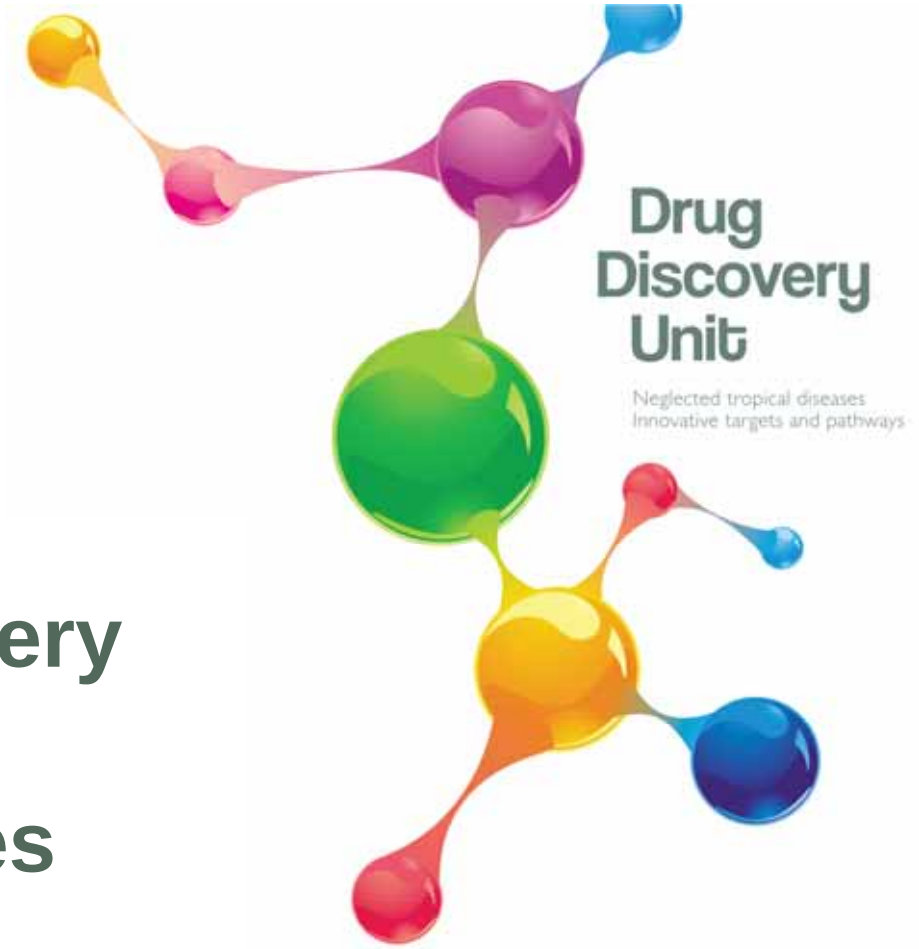
Kinase drug discovery for the neglected diseases



Drug discovery in Dundee

- >90 people
  - >600 years experience in Industry
  - >300 years academic experience
  - Involved in delivering 59 clinical candidates
  - 8 marketed drugs

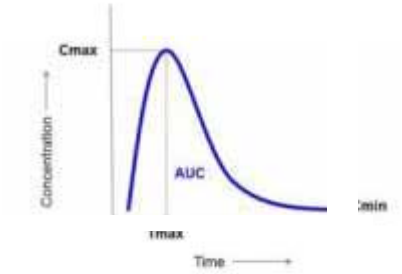
Drug Discovery Capabilities

Target Selection

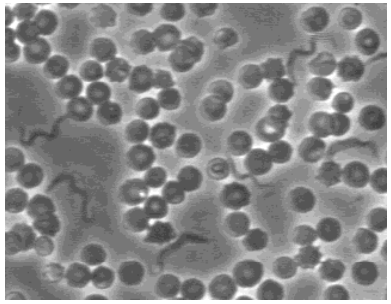
Validation
Druggability
Assay Feasibility
Toxicity
Resistance potential
Structural Information

384 MTS/HTS Robotics

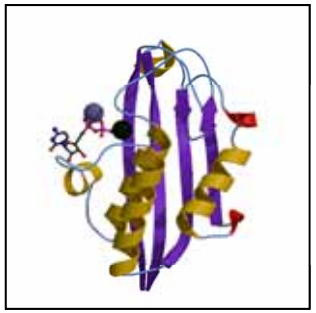
Compound Sets



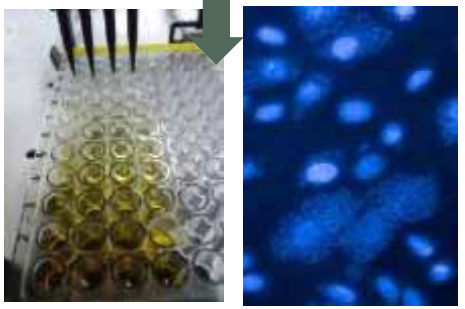
DMPK



in vitro models

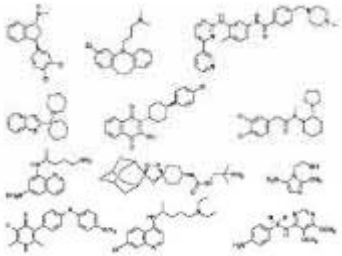


Structural Biology

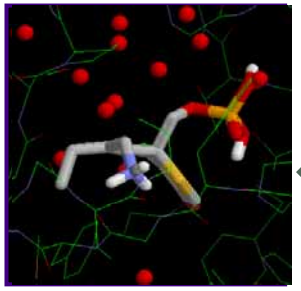


Target or cell screen

in vivo models



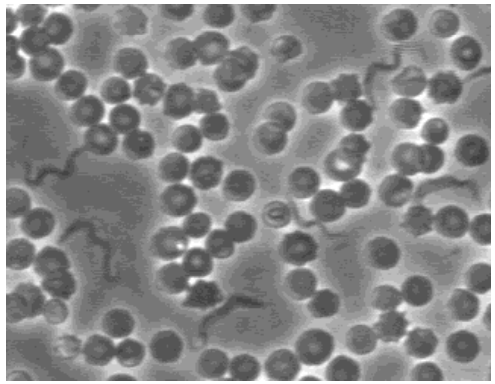
Medicinal & Computational Chemistry



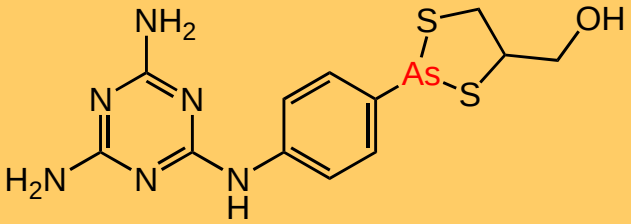
Data Management

Human African Trypanosomiasis

•Caused by protozoan parasite *Trypanosoma brucei* which lives in the blood stream
•Invades CNS



Fatal unless treated:
recorded deaths/ year ~50,000



melarsoprol

Protein Kinases

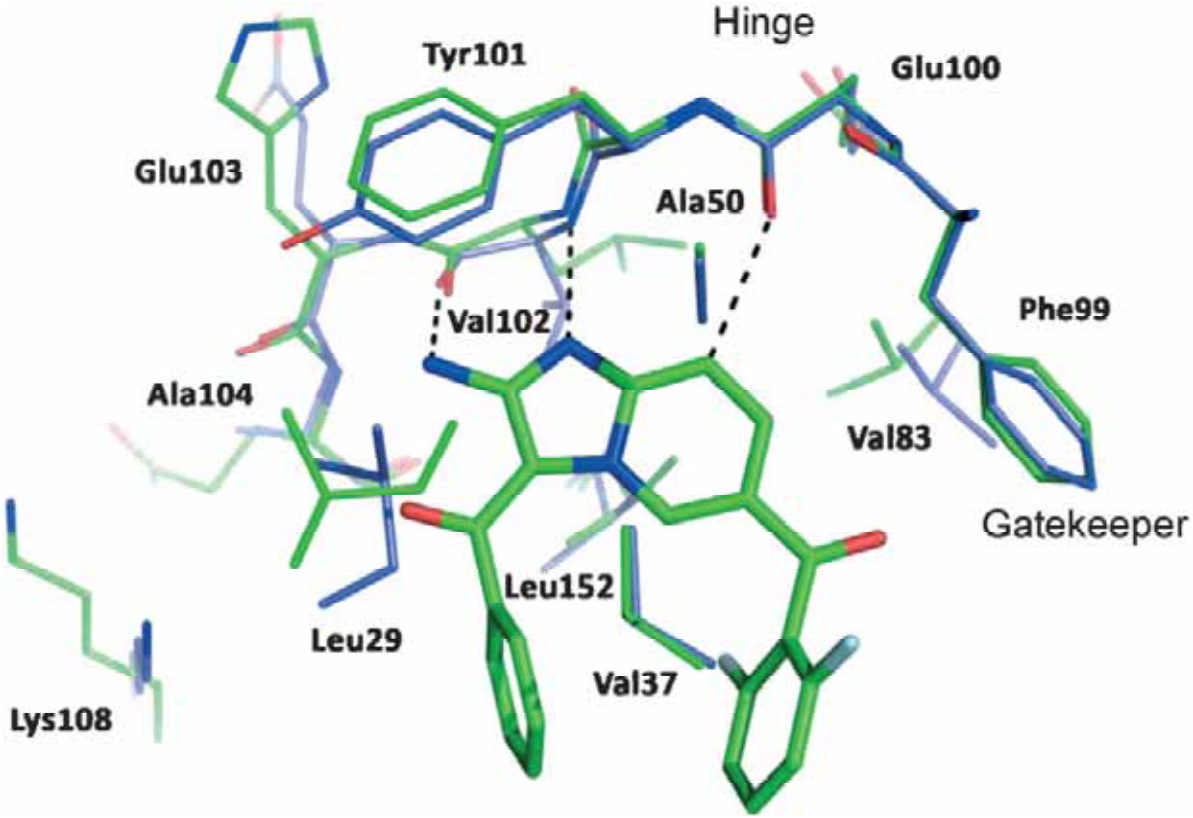
- Kinases known to be druggable

- ~190 orthologous protein kinases in each organism
  - Relative expansion of cell cycle and stress response lineage
  - Absence of dedicated tyrosine kinases
  - >15% unique to trypanosomatids

- Strategy
  - ~7000 kinase focused set, covering >150 scaffolds
  - Reverse genetic approach with genetically validated targets
    - CRK3, PK50, PK53, GSK-3β, Plk (CK1; Auk1; vsp34)
  - Phenotypic approach

Mottram, J. C. et. al. *BMC Genomics,* **2005,** 404-409; Mottram, J. C. et. Al. *BBA-Proteins Proteomics,* **2005**, 151-159; Doerig, C. et. Al. *BBA-Proteins Proteomics,* **2004**, 155-168

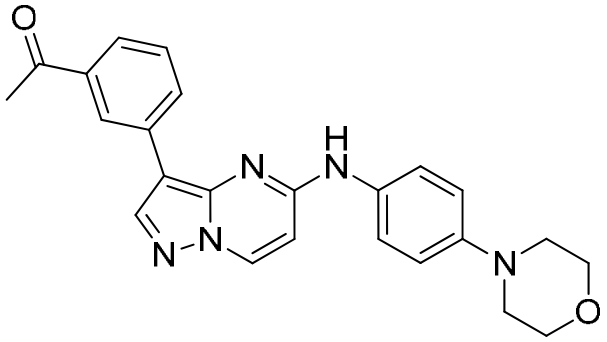
L. Mexicana CRK3 overlaid with human CDK2



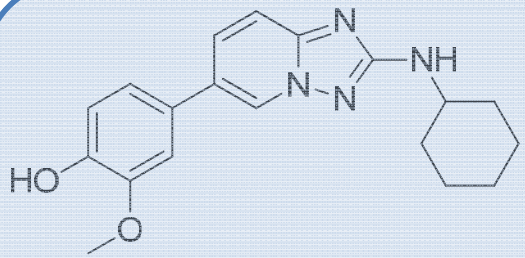
- Leishmania CRK3 homology model (blue carbon atoms)
- Crystal structure of HsCDK2 (green carbon atoms, PDB code 1PYE[19]) in complex with a ligand (bright-green carbon atoms)
- Phe82, Leu 83, His 84, and Gln 85 in CDK2, replaced with Tyr, Val, Glu, and Ala for Leishmania CRK3

Lm-CRK3

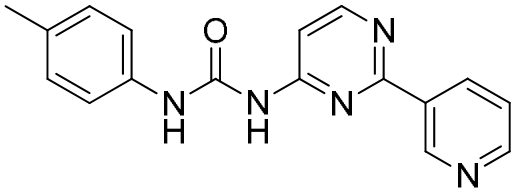
- Screened Kinase set
  - 43 hit compounds suitable for progression
  - 8 compound series
  - 3 progressed



Series 3
Leishmania CRK3 IC50 0.26 μM



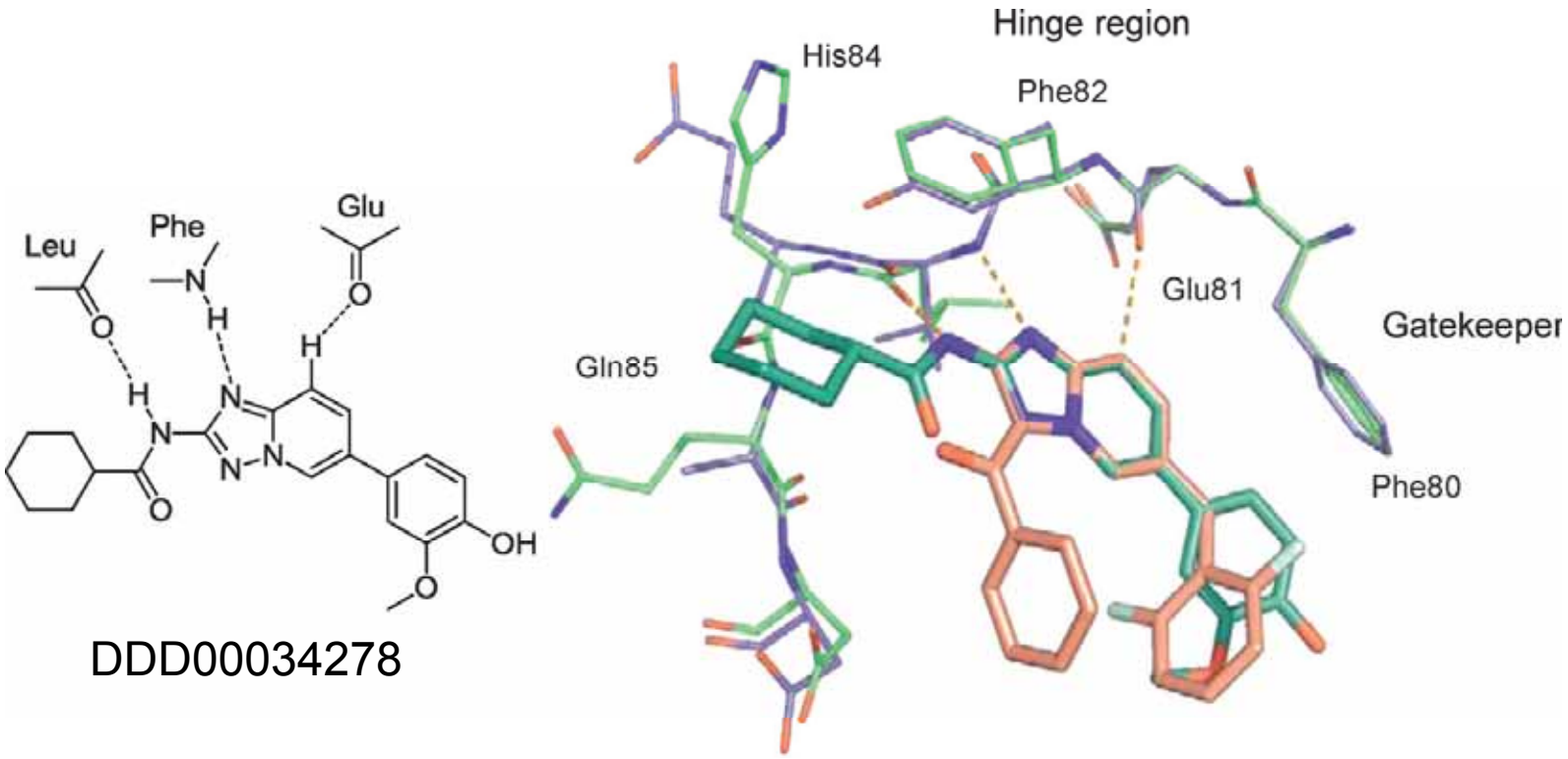
DDD00034278
Series 7
Leishmania CRK3 IC50 0.24 μM



Series 8
Leishmania CRK3 IC50 1.5 μM

Wyatt P. G. et. al., CMC, 2011, 6, 2214 – 2224

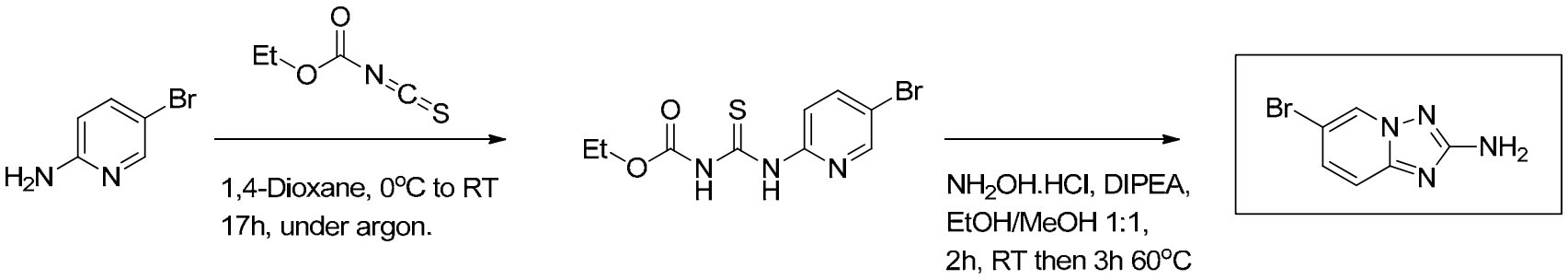
Modelling

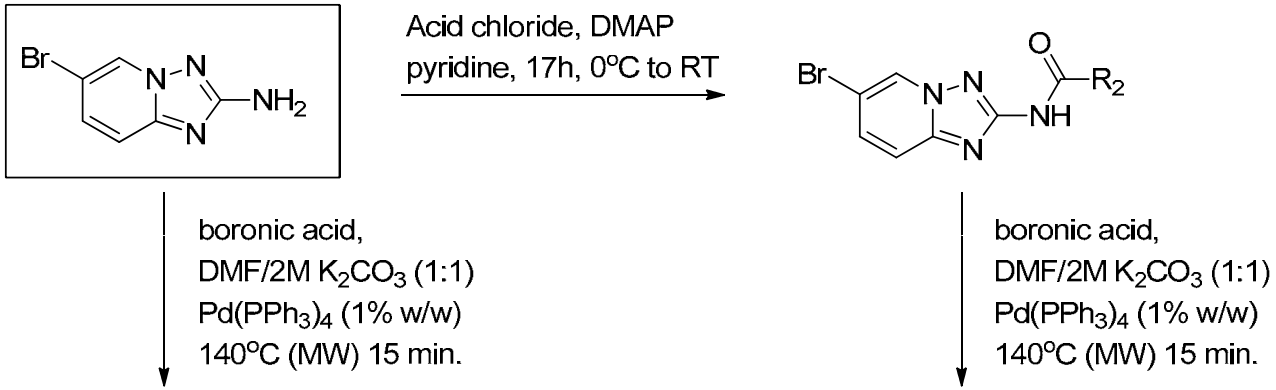


- Overlap of the 5,6 bicyclic core of the known aminoimidazo[1,2-a]pyridine HsCDK2 inhibitor (pink) with hit 1 (cyan). Dashed lines represent the proposed hydrogen bond interaction with the hinge region.

Compound	LmCRK3 pXC_{50}	HsCDK2 pXC_{50}	Calc. LogD	TPSA	MW
34278	6.6	6.4	3.4	94	366

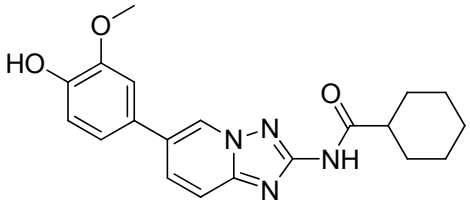
Synthesis


Et
O
N
C
S
Br
H2N
N
1,4-Dioxane, 0°C to RT
17h, under argon.
Et
O
N
H
S
N
H
Br
N
NH2
NH2OH.HCl, DIPEA,
EtOH/MeOH 1:1,
2h, RT then 3h 60°C


Br
N
NH2
Acid chloride, DMAP
pyridine, 17h, 0°C to RT
O
R2
NH
boronic acid,
DMF/2M K2CO3 (1:1)
Pd(PPh3)4 (1% w/w)
140°C (MW) 15 min.
R1
Acid chloride, DMAP
pyridine, 17h, 0°C to RT

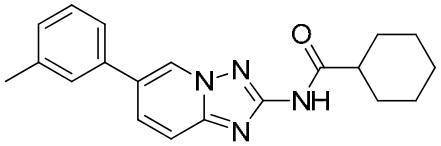
CRK3 - SAR

Un-selective hit



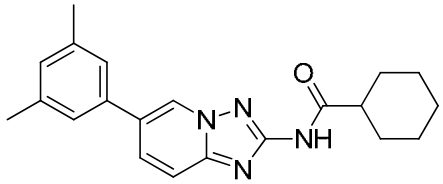
DDD00034278
pXC_{50} (LmCRK3) 6.6
pXC_{50} (HsCDK2) 6.4

3-Methyl phenyl

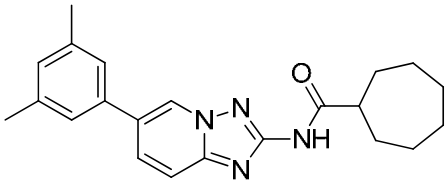


DDD00067048
pXC_{50} (LmCRK3) 7.1
pXC_{50} (HsCDK2) <4.0

3,5-Dimethyl phenyl

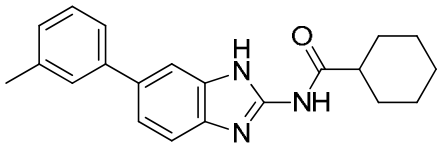


DDD00071584
pXC_{50} (LmCRK3) 7.7
pXC_{50} (HsCDK2) <4.0



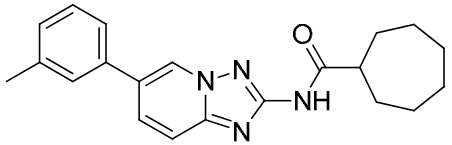
DDD00073284
pXC_{50} (LmCRK3) >8.3
pXC_{50} (HsCDK2) <4.0

Low nM
>10000 fold selective



DDD00073244
pXC_{50} (LmCRK3) 7.1
pXC_{50} (HsCDK2) 5.1

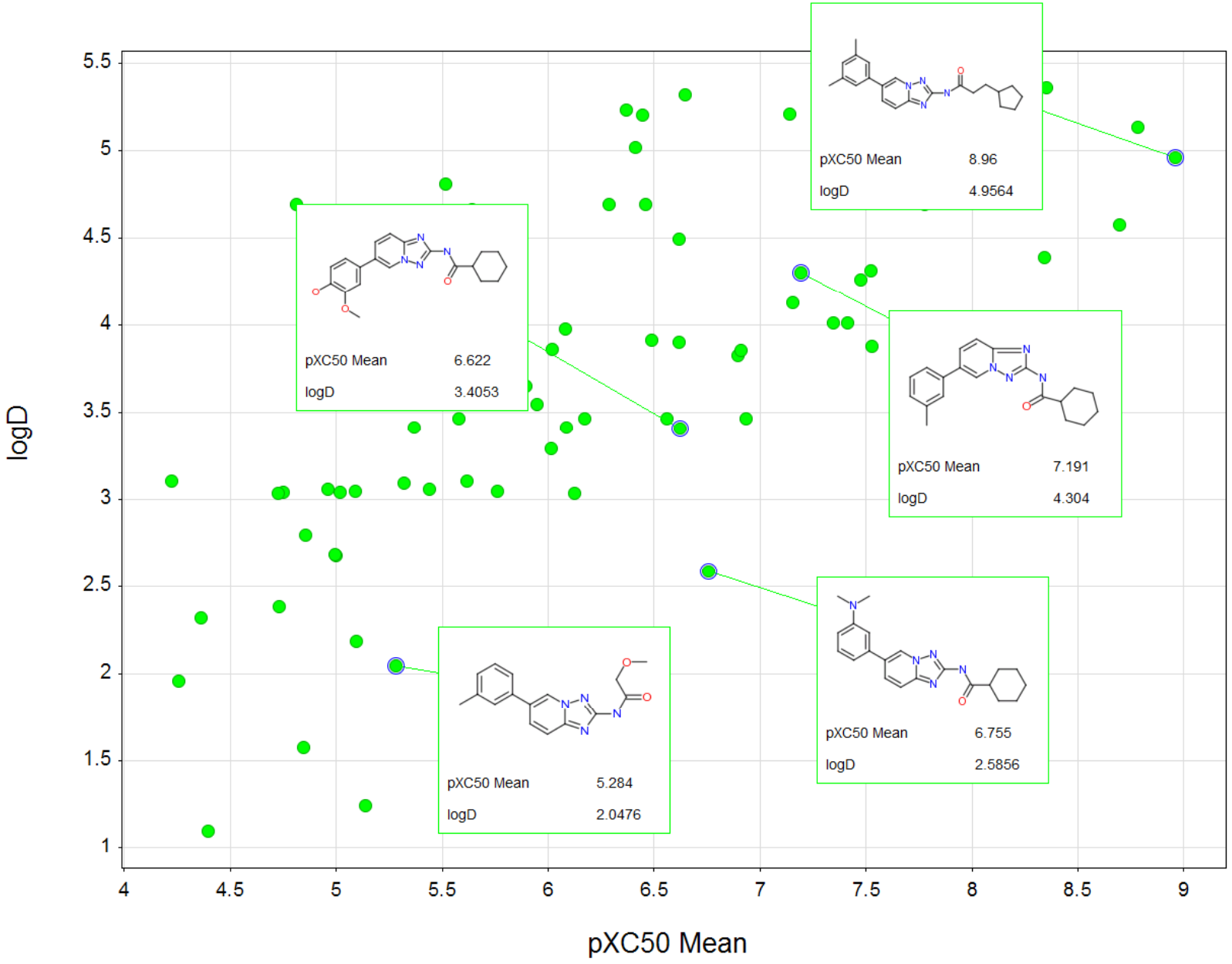
Heterocyclic core



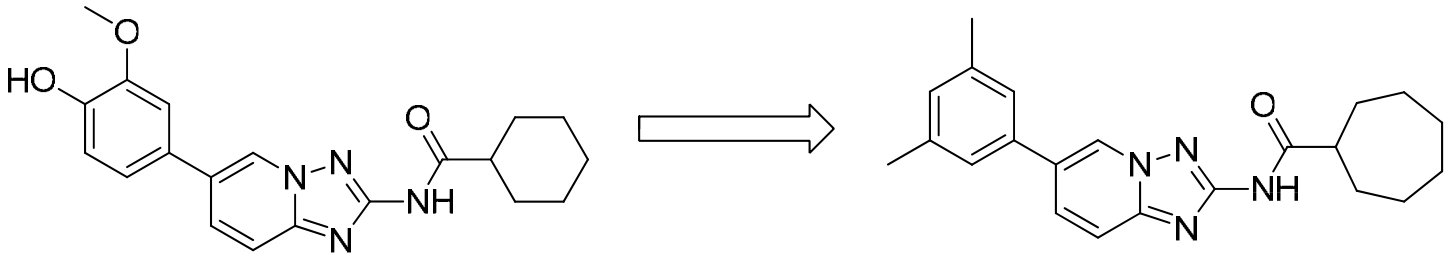
DDD00072725
pXC_{50} (LmCRK3) 8.1
pXC_{50} (HsCDK2) <4.0

Increase ring size

Plot logP vs activity series 7A



Lm-CRK3



DDD00034278

Unselective hit

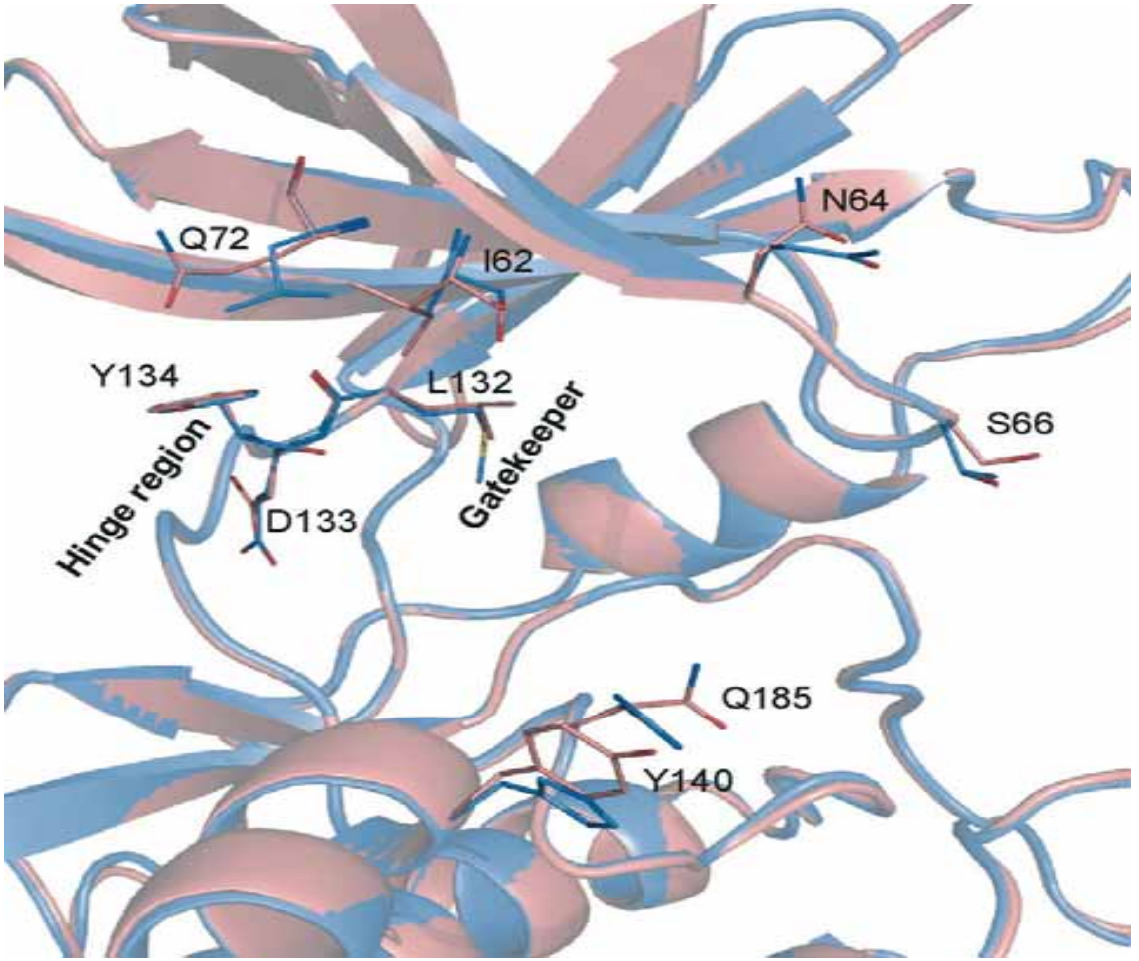
DDD00073284

Selective, low nanomolar inhibitors

DDD00073284						
MW	LogP	TPSA	LmCRK3 (pXC_{50})	HsCDK2 (pXC_{50})	L. donovani (pXC_{50})	T. brucei (pXC_{50})
362	5.4	64	>8.5	<4.0	<4.3	<4.3

Wyatt P. G. et. al., CMC, 2011, 6, 2214 – 2224

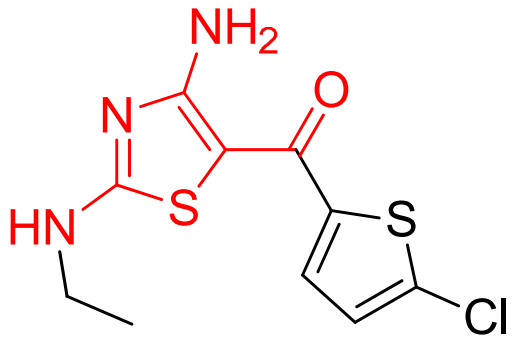
TbGSK3 Homology model



- Pink: HsGSK3b crystal structure (PDB code 1R0E)
- Homology model of TbGSK3 in blue.
- Binding pocket residues of TbGSK3 that differ from those of HsGSK3b are represented as sticks.

*Tb*GSK-3: Series 1

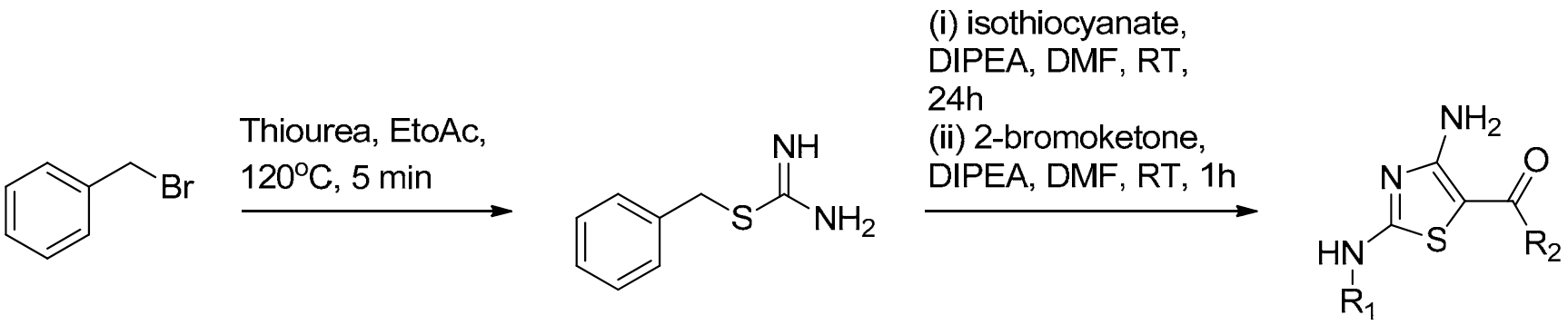
- TbGSK3β: active protein and HTS assay from UoW
- 13% hit rate
- 8 hit series



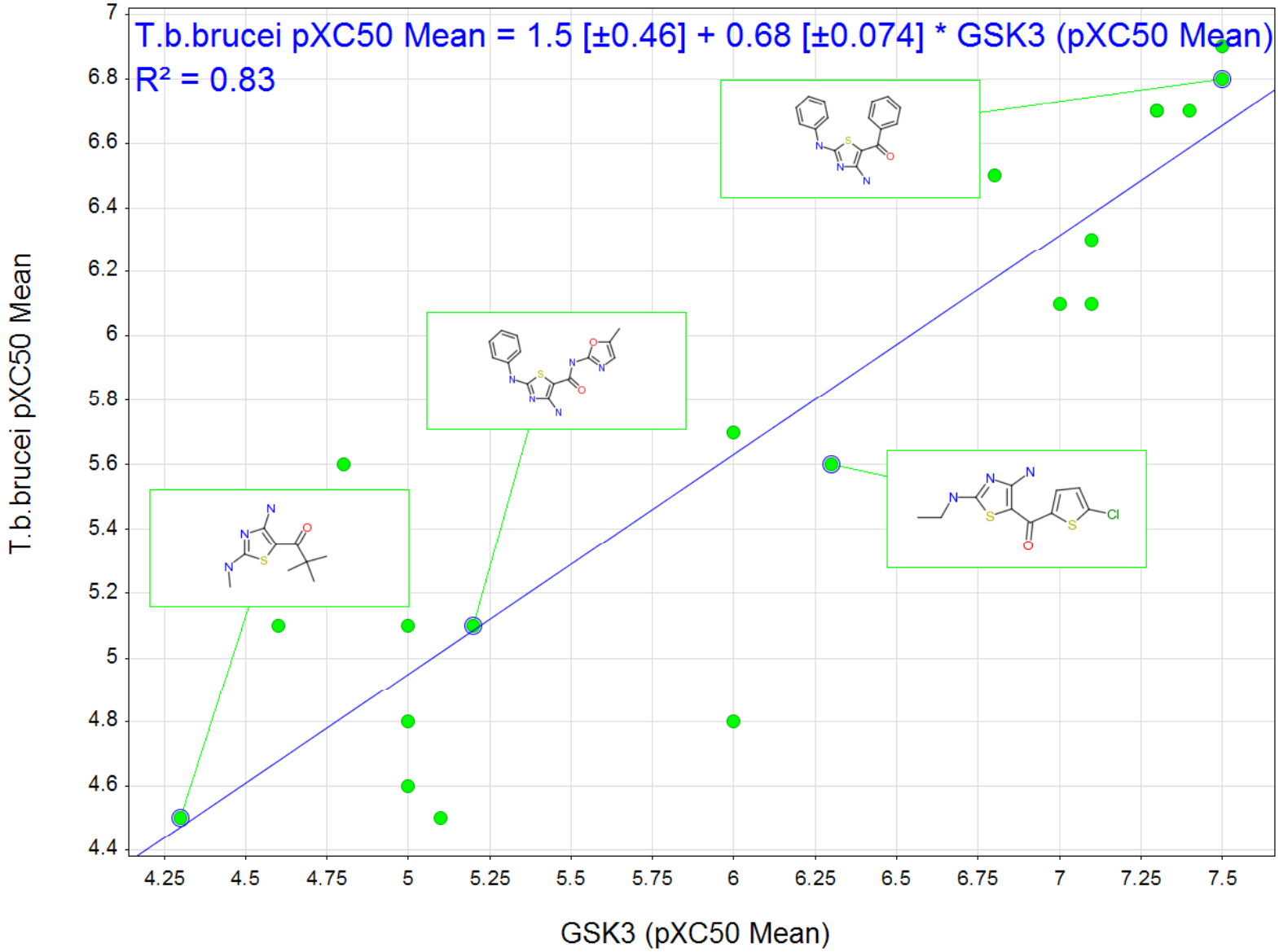
Series 1
DDD00065658
2,4-diaminothiazol-5-carbaldehyde

TbGSK3 IC_{50} 0.4 μM
Ligand efficiency 0.52

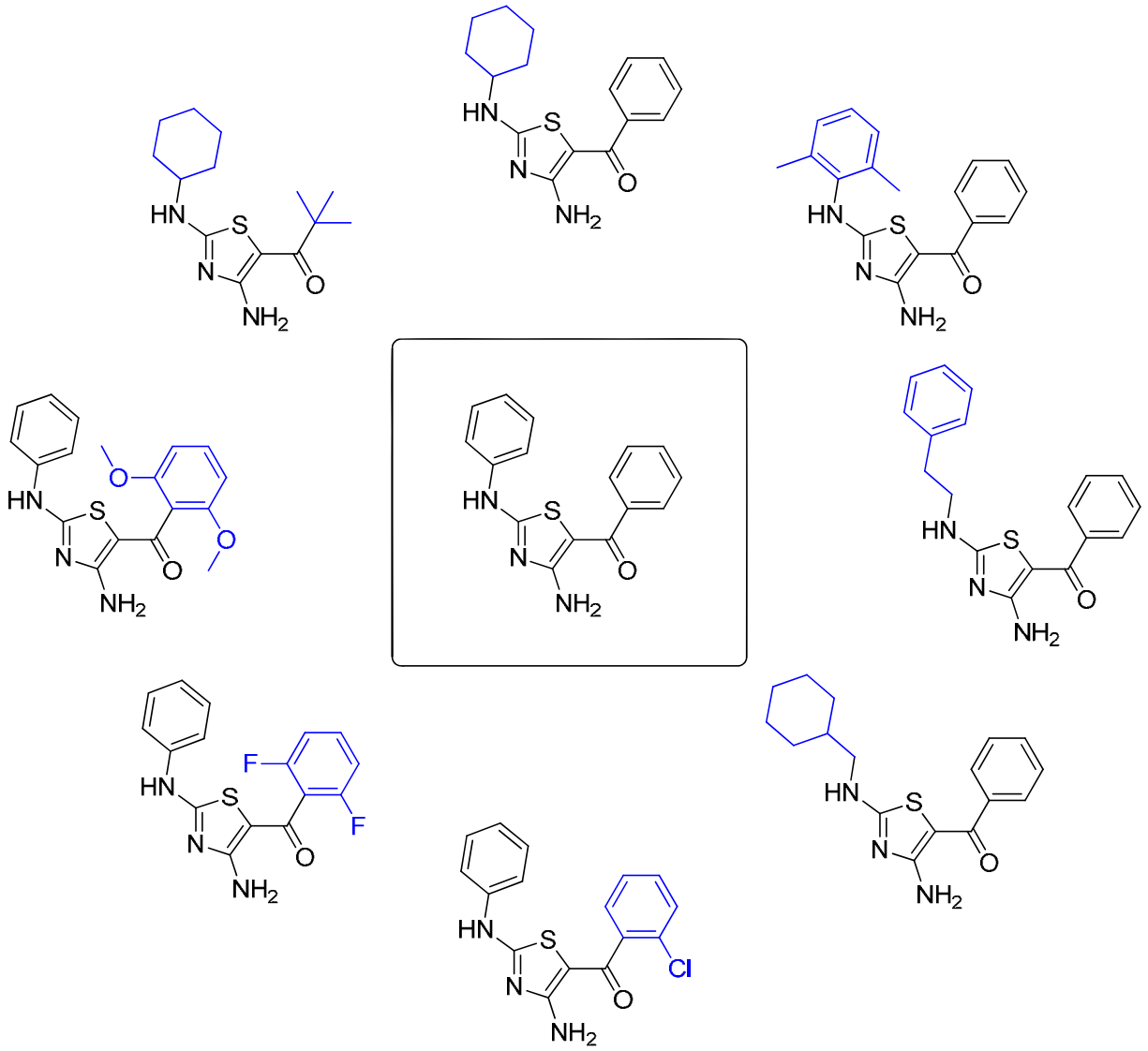
A convenient 2 pot synthesis



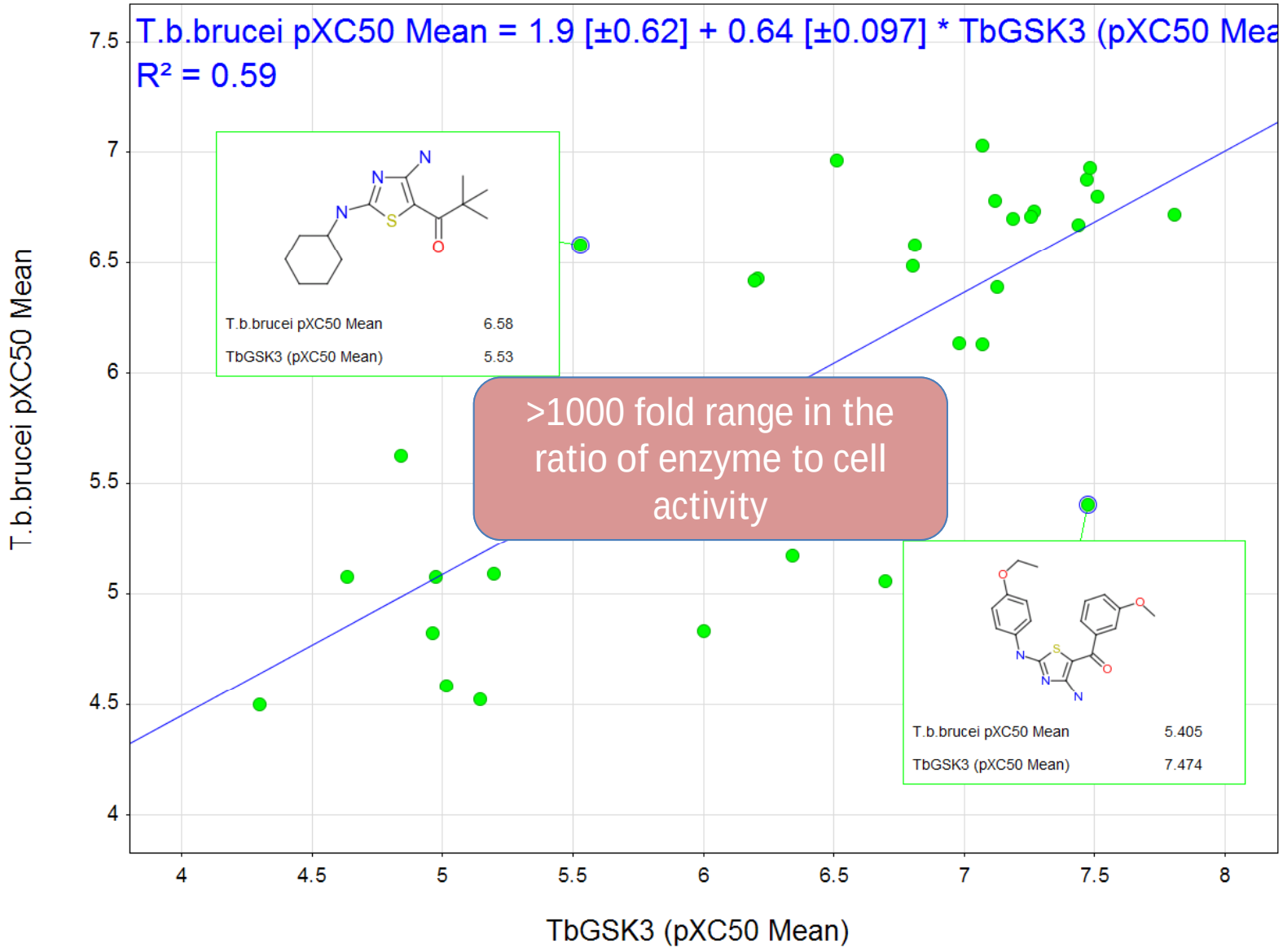
GSK3 Early SAR



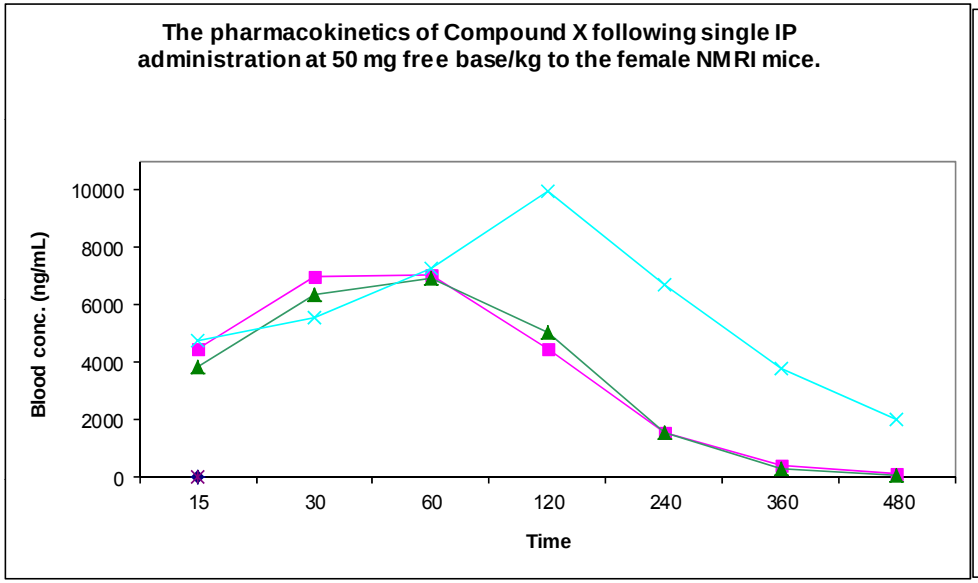
Exploring steric tolerance

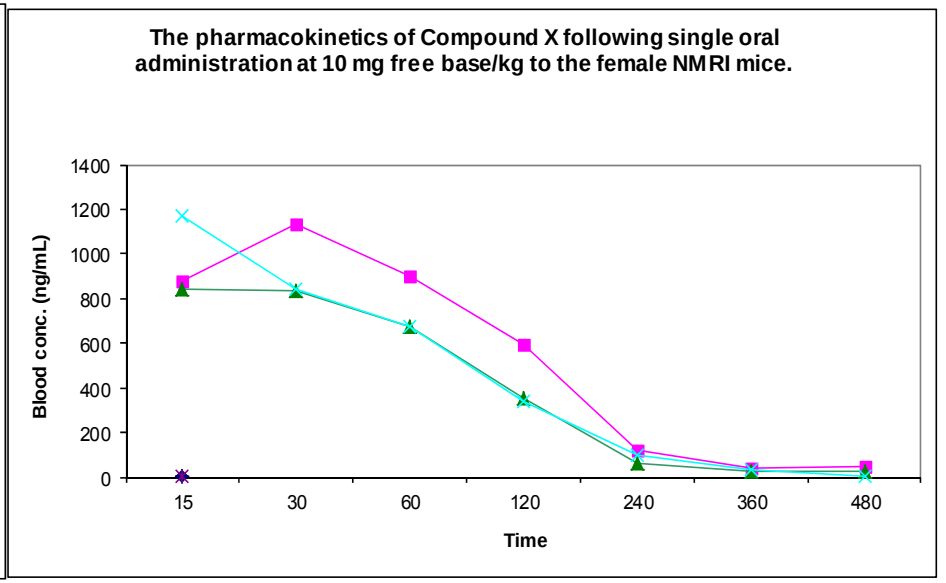


Breakdown in correlation



Compound X Mouse Pharmacokinetics



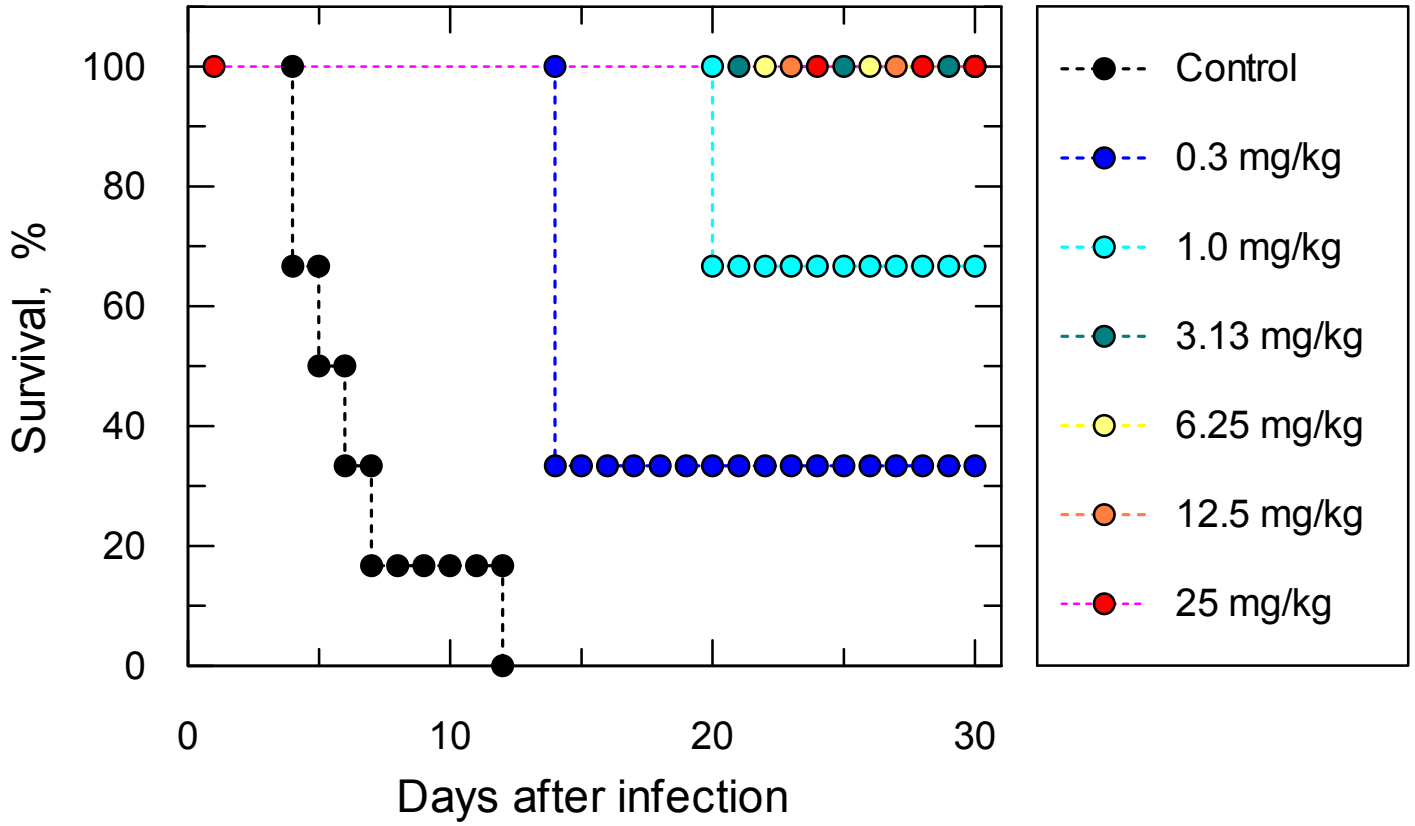


T.Brucei EC_{50} 0.001 µM
MRC-5 EC_{50} 1.4 µM
Cl_{int} (mouse) = 6.3 mL/min/g
Mouse plasma FU = 11 %

EC_{50} = 0.5 ng/mL
EC_{99} = ~1 ng/mL (Hill slope 5.8)

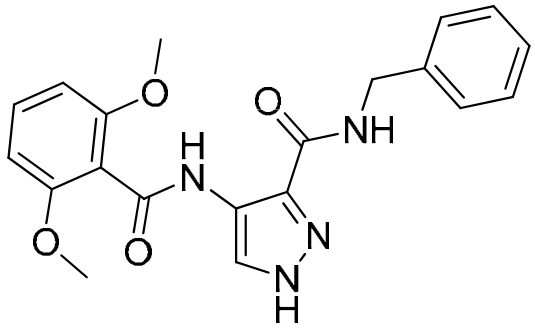
Based on IP and oral PK profiles, considering need to maintain free EC_{99} levels, Compound X should be active at 1mg/kg IP bid and 10mg/kg bid PO

Survival of mice after T. b. brucei (S427) infection and twice daily i.p. treatment with Compound X

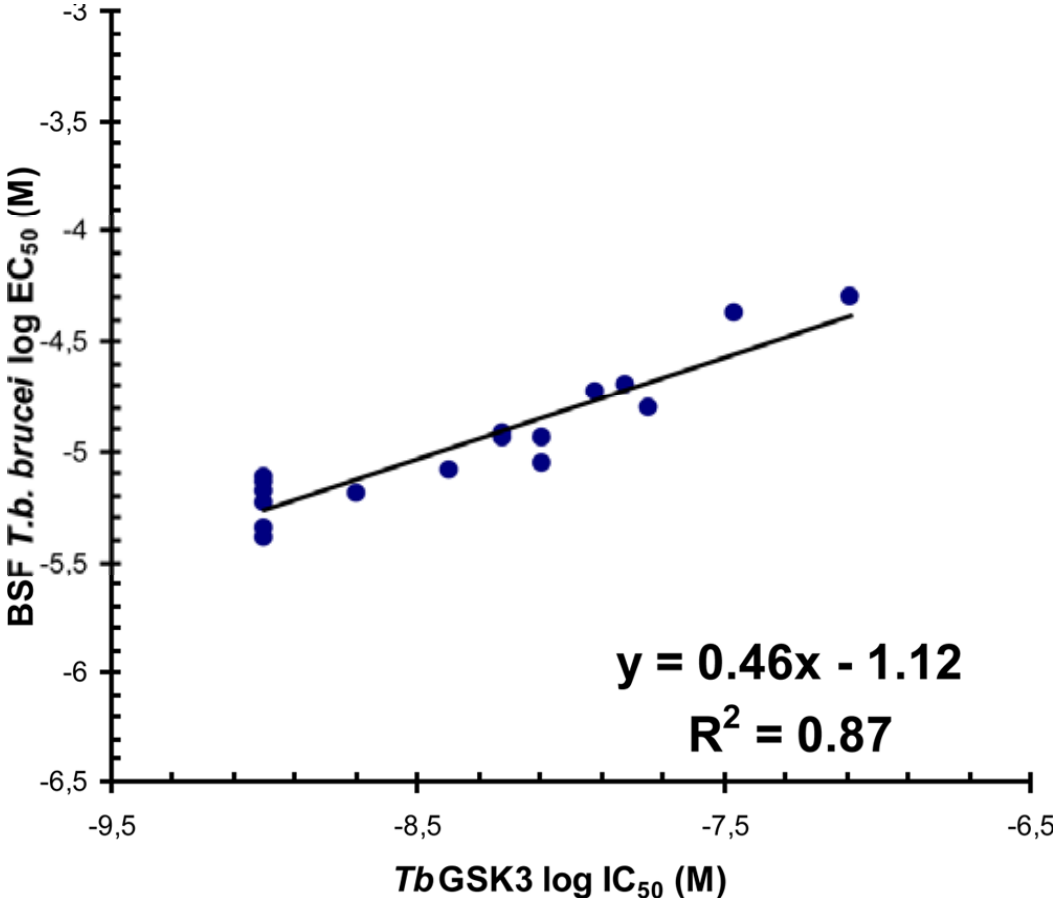


MED: 3 mg/kg bid IP for 4 days (Oral: 10 mg/kg)
MTD: 50 mg/kg bid for 4 days

TbGSK3 selective series



TbGSK3 (IC_{50}) = 1 nM
HsGSK3 (IC_{50}) = 330 nM
T.b.brucei EC_{50} = 6 μM
MRC5 EC_{50} >50 μM



J. Med. Chem. 2014, 57, 7536−754. Robert Urich et. al.

“Merck Serono and MMV Sign Agreement to Develop Potential Antimalarial Therapy”

- Merck Serono, the biopharmaceutical business of Merck, and MMV announced today that an agreement has been signed for Merck Serono to obtain the rights to the investigational antimalarial compound DDD107498

- DDD107498 originated from … the University of Dundee Drug Discovery Unit, led by Prof. Ian Gilbert and Dr. Kevin Read.

- http://www.mmv.org/newsroom/press-releases/merck-serono-and-mmv-sign-agreement-develop-potential-antimalarial-therapy

Conclusions

- Possible to develop potent & selective inhibitors of parasite protein kinases.
- Potent inhibitors of genetically validated protein kinases do not always give potent anti-trypanocidal compounds.
- Phenotypic screening is a productive strategy in neglected disease drug discovery

Acknowledgements

Management team Paul Wyatt, Julie Frearson, Ruth Brenk, Alan Fairlamb, Mike Ferguson, Ian Gilbert, Kevin Read

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Data Management
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Alastair Pate

Biology
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Irene Hallyburton
Rafaella Grimaldi
Leah Torrie
Ondrej Smid
Emma Shanks
David Blair
Stuart McElroy
Lorna Campbell
James Robert

Structural Biology
David Robinson

DMPK
Suzanne Norval
Laste Stojanovski
Robert Kime
Fred Simeons
Jennifer Riley

All of our Collaborators!