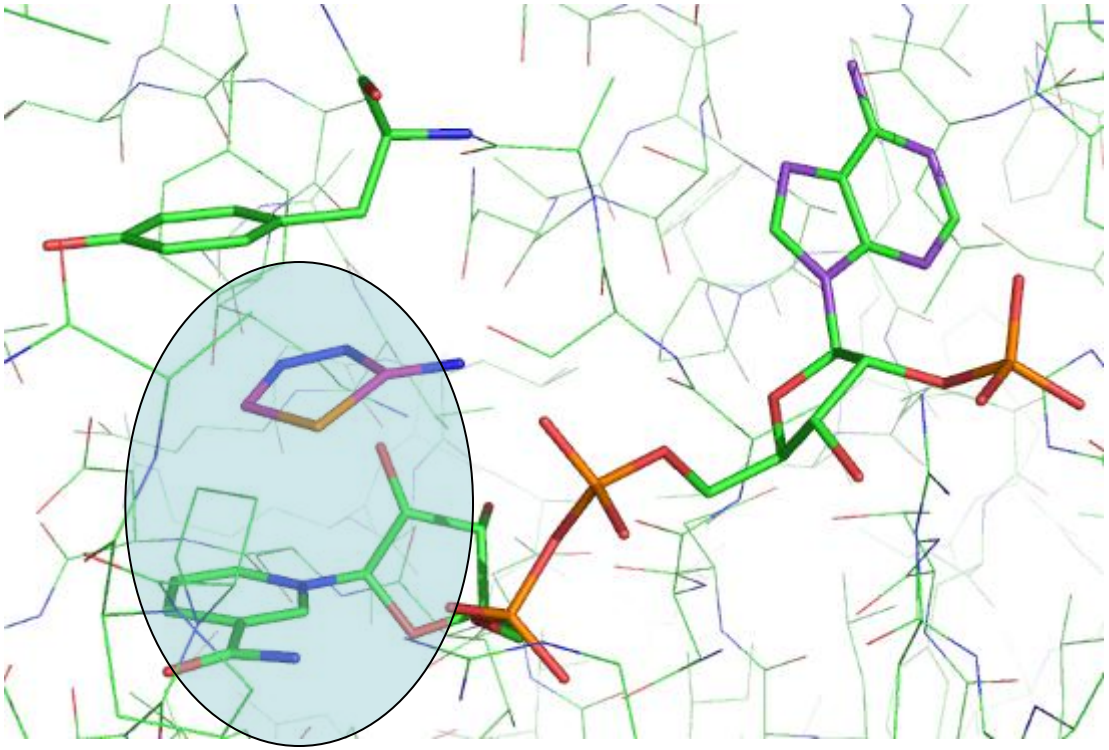
SHOWED INHIBITION ACTIVITY

WITH Ki 90-600 µM



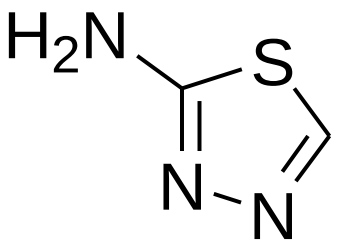
lead 1
(2-amino-1,3,4-thiadiazole)

Leishmania major (1E92)

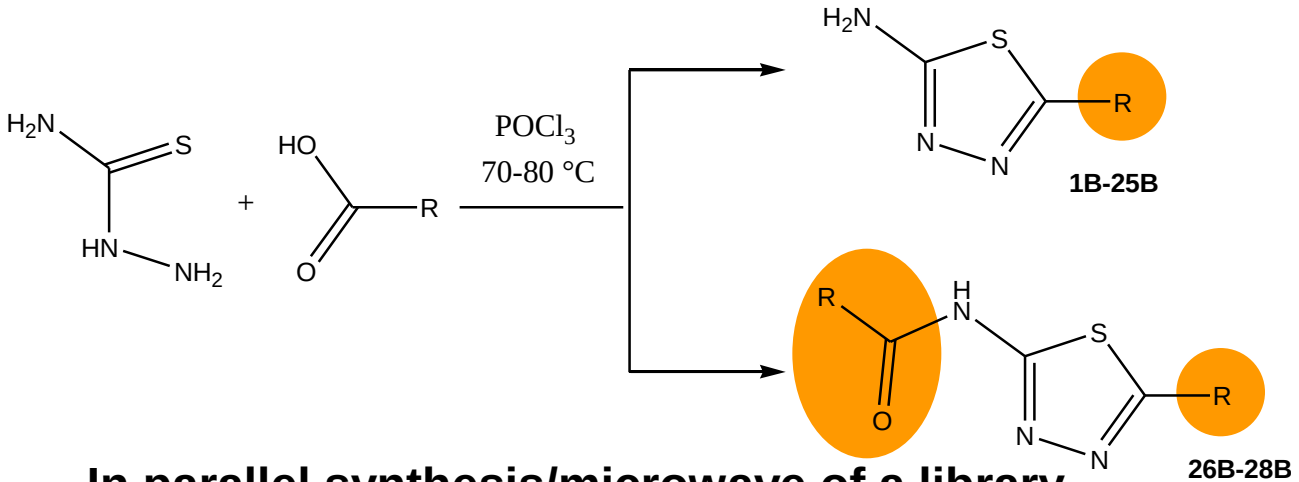


Docking model of Lead 1 with LmPTR1

			LmPTR1 IC50
virtual screening	**ACD database**	~ 350000	
	structure-based virtual screening: LUDI	21394	
	filtering 1: % contacts, number of H bonds, calculated score	724	
	filtering 2: visual comparison to LmPTR1 and hDHFR, number and type of interactions	93	
in vitro tests	**in vitro testing**: LmPTR1, LmDHFR, hDHFR	53	
	active against LmPTR1	**6**	0.39- 5.6 mM
		lead 1	5.6 mM
derivates of lead 1	designing and synthesis of derivates of lead 1	26	
	testing of derivates of lead 1	**7**	22 – 309 µM

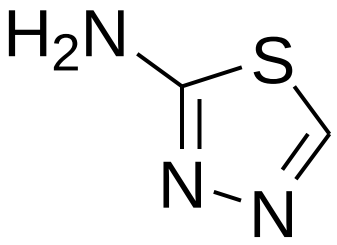


lead 1
(2-amino-1,3,4-thiadiazole)

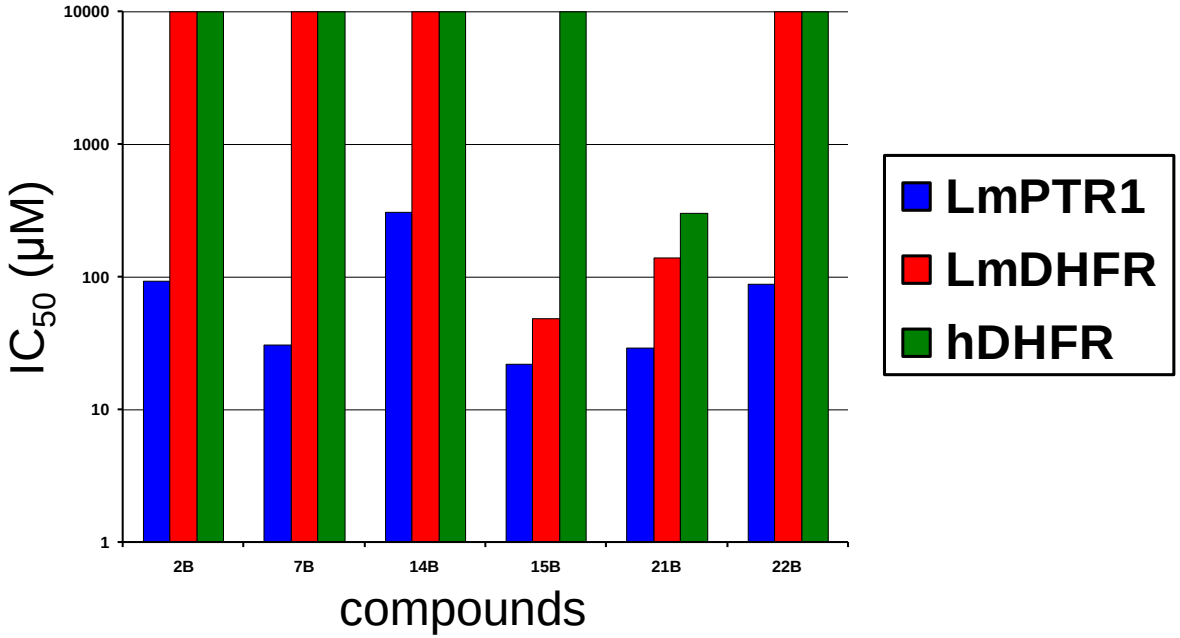


In parallel synthesis/microwave of a library of 2-amino-thiadiazole derivatives

			LmPTR1 IC50
virtual screening	**ACD database**	~ 350000	
	structure-based virtual screening: LUDI	21394	
	filtering 1: % contacts, number of H bonds, calculated score	724	
	filtering 2: visual comparison to LmPTR1 and hDHFR, number and type of interactions	93	
in vitro tests	**in vitro testing**: LmPTR1, LmDHFR, hDHFR	53	
	active against LmPTR1	**6**	0.39- 5.6 mM
		lead 1	5.6 mM
derivates of lead 1	designing and synthesis of derivates of lead 1	26	
	testing of derivates of lead 1	**7**	22 – 309 µM

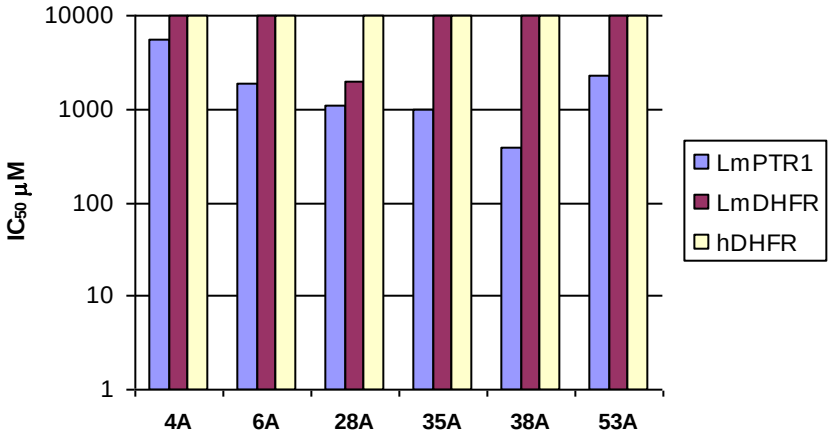


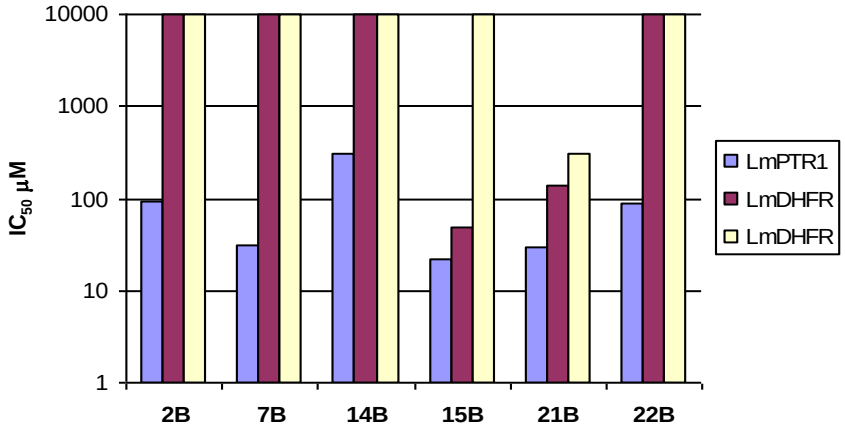
lead 1 derivatives
(2-amino-1,3,4-thiadiazole)



Enzymatic assays of 2-amino-thiadiazole derivatives

From scaffold to lead?





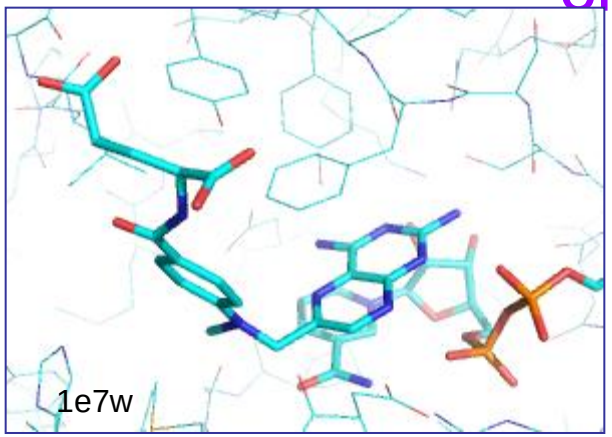
Hits selection → **Chemical modification** → **1° round Library**

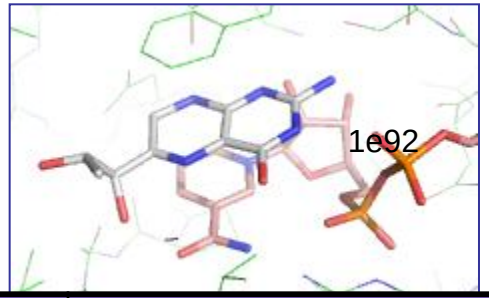
			LmPTR1 IC50
virtual screening	**ACD database**	~ 350000	
	structure-based virtual screening: LUDI	21394	
	filtering 1: % contacts, number of H bonds, calculated score	724	
	filtering 2: visual comparison to LmPTR1 and hDHFR, number and type of interactions	93	
in vitro tests	**in vitro testing**: LmPTR1, LmDHFR, hDHFR	53	
	active against LmPTR1	**6**	0.39- 5.6 mM
		lead 1	5.6 mM
derivates of lead 1	designing and synthesis of derivates of lead 1	26	
	testing of derivates of lead 1	**7**	22 – 309 µM
computational drug design	docking of derivates of lead 1 but no discrimination between active and inactive is possible	26	
	comparison of docking poses and MIFs		

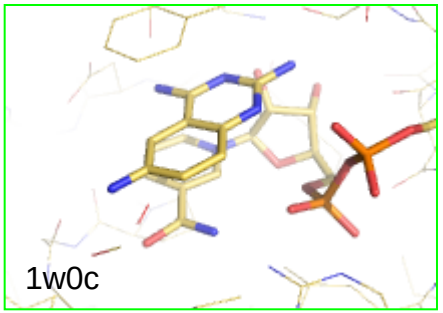
Suggest for further lead development

GRID

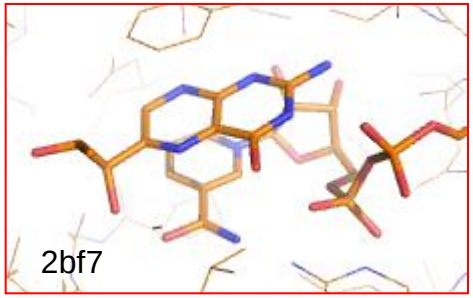
Leishmania major PTR1 X-ray crystal structures: cross docking studies

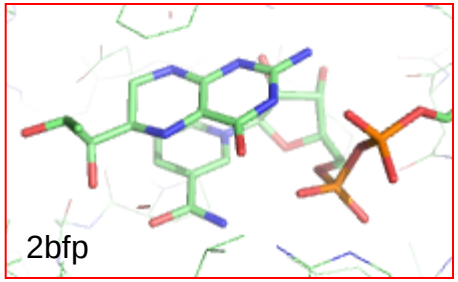


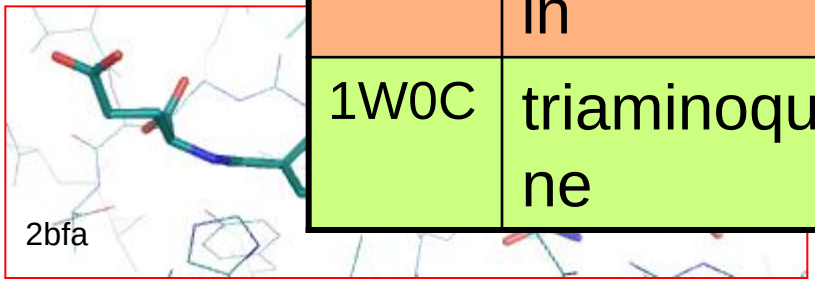


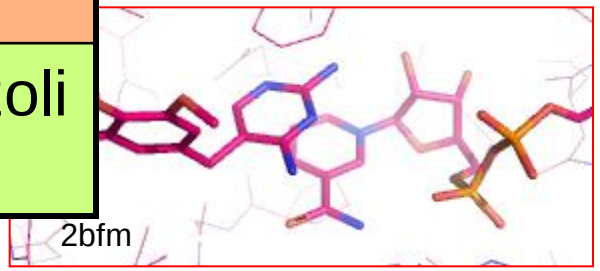


1E92	dihydrobiopterin
1E7W	methotrexate
2BF7	biopterin
2BFA	CB3717
2BFM	trimethoprim
2BFP	tetrahydrobiopterin
1W0C	triaminoquinazoline

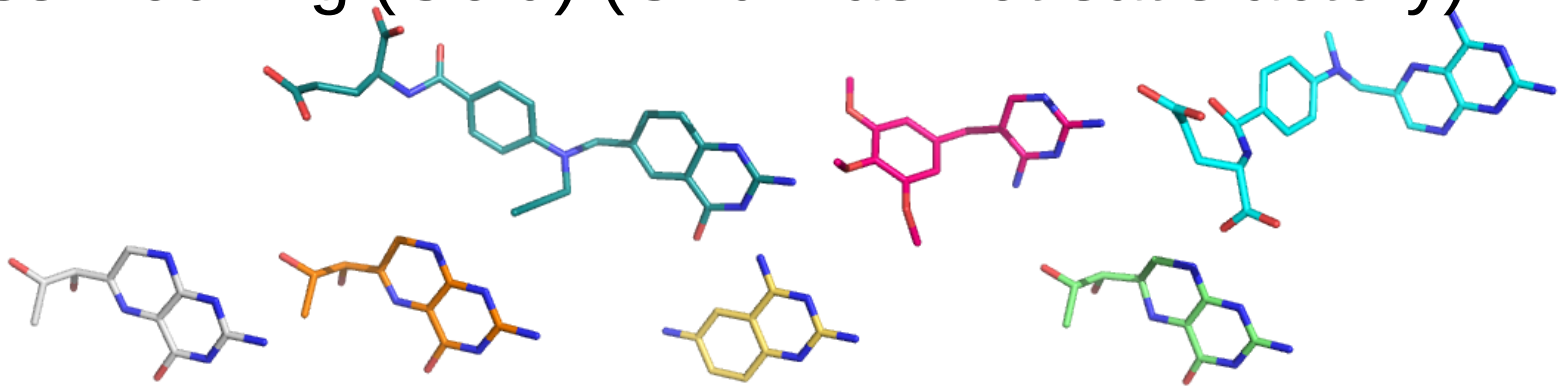








Cross Docking (Gold) (Grid was not satisfactory)



receptor	1E92	2BF7	2BFA	1W0C	2BFM	2BFP	1E7W
1E92							
2BF7							
2BFA							
1W0C							
2BFM							
2BFP							
1E7W							

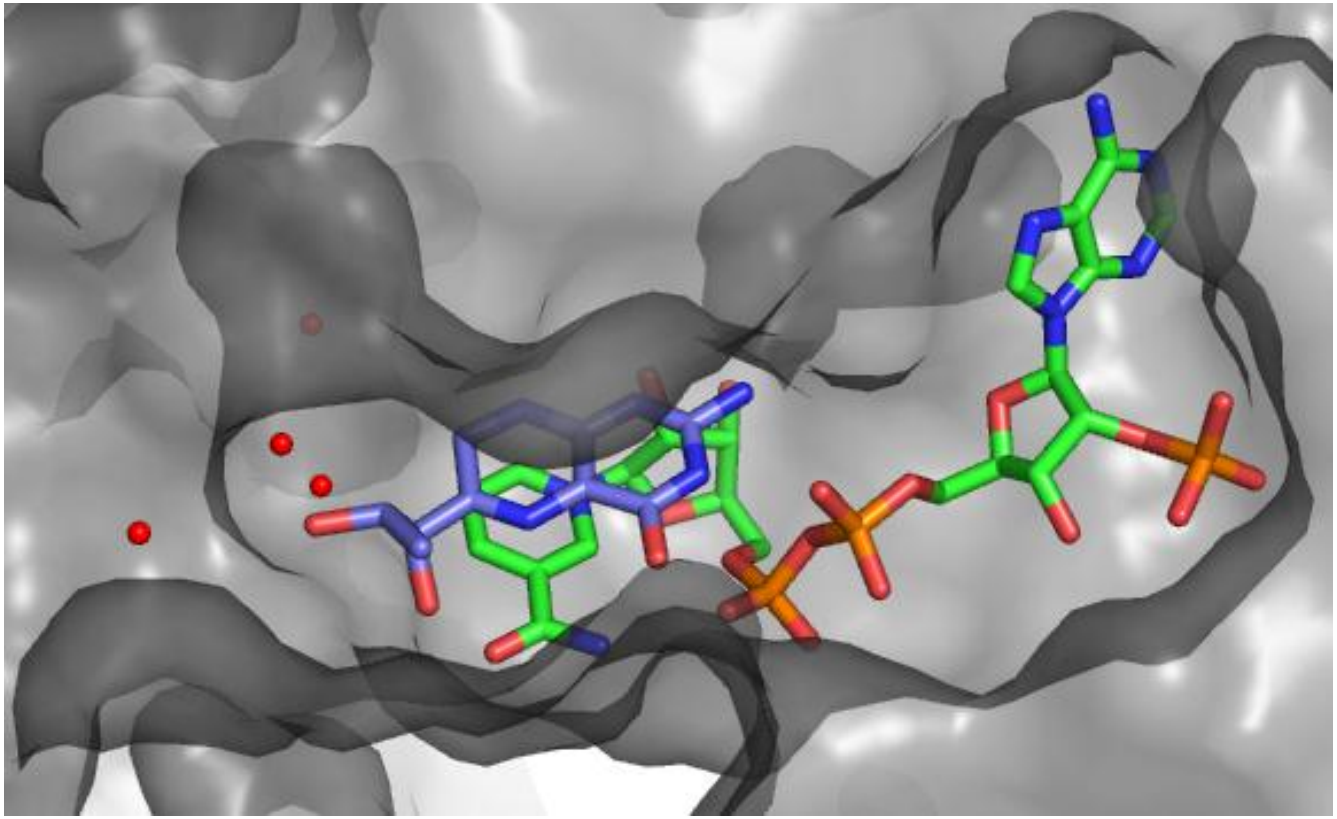
RMSD <3Å <5Å >5Å

Classical docking couldn‘t score well the experimental inhibition data.

Improving the docking results and dock scoring using conserved water molecules for docking.

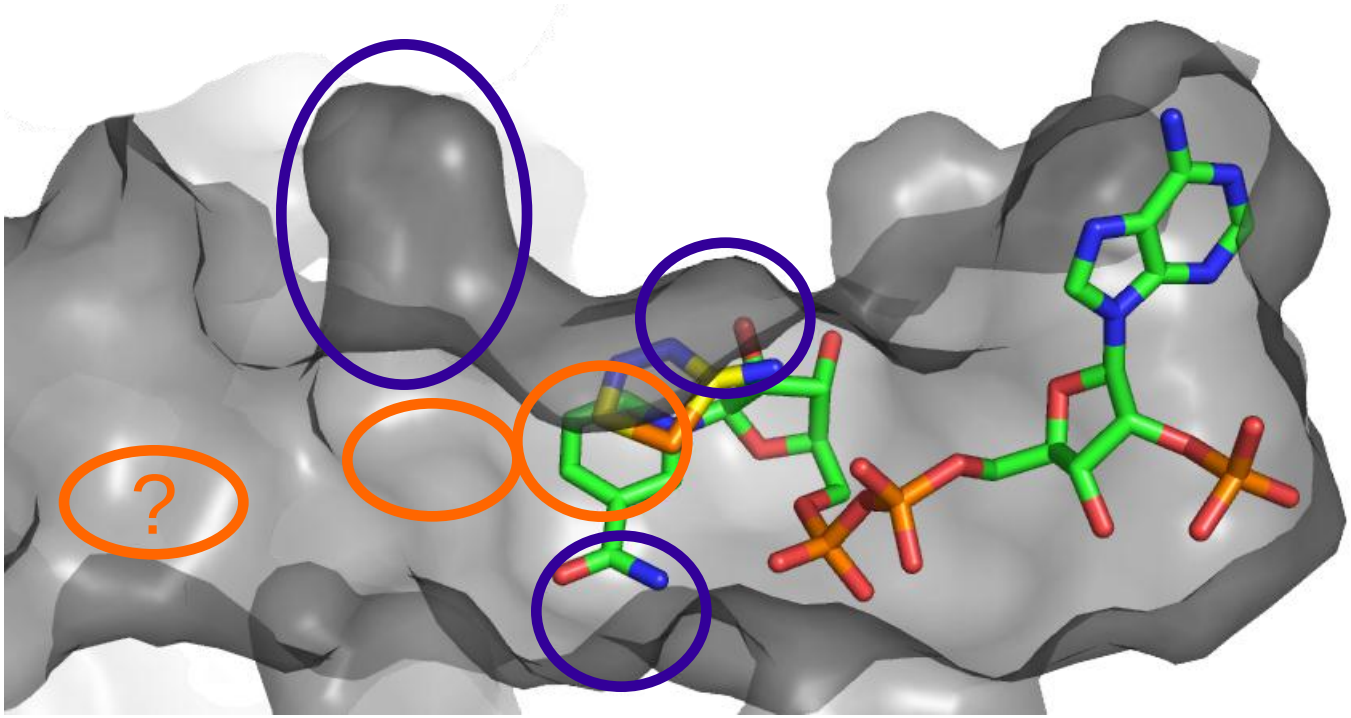
comparing water molecules given in the crystal structures using cluster analysis (WatCH)

→ 4 conserved water molecules close by the active site



Compound Design

Molecular probing: **identification of favourable binding site for specific probes**
(H2O, CH3, aromatic, OH, COOH, NH2, NH3+…..)
(GRID, all pdb structures)



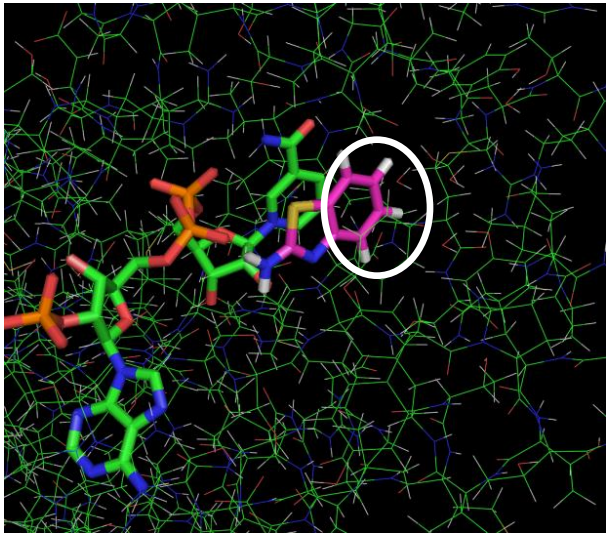
Hydrophobic

Hydrophilic

			LmPTR1 IC50
virtual screening	**ACD database**	~ 350000	
	structure-based virtual screening: LUDI	21394	
	filtering 1: % contacts, number of H bonds, calculated score	724	
	filtering 2: visual comparison to LmPTR1 and hDHFR, number and type of interactions	93	
in vitro tests	**in vitro testing**: LmPTR1, LmDHFR, hDHFR	53	
	active against LmPTR1	**6**	0.39- 5.6 mM
		lead 1	5.6 mM
derivates of lead 1	designing and synthesis of derivates of lead 1	26	
	testing of derivates of lead 1	**7**	22 – 309 µM
computational drug design	docking of derivates of lead 1 but no discrimination between active and inactive is possible	26	
	comparison of docking poses and MIFs		
	design of lead 2		
in vitro tests	vitro testing of lead 2	**lead 2**	1.8 mM
derivates of lead 2	design derivates of lead 2	4	
	testing derivates of lead 2	**4**	40 – 1000 µM

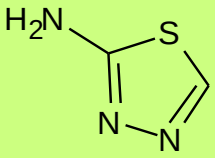
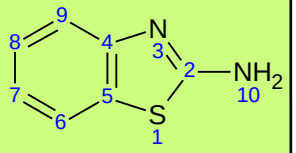
Testing of the best compounds from Lead 1 and Lead 2 aginst Leishmania parasites

Hopping to a new compound class:benzothiazoles



Lead 1 vs Lead 2

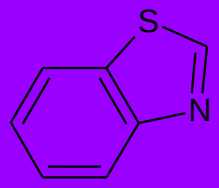
L. major PTR1

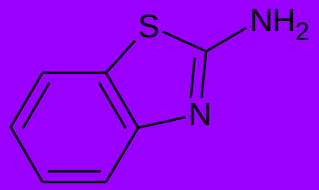
Hits	IC_{50} mM	Ki µM
	5.6	436
	1.8	143

No inhibition of *L. major* DHFR or human DHFR

From virtual screening on known and available compounds we may identify conventional drugs showing off target or out target effects

Database Search

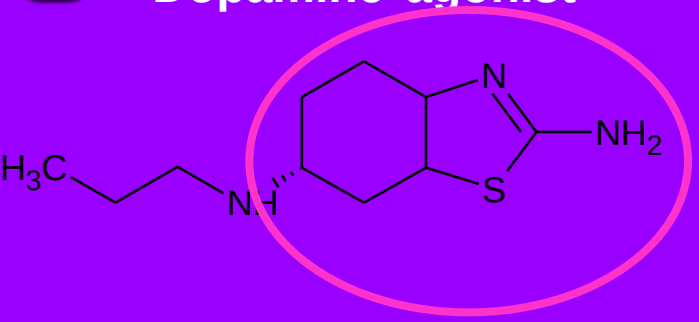




- **PubChem**
- **ZINC**
- **BindDB**
- **ChemBank**
- **DrugBank**
- **SciFinder**

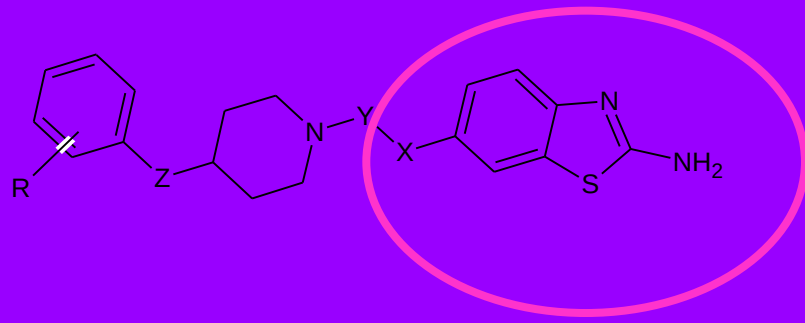
Pramipexole

- Parkinson, restless legs syndrome
- Dopamine agonist

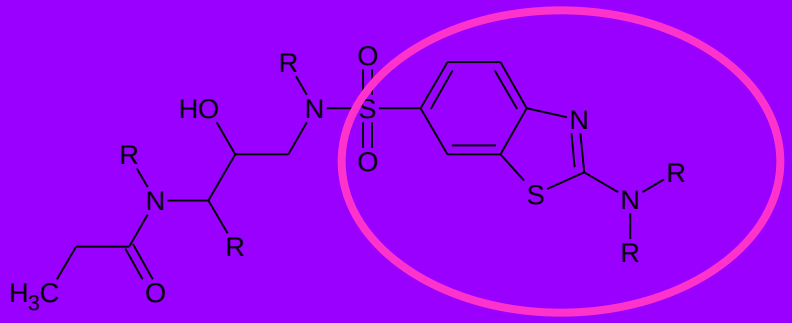


Patent US 6,407,122 B1

- Treatment of neurodegenerative disorders

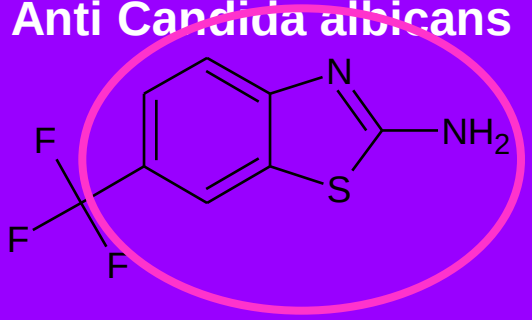


Patent CA 2492832



- HIV protease inhibitor

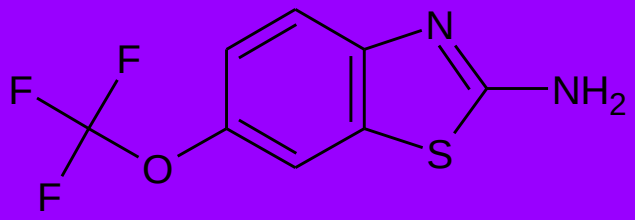
Anti Candida albicans



- Inhibitor of mitotic kinesin Kip1p

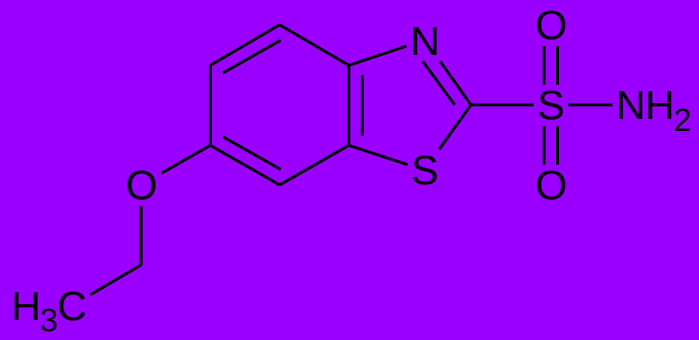
Riluzole

- **Amyotrophic Lateral Sclerosis**
- **Glutamate antagonist ?**



Ethoxzolamide

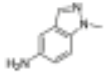
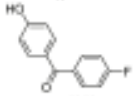
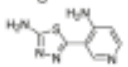
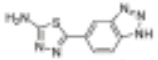
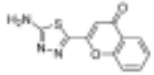
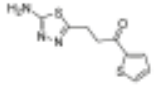
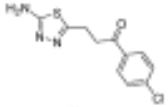
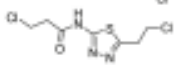
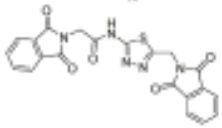
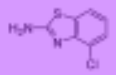
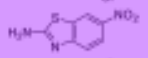
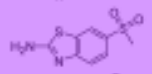
- **Glaucoma, as diuretic**
- **Carbonic anhydrase inhibitor**

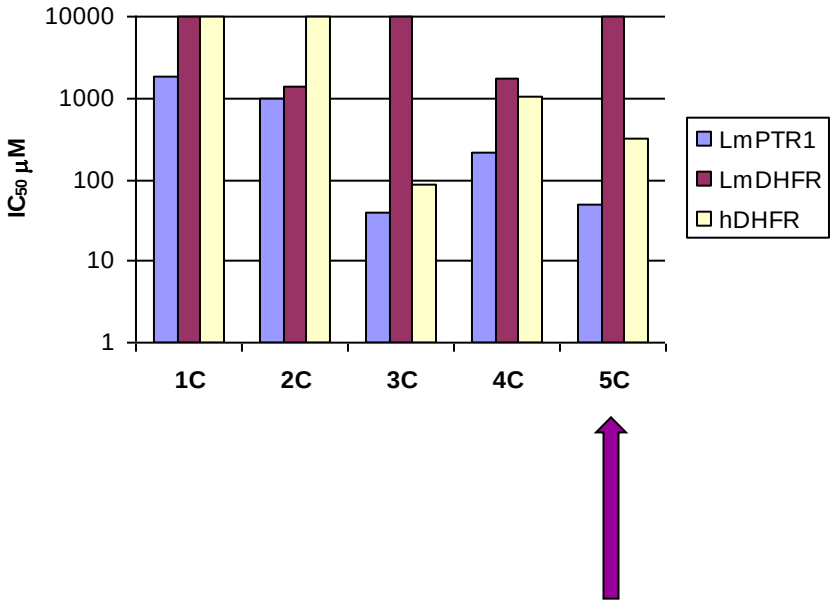


DB search – conclusions

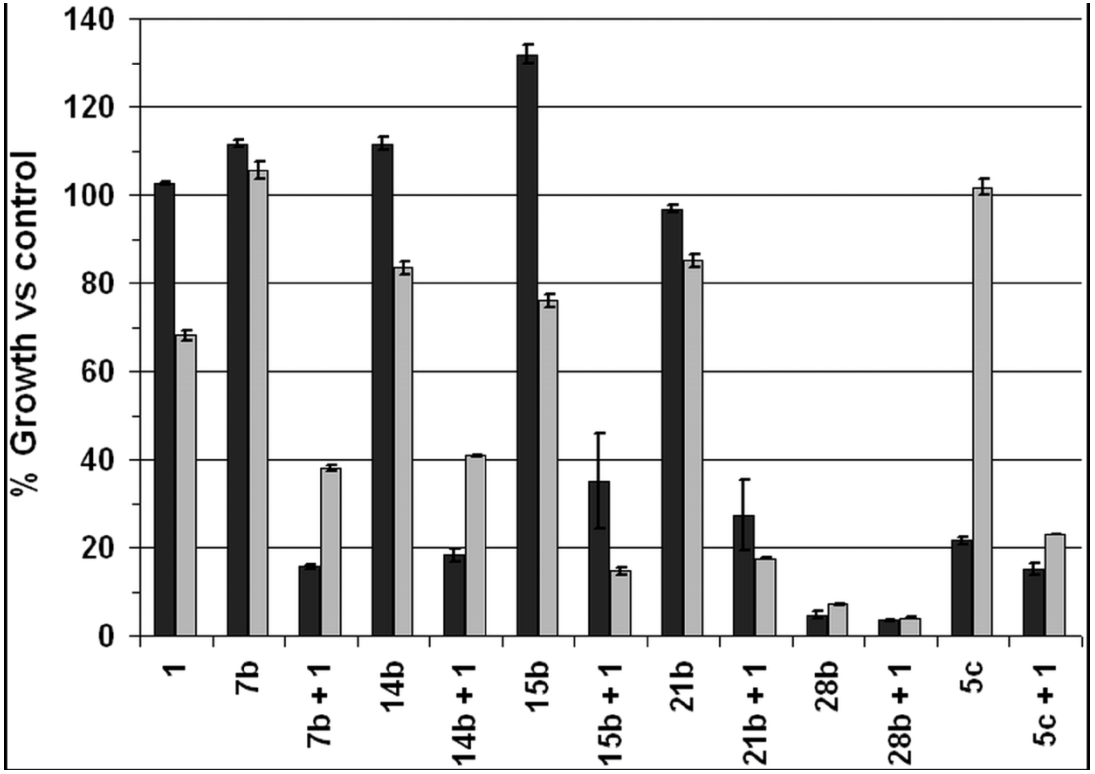
- **Most extensions at amino group**
- **~ 150 compounds with lead-2**
- **Several drugs with benzothiazole Structure.**

Benzothiazoles

Code	Structure	LmPTR1 IC_{50} (μM) [Ki (μM)]	LmDHFR IC_{50} (μM)	hDHFR IC_{50} (μM)
4a		5600 [436]	NI[a]	NI[b]
6a		1900	NI[a]	NI[a]
28a		1100	2000[a]	NI[a]
35a		1000	NI[c]	NI[d]
38a		390	NI[a]	NI[a]
53a		2300	NI[c]	NI[c]
7b		31 [2]	NI[c]	NI[c]
14b		309 [24]	NI[c]	NI[c]
15b		22 [2]	1300	NI[c]
21b		29 [2]	139[c]	300
22b		89 [7]	NI[c]	NI[c]
28b		93 [7]	NI[c]	NI[a]
29b		116 [9]	NI[c]	NI[ca]
1c		1800 [143]	NI[a]	NI[a]
2c		1000 [79]	1390[a]	NI[a]
3c		40 [3]	NI[c]	89[c]
4c		212 [16]	1780[a]	1040[a]
5c		50 [4]	NI[b]	312[b]



Antiparasitic activity against Lmajor and Lmexicana



Growth of L. mexicana (in black) and L. major (in gray) parasites in the presence of 30 µg/mL 1 and/or thiadiazole/ benzothiazole compounds at a concentration of 50 µg/mL. The growth values are expressed as percentages calculated with respect to the growth of parasites without 1 and thiadiazole/benzothiazole compounds

Biological studies on Riluzole

Riluzole shows similar activity in both *Leishmania* and no synergic effect appears when used in combination with Pyrimethamine

Guerrieri D., P.Michels, M.P.Costi et al in prep.

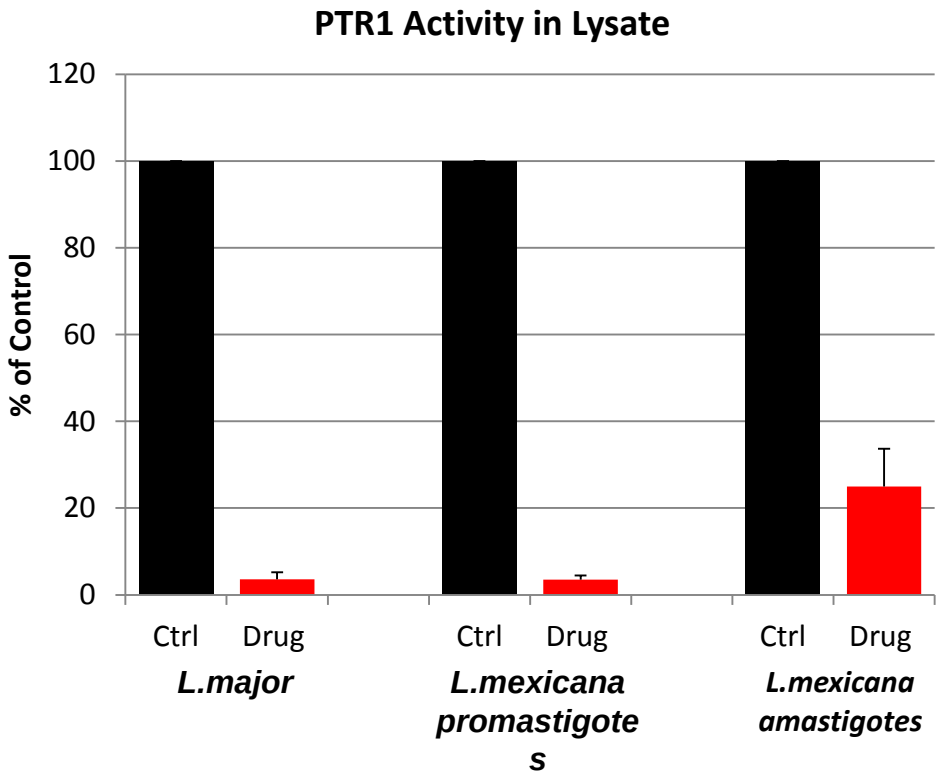
NADPH reduction in *Leishmania* Lysate – substrate: biopterin

L.major		
	%	±
Ctrl	100	0
Rilu	3.6	1.6

L.mexicana promastigotes		
	%	±
Ctrl	100	0
Rilu	3.5	1.0

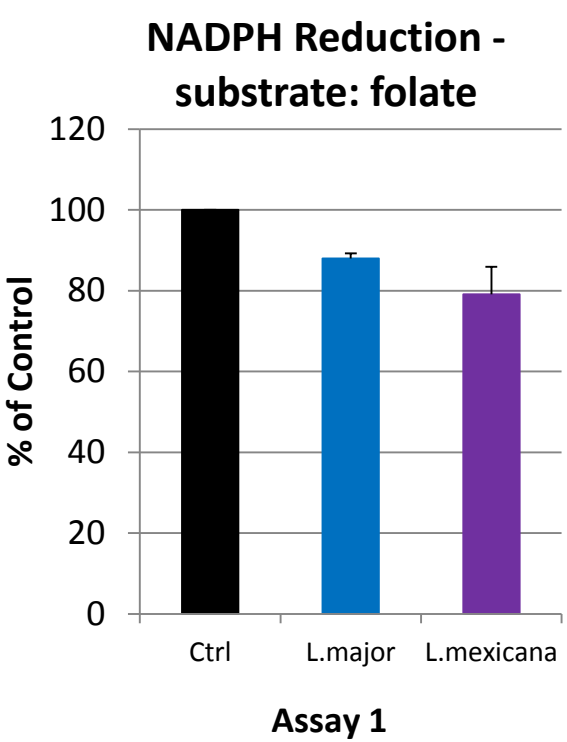
L.mexicana amastigotes		
	%	±
Ctrl	100	0
Rilu	25.0	8.7

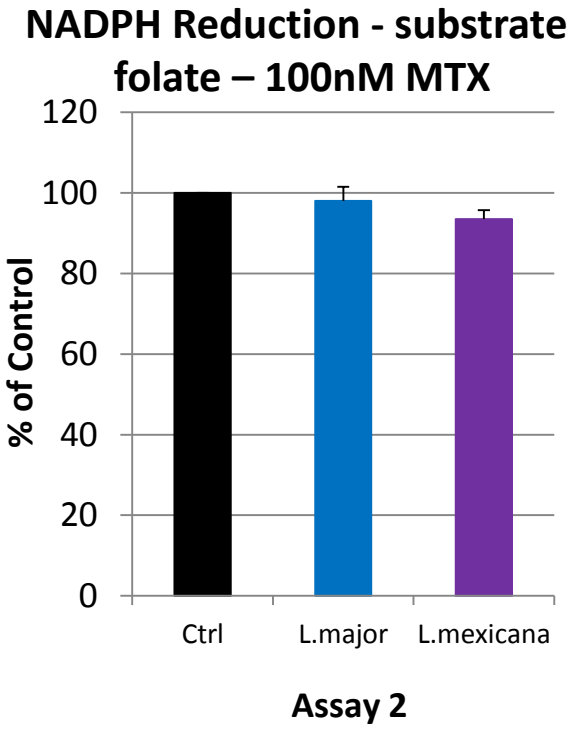
NADPH reduction by parasite lysate is inhibited by adding Rilu to the reaction mix

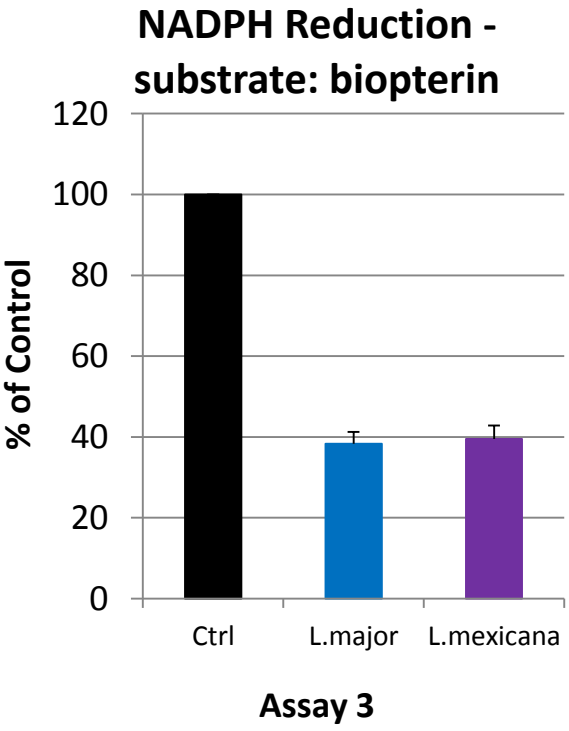


PTR1 levels are increased in amastigote-like parasites with respect to promastigotes.

NADPH reduction after incubation with Rilu (IC50conc) treated *Leishmania* Lysate

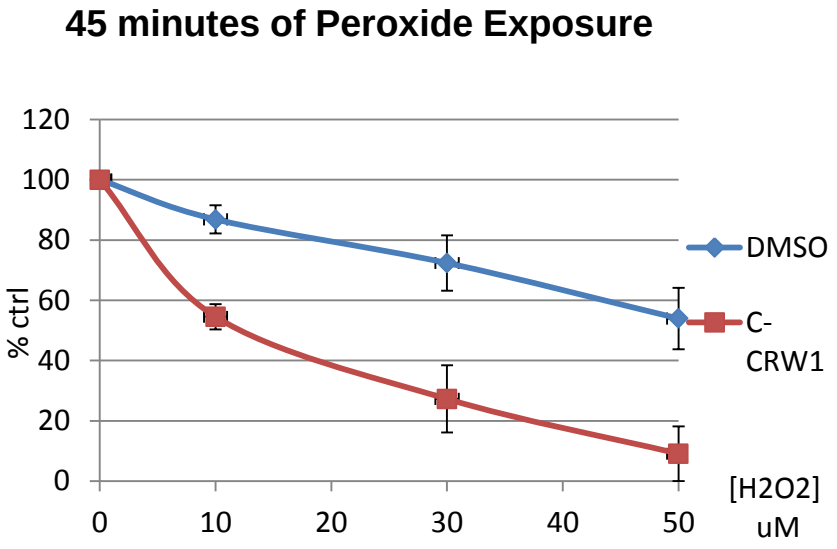




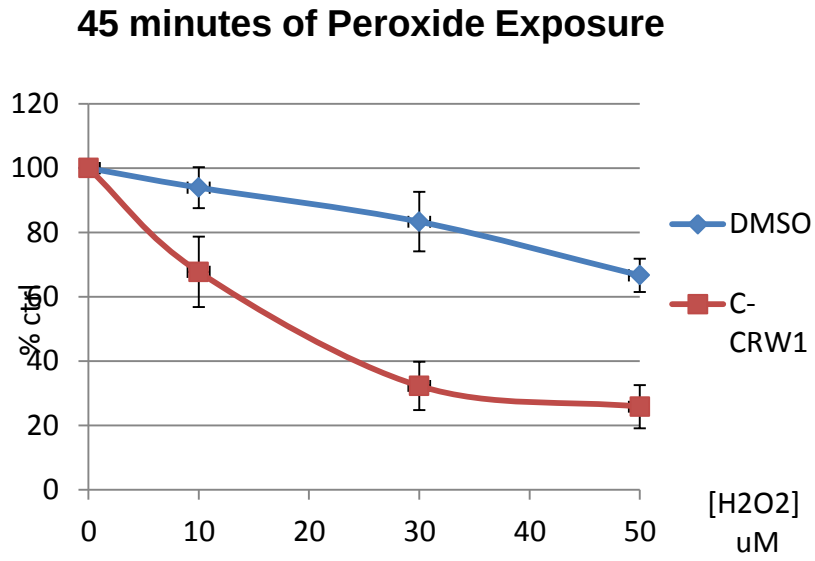


Rilu keeps its inhibitory activity after 48h cell treatment, most notably reduction appears when using Biopterin as substrate

1. Increase of oxidative stress sensitivity on *Leishmania.* 48 h pre-treatment with Rilu



Leishmania major



Leishmania mexicana

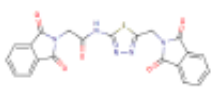
Parasites treated with Rilu are more sensitive to oxidative stress

2. Effect of Riluzole on PTR1 expression.
PTR1 expression is not affected by 48 hours treatment with Rilu.
PTR1 levels are increased in amastigote-like parasites with respect to promastigotes.

STRUCTURAL STUDIES

Attempts to obtain the X-ray crystal structures of the thiadiazole derivatives with LmPTR1, failed, then we obtained the structure with PTR1 from Tbrucei.

E.Nerini, W.Hunter, MP Costi, P.Michels in prep.



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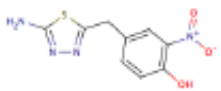
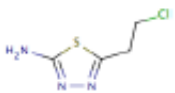


WHF4



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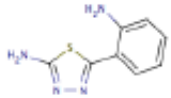

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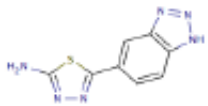

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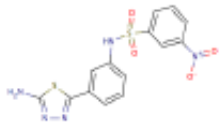
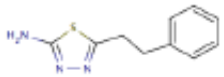


WH18dc



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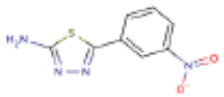

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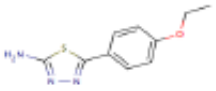

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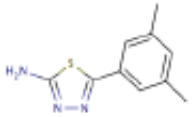

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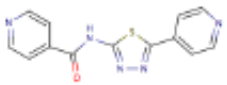
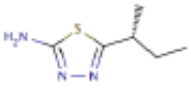
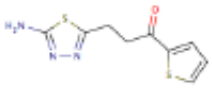


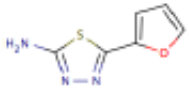
WHF28



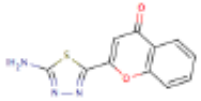
WHF29



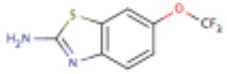
WHF30



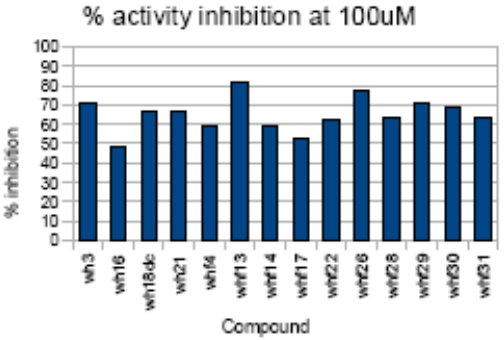
WHF31



WHF18*



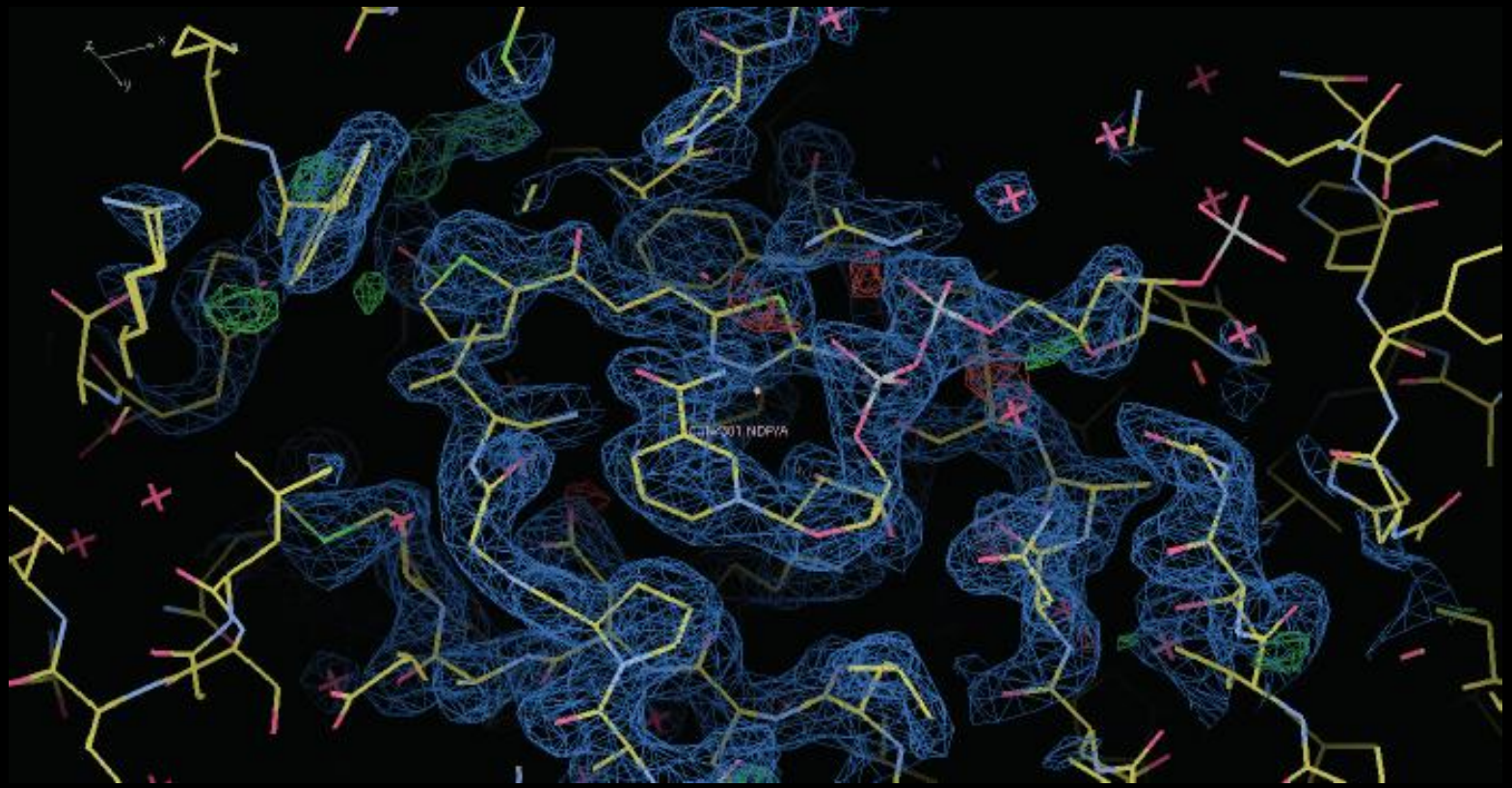
Riluzole*

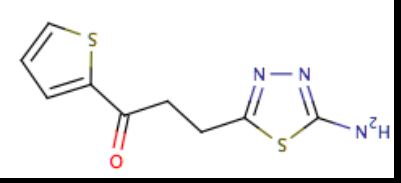


Compounds	IC_{50} in *T.brucei* (µg/ml)	IC_{50} in mam.cells (µg/ml)
WH6	44.14 ± 1.54	23.34 ± 3.93
WH16	5.78 ± 0.57	4.18 ± 0.43
WHF17	No effect	No effect
WHF18	44.84 ± 3.81	43.03 ± 5.15
WHF22a	46.07 ± 2.09	57.13 ± 3.79
WHF22b	36.79 ± 3.81	51.50 ± 5.52
WHF30	No effect	34.36 ± 3.72
Riluzole	26.74 ± 1.23	27.88 ± 3.17
PYR	0.82 ± 0.03	16.23 ± 2.47

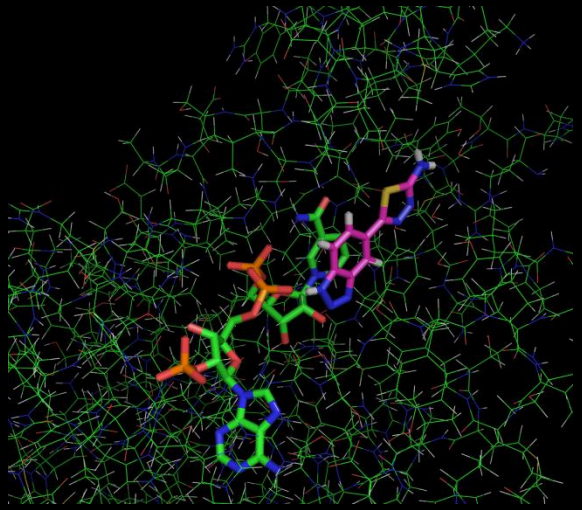
No effect: IC_{50} was not reached at the highest concentration tested (100 µg/ml).

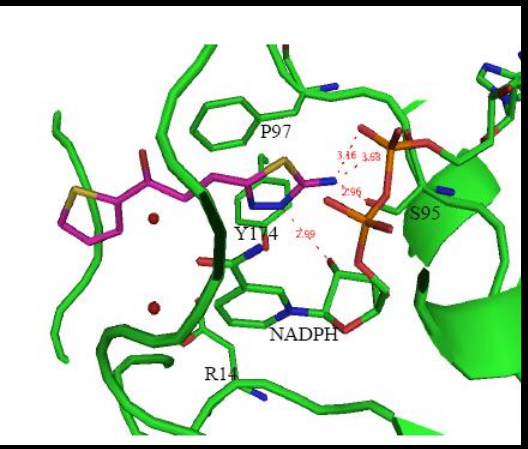
X-RAY CRYSTALLOGHRAPHY
WHF30-Trypanosoma brucei X-ray complex

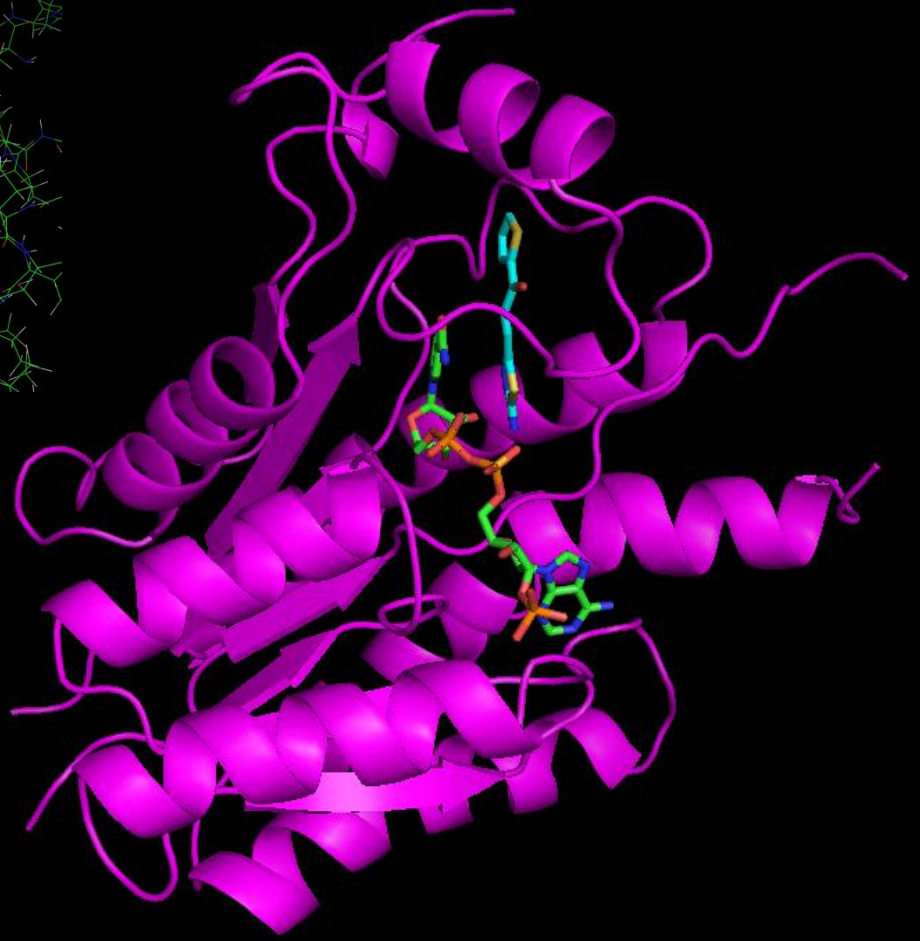


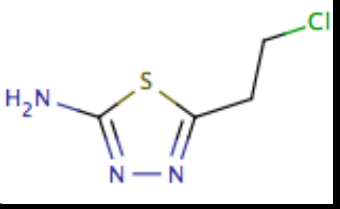


Active site of *Tb*PTR1. Compound WHF30, through its thiadiazole ring, is sandwiched between the nicotinamide ring of cofactor NADPH and Phe97.



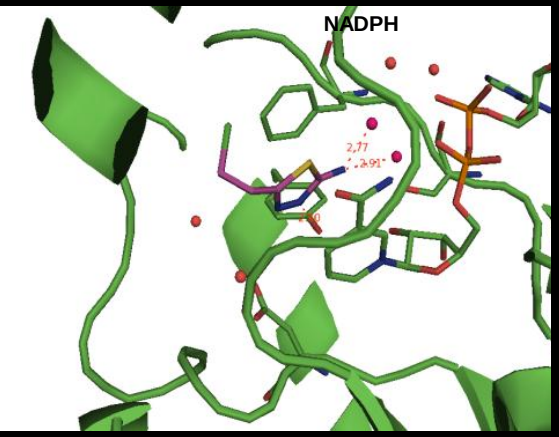


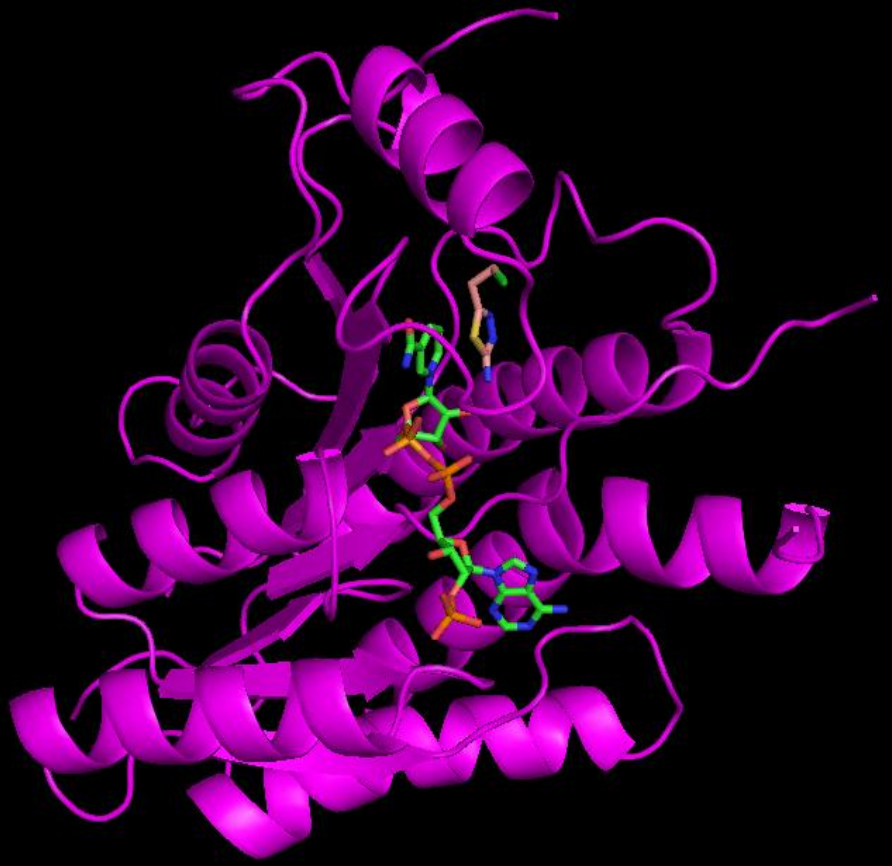




Compound WH16 in the active site of TbPTR1

and its interactions with the enzyme. The thiadiazole ring is responsible of the main interactions. Distance with the two water molecules and Tyr174 are in red dashed lines. WH16 is in pink sticks, the enzyme is green sticks, both are coloured by atom types.





Key interactions are conserved and confirm the proposed finding by J.Mol.Mod2011, Dube D. in Pharmacofore studies on PTR1 ligands.

Conclusions

We have identified two classes of compounds that inhibit specifically PTR1 in the micromolar range (no DHFR or human enzymes inhibition). Some of them are not toxic against MRC5 human cells.

We have confirmed the initial finding that specific inhibiton of PTR1 can be synergistic with DHFR inhibition.

Potentially Pyrimetamine can be used as a drug also in Leishmania, in combination with PTR1 inhibitors.

Same compounds inhibit Lm parasites in sinergy with PYR, while no synergy is observed in T.brucei inhibition.

Riluzole and its derivatives can be explored as drug candidates (LABEL EXTENSION?).

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Virtual Screening
Docking
Molecular Probing
Library Design and Selection

Virtual Screening
1st Library Design
Synthesis
Enzymatic Assays

Parasitology
Human Cell Model Toxicity

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