Supporting Information.

Structure-based Design of Pteridine Reductase Inhibitors Targeting African Sleeping Sickness and the Leishmaniases.

Lindsay B. Tulloch,[1] Viviane P. Martini,[1,2] Jorge Iulek,[1,2] Judith K. Huggan,[3] Jeong Hwan Lee,[3] Colin L. Gibson,[3] Terry K. Smith,[1§] Colin J. Suckling[3] and William N. Hunter[1,*]

[1]Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK.

[2] Universidade Estadual de Ponta Grossa, Departamento de Química, Av. Carlos Cavalcanti, 4748 Uvaranas, 84030-000, Ponta Grossa, Paraná, Brazil.

[3] WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow, G1 1XL, UK.

[§]Present address**:** BMS, University of St Andrews, North Haugh, St Andrews, Fife, KY16 9ST, UK.

Contents.

Three figures depicting the different scaffold based ligands in a *Tb*PTR1 active site. Graphs detailing the trypanocidal activity of pyrimethamine or compound **11** in combination with MTX are given together with three tables of crystallographic information.

Figure legends.

Figure S1. Scaffold I compounds in the active site of *Tb*PTR1. In Figure S1C compound 3 is shown in the MTX-like binding mode.

Figure S2. Scaffold II compounds in the active site of *Tb*PTR1.

Figure S3. Scaffold III compounds in the active site of *Tb*PTR1.

In all figures, atomic positions are colored N blue, O red, P orange, S yellow, C of PTR1 and NADPH grey, C of ligands pale yellow. An exception is made for the side chain of Phe97, which, for the purpose of clarity since it is directly over the ligand binding position, is shown in thin dark grey lines. In several of the structures Cys168 is oxidized to sulfenic acid, in a number of others it is modified by dithiothreitol (DTT). Hydrogen bonds are depicted as dashed lines, water molecules are shown as red spheres. Compounds are labeled according to the assignment in Tables 1, 2 and 3.

Figure S4. Trypanocidal activity of PTR1 and DHFR inhibitors. (A) Changes in pyrimethamine ED_{50} values in combination with varying concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5 μM) of MTX. (B) Changes in **11** ED_{50} values in combination with varying concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5 μM) of MTX. Values are means ± std. dev. (n = 4).

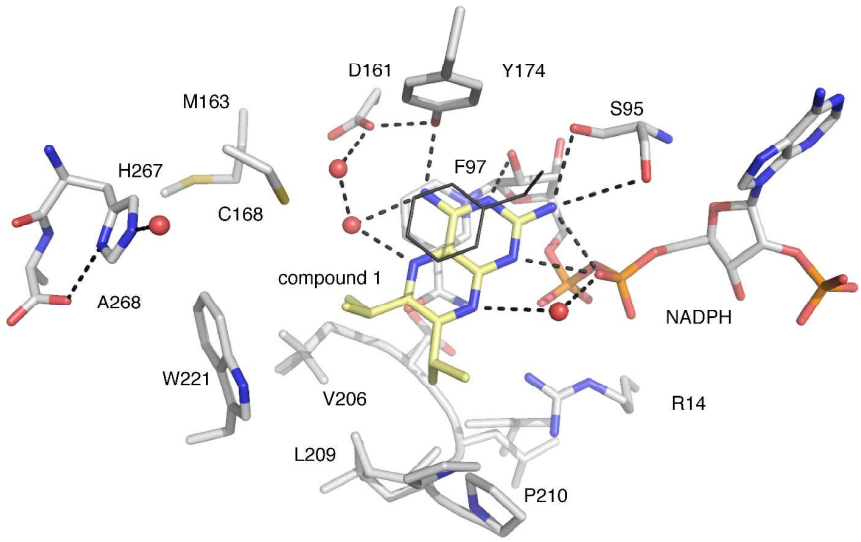

Figure S1A

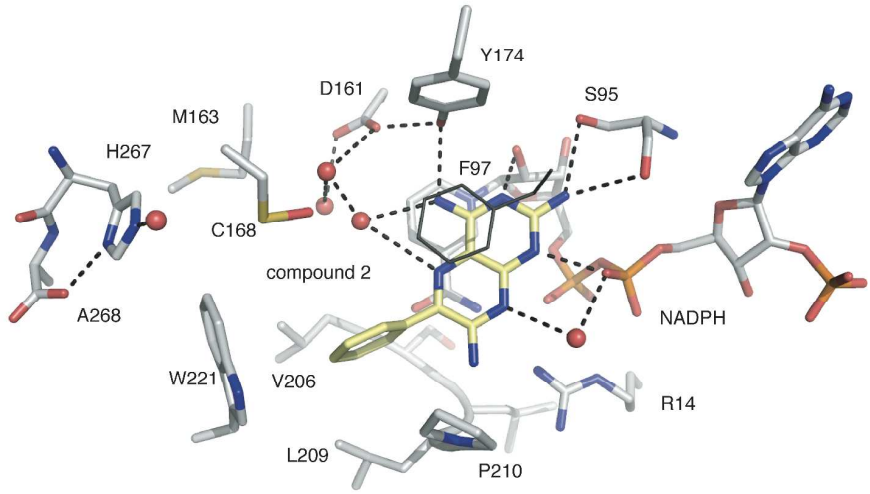

Figure S1B

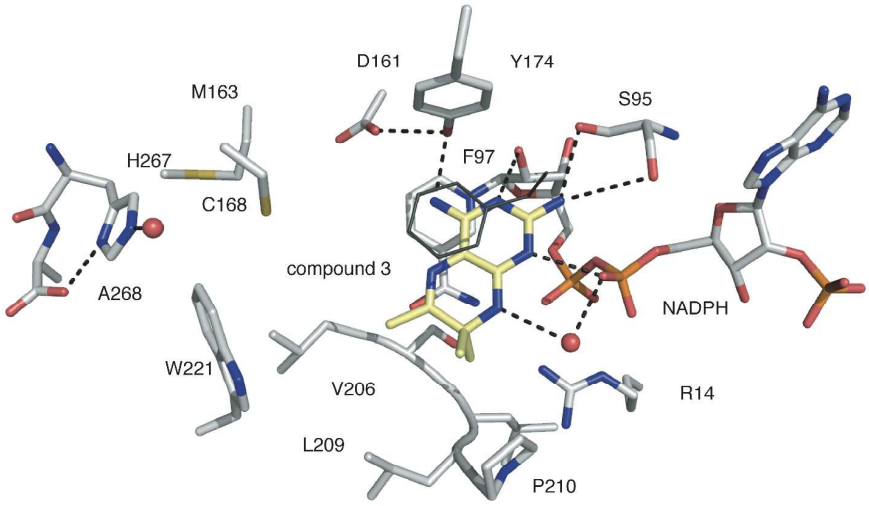

Figure S1C

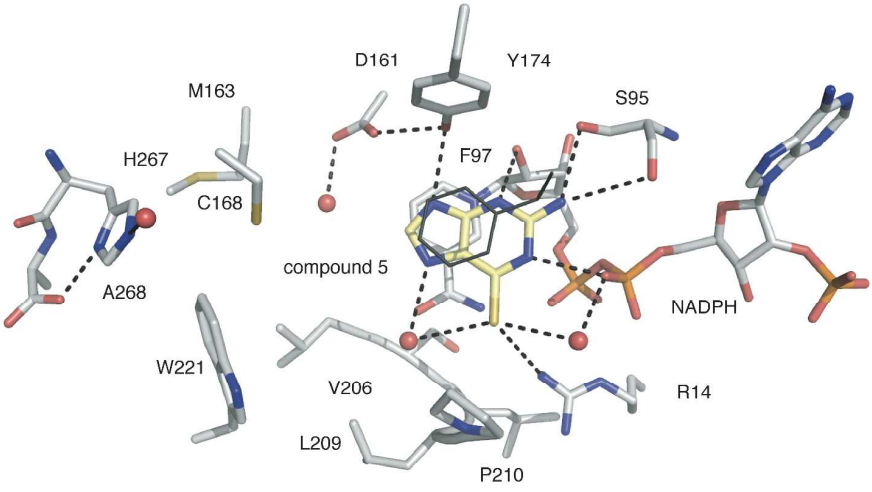

Figure S2A

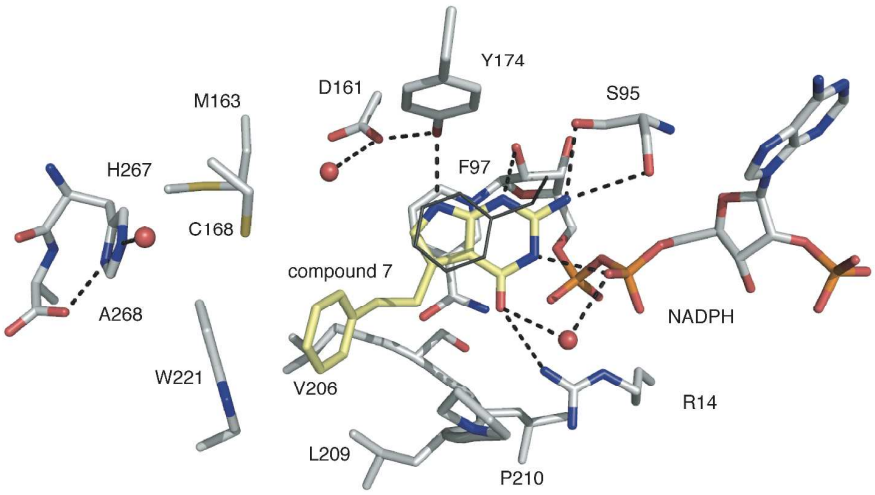

Figure S2B

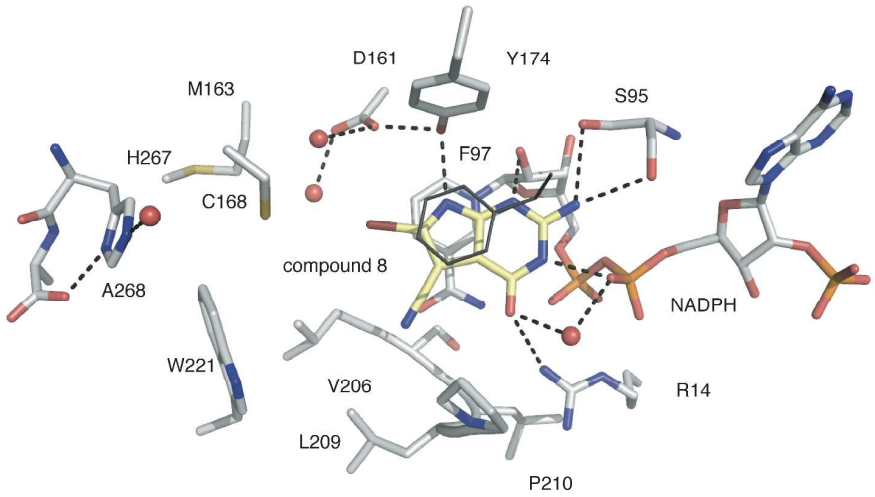

Figure S2C

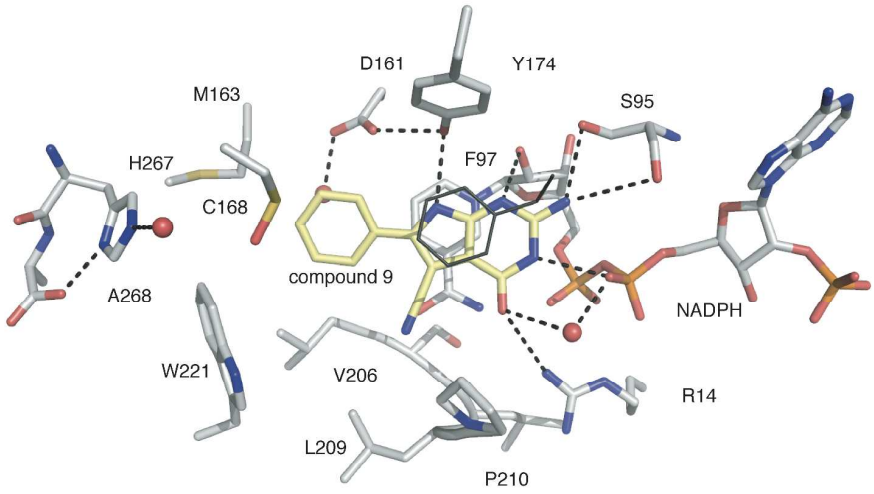

Figure S2D

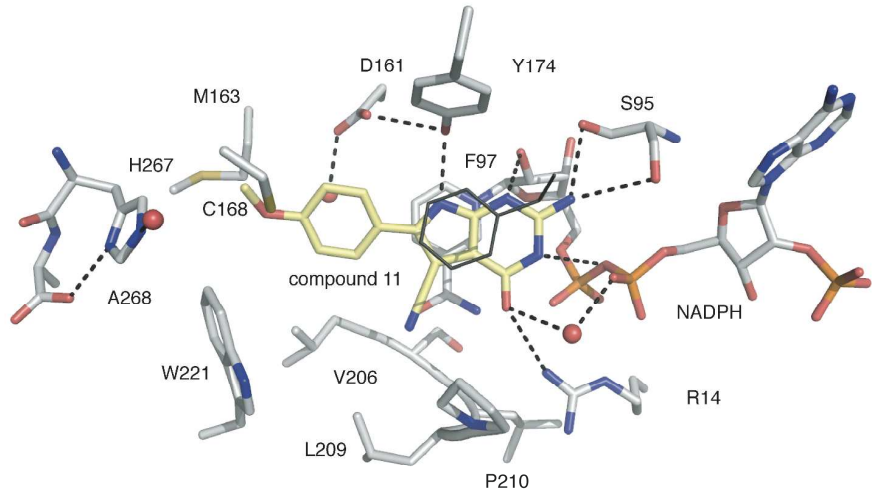

Figure S2E

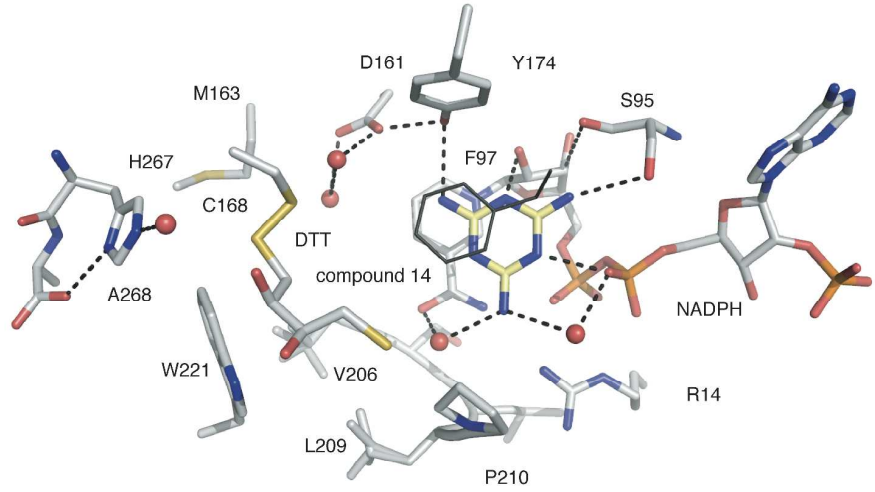

Figure S3A

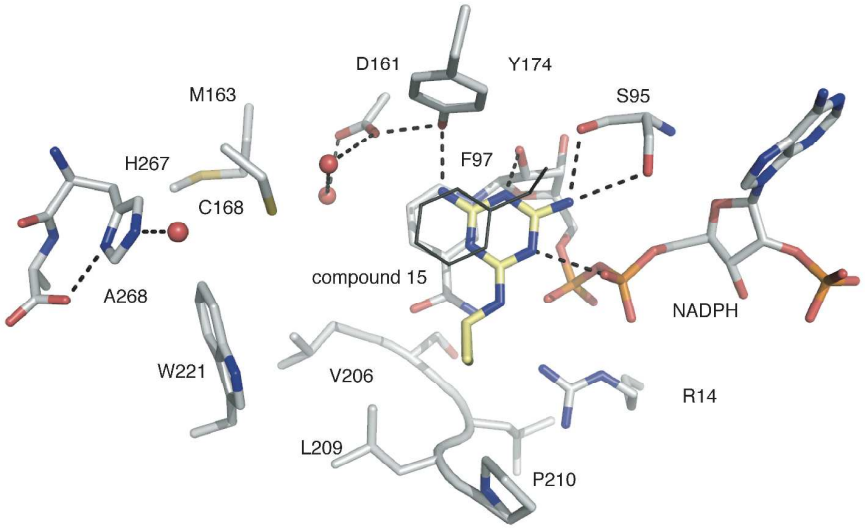

Figure S3B

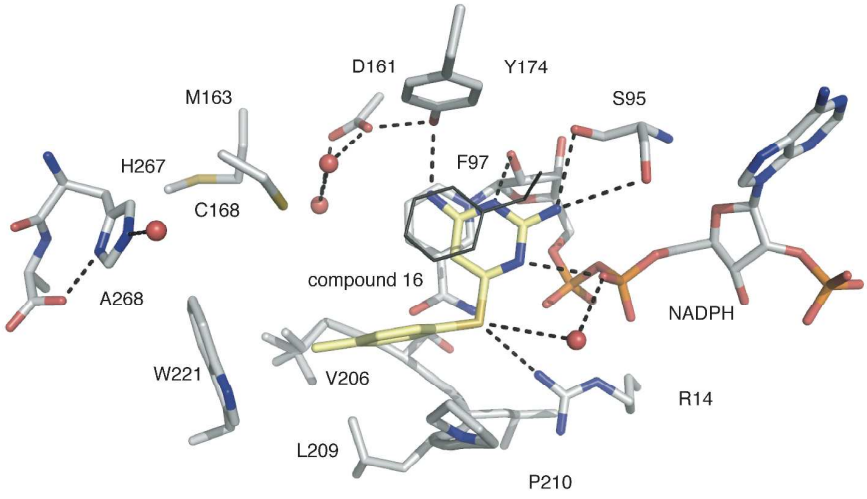

Figure S3C

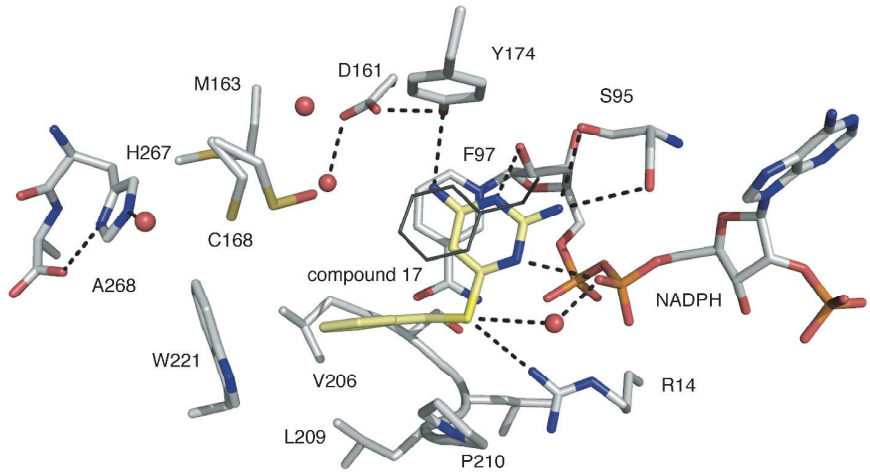

Figure S3D

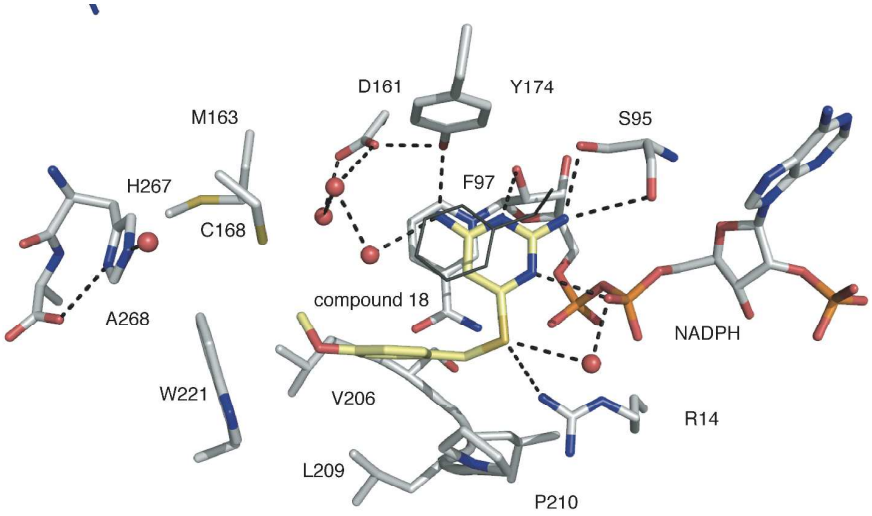

Figure S3E

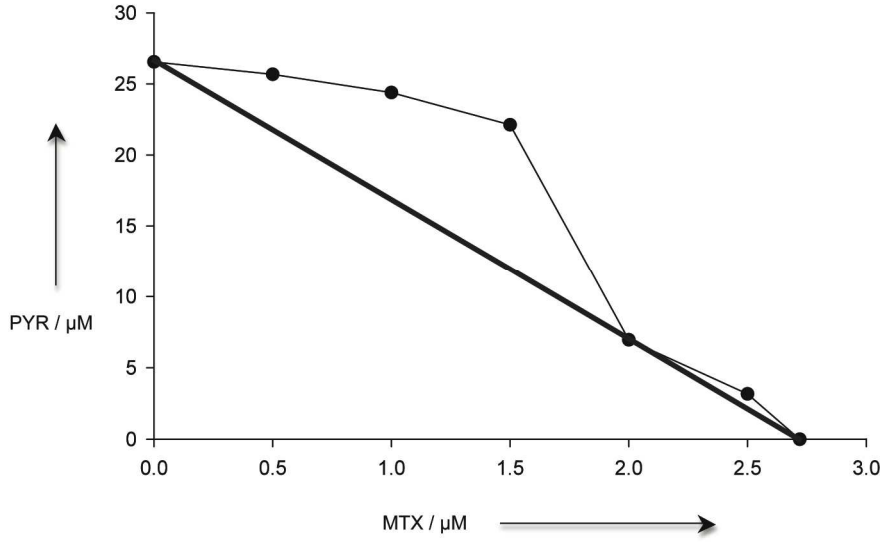

Figure S4A

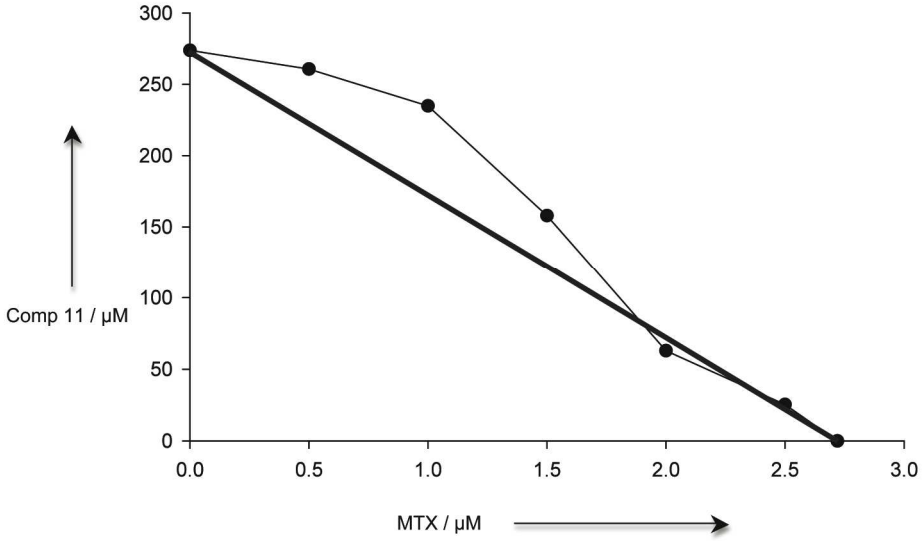

Figure S4B

Supplemental Table S1 – *Tb*PTR1-Folate and *Tb*PTR1-Scaffold I inhibitors

Ligand		**Folate**				**Compound 1**				**Compound 2**				**Compound 3**			
PDB Code		3BMC				3JQ6				3JQ7				3JQ8			
Crystal Statistics																	
Resolution range	(Å)	2.60 – 37.70				1.80 – 30.70				1.90 – 74.30				1.95 – 67.40			
Space Group		$P2_1$				$P2_1$				$P2_1$				$P2_1$			
Unit cell dimensions:																	
a, b, c	(Å)	74.44	86.60	81.59		74.14	89.47	84.34		74.54	90.03	82.36		74.10	89.15	84.19	
β	(°)	115.3				115.5				115.5				115.5			
Scaling Statistics																	
Data collection location		In-House				ESRF ID29				ESRF ID23-1				ESRF ID29			
No. Measurements		51,646				396,003				256,628				303,151			
Unique reflections		23,556				92,014				90,613				70,867			
Redundancy		2.2 (2.3) [a, b]				4.3 (4.3) [a]				2.8 (2.9) [a]				4.3 (4.8) [a]			
Completeness	(%)	81.1 (74.0) [a]				99.9 (100.0) [a]				99.7 (100.0) [a]				98.5 (97.6) [a]			
< I / σ(I) >		13.7 (3.0) [a]				13.5 (3.5) [a]				11.6 (2.6) [a]				10.4 (4.0) [a]			
R_{merge}	(%)	6.8 (37.7) [a]				8.0 (36.4) [a]				3.6 (25.0) [a]				6.6 (22.0) [a]			
Refinement Statistics																	
R_{work} (%) / No of reflections		18.9 / 22,315				15.0 / 87,338				15.2 / 86,018				24.0 / 66,848			
R_{free} (%) / No of reflections		22.4 / 1,241				19.0 / 4,676				19.1 / 4,595				28.7 / 4,019			
R.M.S.D bond lengths	(Å)	0.009				0.011				0.014				0.007			
R.M.S.D bond angles	(°)	1.460				1.305				1.501				1.101			
DPI [c]		0.42				0.12				0.11				0.24			
Ramachandran:																	
Core region	(%)	88.7				88.7				88.6				88.8			
Allowed region	(%)	10.9				10.9				10.9				10.8			
Generous region	(%)	0.5				0.5				0.6				0.3			
Model Statistics																	
Protein residues:																	
Total		990				981				975				979			
Overall	*B*-factor	30.7				16.0				27.6				14.6			
No. in subunits	A-D	248	247	247	248	246	244	245	246	247	244	237	247	246	246	240	247
B-factor	A-D	30.8	30.7	30.7	30.8	15.9	16.3	15.6	16.3	27.0	26.1	27.9	27.9	15.1	15.6	16.3	14.8
Additional groups:																	
Solvent	No/Av. *B*-factor	255 / 29.2				1023 / 29.5				611 / 39.8				976 / 22.4			
$NADP^+$	*B*-factor A-D	30.9	30.9	30.9	30.9	12.4	14.5	13.7	14.0	23.1	21.2	33.2	25.9	12.6	12.3	13.5	11.9
Ligand, conf. a	*B*-factor A-D	31.1	31.1	31.1	31.2	25.7	29.5	22.1	25.2	32.1	28.6	81.0	36.2	18.6	18.6	18.6	18.6
Ligand, conf. b	*B*-factor A-D	-				-				-				18.6	-	-	18.6
DTT [d]	*B*-factor A-D	-				47.4	47.9	55.9	59.2	-	49.9	-	-	-	-	18.6	-
Acetate	*B*-factor E	-				-				29.5				-			
Cys168 modification [e]	A-D	CSX	-	-	-	DTT	DTT	DTT	DTT	CSX	DTT	CSX	CSX	-	CSX	DTT	-

[a] Values in parentheses refer to those of the highest resolution shell.
[b] Incomplete data collection due to hardware problem.
[c] DPI – Diffraction-component Precision Index.
[d] DTT – linear DTT.
[e] Cys168 oxidized to sulfenic acid (CSX), covalently bound to DTT (DTT), or unmodified (-)

Supplemental Table S2 – *Tb*PTR1-Scaffold II inhibitors

Ligand	**Compound 5**	**Compound 7**	**Compound 8**	**Compound 9**	**Compound 11**	**Compound 13**
PDB Code	3JQA	3JQB	3JQC	3JQD	3JQE	3JQ9
Crystal Statistics						
Resolution range (Å)	1.90 – 74.50	2.40 – 45.20	1.80 – 75.60	1.60 – 39.00	2.00 – 34.30	2.30 – 89.10
Space Group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Unit cell dimensions:						
a, b, c (Å)	75.00 90.83 82.64	74.67 90.43 82.56	73.86 89.11 84.25	74.64 90.73 84.64	74.58 89.42 82.37	74.25 89.20 84.57
® (°)	115.5	115.5	116.1	115.3	115.6	115.7
Scaling Statistics						
Data collection location	ESRF ID23-2	ESRF ID23-2	ESRF BM14	ESRF ID23-2	In-House	ESRF ID23-1
No. Measurements	233,755	97,866	254,618	330,090	311,305	114,819
Unique reflections	78,741	38,283	84,262	108,123	62,121	40,166
Redundancy	3.0 (3.3) [a]	2.6 (2.6) [a]	3.0 (2.7) [a]	3.1 (2.9) [a]	5.0 (4.9) [a]	2.9 (3.0) [a]
Completeness (%)	99.9 (99.9) [a]	98.7 (99.2) [a]	92.9 (65.2) [a]	81.7 (84.9) [a]	94.4 (88.1) [a]	90.9 (64.3) [a]
< *I* / *f*(*I*) >	9.9 (3.1) [a]	9.4 (6.6) [a]	11.9 (1.8) [a]	10.2 (3.0) [a]	24.4 (9.1) [a]	8.0 (2.2) [a]
R_{merge} (%)	5.7 (32.0) [a]	11.0 (12.9) [a]	4.7 (37.7) [a]	8.9 (33.2) [a]	8.2 (22.1) [a]	7.3 (36.1) [a]
Refinement Stats						
R_{work} (%) / No. reflect.	14.7 / 74,742	19.2 / 36,312	16.8 / 79,975	17.9 / 102,629	15.2 / 58,920	23.1 / 38,112
R_{free} (%) / No. reflect.	18.9 / 3,999	25.2 / 1,971	20.4 / 4,287	21.6 / 5,494	21.3 / 3,201	28.6 / 2,054
R.M.S.D bond leng. (Å)	0.013	0.007	0.008	0.007	0.012	0.006
R.M.S.D bond ang. (°)	1.342	1.004	1.424	1.154	1.431	1.115
DPI [c]	0.12	0.66	0.14	0.12	0.19	0.64
Ramachandran:						
Core region (%)	88.8	89.3	89.0	88.9	89.2	88.7
Allowed region (%)	10.7	10.3	10.5	10.4	10.3	10.4
Generous region (%)	0.5	0.5	0.6	0.7	0.6	0.6
Model Statistics						
Protein residues:						
Total	987	986	990	985	987	987
Overall *B*-factor	23.3	6.1	17.2	13.7	15.6	41.4
No. in subunits A-D	247 246 247 247	247 246 246 247	247 248 248 247	247 246 246 246	247 246 247 247	247 246 247 247
B-factor A-D	22.9 22.7 23.0 22.8	7.6 6.8 8.1 7.9	17.2 17.6 17.6 17.3	14.5 13.9 14.0 14.3	15.7 15.6 15.9 15.8	41.2 41.3 42.0 41.7
Additional groups:						
Solvent No/Av. *B*-factor	638 / 35.3	358 / 11.6	731 / 27.9	1197 / 28.3	824 / 21.3	214 / 32.1
$NADP^+$ *B*-factor A-D	21.9 20.1 26.2 22.1	3.6 3.5 13.8 5.2	15.5 15.3 15.6 15.7	13.8 12.0 12.3 12.9	15.4 15.3 15.7 15.3	34.3 32.2 36.5 35.4
Ligand *B*-factor A-D	21.8 21.3 34.4 27.2	28.8 30.4 44.5 -	16.6 16.8 16.5 19.8	23.5 25.6 25.6 27.7	28.4 27.5 31.7 30.1	45.5 39.6 30.8 48.7
DTT [d] *B*-factor A-D	-	- - - 65.9	-	-	-	-
Cys168 mod. [e] A-D	- CSX - CSX	CSX CSX CSX DTT	-	- CSX CSX CSX	-	- - CSX CSX

[a] Values in parentheses refer to those of the highest resolution shell.
[b] Incomplete data collection due to radiation damage.
[c] DPI – Diffraction-component Precision Index.
[d] DTT – linear DTT.
[e] Cys168 oxidized to sulfenic acid (CSX), covalently bound to DTT (DTT), or unmodified (-)

Supplemental Table S3 – *Tb*PTR1-Scaffold III inhibitors																					
Ligand		**Compound 14**				**Compound 15**				**Compound 16**				**Compound 17**				**Compound 18**			
PDB Code		3JQF				3BMN				3BMO				3BMQ				3JQG			
Crystal Statistics																					
Resolution range	(Å)	1.60 – 23.40				2.00 – 75.60				1.60 – 74.50				1.70 – 74.50				1.90 – 53.80			
Space Group		$P2_1$				$P2_1$				$P2_1$				$P2_1$				$P2_1$			
Unit cell dimensions:																					
a, b, c	(Å)	74.55	90.24	82.41		73.84	87.50	84.23		74.73	90.99	82.82		74.72	91.16	82.83		74.53	89.85	82.44	
®	(°)	115.6				116.1				115.8				115.7				115.5			
Scaling Statistics																					
Data collection location		SRS 14.1				ESRF BM14				SRS 14.1				SRS 14.1				ESRF ID23-2			
No. Measurements		358,472				244,417				437,070				522,876				180,679			
Unique reflections		119,467				63,759				125,691				94,013				74,417			
Redundancy		3.0 (3.2) [a]				3.8 (3.6) [a]				3.5 (3.3) [a]				5.6 (5.5) [a]				2.4 (2.7) [a]			
Completeness	(%)	92.5 (88.0) [a]				94.9 (88.1) [a]				96.0 (99.4) [a]				85.8 (90.6) [a]				96.5 (96.6) [a]			
< *I* / *f*(*I*) >		12.4 (2.4) [a]				14.3 (4.3) [a]				16.9 (3.8) [a]				21.3 (9.5) [a]				5.9 (1.9) [a]			
R_{merge}	(%)	3.4 (34.7) [a]				10.3 (36.7) [a]				4.7 (39.3) [a]				5.5 (14.7) [a]				9.2 (29.0) [a]			
Refinement Stats																					
R_{work} (%) / No. reflect.		14.5 / 113,438				20.6 / 61,175				11.8 / 119,087				13.1 / 88,770				21.1 / 70,662			
R_{free} (%) / No. reflect.		18.6 / 6,029				26.7 / 2,584				15.0 / 6,604				17.8 / 5,243				26.7 / 3,755			
R.M.S.D bond leng.	(Å)	0.017				0.006				0.016				0.019				0.014			
R.M.S.D bond ang.	(°)	1.601				2.028				1.644				1.731				1.531			
DPI [b]		0.08				0.23				0.07				0.10				0.20			
Ramachandran:																					
Core region	(%)	89.0				86.9				89.8				88.5				88.7			
Allowed region	(%)	10.5				12.6				9.9				11.0				10.9			
Generous region	(%)	0.5				0.6				0.3				0.5				0.5			
Model Statistics																					
P**rotein residues:**																					
Total (number)		978				999				992				999				986			
Overall (*B*-factor)		19.9				26.4				12.9				10.9				15.7			
No. in subunits	A-D	245	244	244	245	249	250	249	251	251	245	248	248	250	250	249	250	247	246	246	247
B-factor	A-D	18.9	19.0	20.5	20.4	26.2	26.9	26.2	26.1	12.7	11.4	13.8	13.8	10.7	10.3	11.1	11.6	16.5	16.8	17.6	17.5
Additional groups:																					
Solvent No/Av. *B*-factor		1080 / 38.5				540 / 31.3				1251 / 33.1				1316 / 27.6				612 / 24.5			
$NADP^+$ *B*-factor	A-D	16.1	15.2	17.0	17.3	21.2	20.9	20.7	20.4	9.5	8.7	10.2	10.9	6.9	6.2	7.4	8.1	13.8	14.4	17.0	18.9
Ligand *B*-factor	A-D	16.5	14.9	17.0	17.3	23.5	33.2	24.0	24.3	10.6	12.3	14.1	13.0	12.8	12.7	16.6	16.5	24.4	17.5	30.6	40.4
DTT [c] *B*-factor	A-D	45.9	47.9	45.8	47.2	-				47.3	46.0	43.8	46.8	50.0	-	-	35.8	-			
DTO [d] *B*-factor	A-D	34.4	32.9	31.9	36.8	-				31.8	18.8	22.2	29.0	-				-			
Acetate *B*-factor	E	22.5	26.0	27.8	16.9	26.3	37.0							13.4 24.4 24.3 22.7 17.3 25.2 26.3				11.3 17.5			
Glycerol *B*-factor	F	34.6 32.3				41.3 25.6 30.7 31.7 42.5 36.4				18.9 24.4 29.9				41.6 30.9 29.5				-			
Cys168 mod. [e]	(A-D)	DTT	DTT	DTT	DTT	CSX	-	-	-	DTT	DTT	DTT	DTT	DTT	CSX	CSX	DTT	-			

[a] Values in parentheses refer to those of the highest resolution shell.
[b] DPI – Diffraction-component Precision Index.
[c] DTT – linear DTT.
[d] DTO – oxidized circular DTT.
[e] Cys168 oxidized to sulfenic acid (CSX), covalently bound to DTT (DTT), or unmodified (-)