

Supplementary Materials for

In silico, in vitro, X-ray crystallography, and integrated strategies for discovering spermidine synthase inhibitors for Chagas disease

Authors

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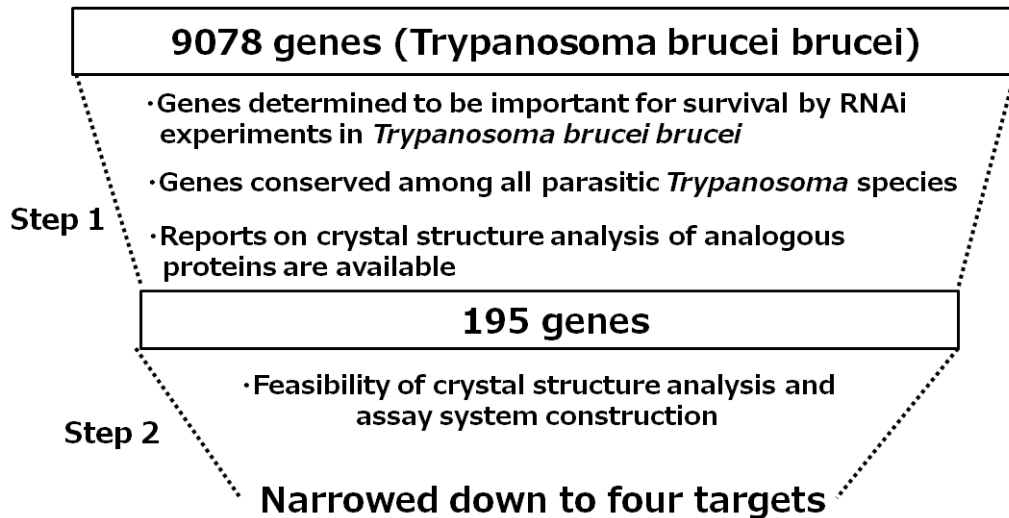
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Supplementary Figure 1. Search method for target proteins using iNTRODB.

a

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>Tb09.v1.0380 |||spermidine synthase, putative|Trypanosoma
brucei|chr 9|||Manual
Length = 298

Score = 431 bits (1107), Expect = e-154, Method: Compositional matrix adjust.
Identities = 199/296 (67%), Positives = 239/296 (80%), Gaps = 1/296 (0%)

Query: 1  MPGSELISGGWFREENDQWPGQAMSLRVEKVLVDAPTKFQHLTIFESDPKGPWGTVMALD 60
          MPG L++ GWFREEN QWPGQAMS +VE+VL+D PTKFQHL+IFE+DPKGPWGTVM LD
Sbjct: 1  MPGPGLLADGWFREENGQWPGQAMSFKVEEVLHDTPTKQHLTIFETDPKGPWGTVMTLD 60

Query: 61  GCIQVTDYDEFVYHEVLGHTSLCSHPKXXXXXXXXXXXXXXXXXXXXHGTVEHCDLVDID 120
          GCIQ+TD+DEFVYHE+L HT LC+HP P HGTV+ C LVDID
Sbjct: 61  GCIQLTDFDEFVYHEMLSHTPCAHPDVPDVLIIIGGGGGVMREVLRHGTVKRCVLDID 120

Query: 121  GEVMEQSKQHFQISRSLDPRATVRVDDGLAFVRQTPDNTYDVIIDTTDPAGPASELF 180
          G+V+E SK++FPQIS +DPRA VRV DG+AFVR+ ++DVIIDTTDP GPA+ELF
Sbjct: 121  GDVIEASKKYFPQISSGFSRPRADVRVGDGVAFAVREAASESFDVVIIDTTDPDGPAAELF 180

Query: 181  GEAFYKHLRILKPDGICCNQGESIWLNLELIEKMSRFIR-ETGFASVQYALMHVPTYPC 239
          GE FY+ VLRLKP GICCNQGES+WLN LIE M+ FI+ + GFASV+YA+++ PTYPC
Sbjct: 181  GEKFRYDVLRLKPRGICCNQGESVWLNRLIEGMADFIKNKVGFAVYKAMIYPTYPC 240

Query: 240  GSIGTLCISKAGVDVTKPLRPVEDMPFAKDLKYYDSEMHKASFALPRFARHINNS 295
          GSIG+LISCK AGVDVT+P+RPVE MPFA +LKYYS++HKA+F LPRFARH+N +
Sbjct: 241  GSIGSLICSKVAGVDVTQVPRVPESEMPFAGELKYYSDVHKAFAVLPFRFARHLNQN 296
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b

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>LmjF.04.0580 | organism=Leishmania_major_strain_Friedlin |
product=spermidine synthase |
location=LmjF.04:239178-240080(-) | length=300
Length = 300

Score = 380 bits (977), Expect = e-134, Method: Compositional matrix adjust.
Identities = 180/299 (60%), Positives = 218/299 (72%), Gaps = 6/299 (2%)

Query: 1  MPGSELISGGWFREENDQWPGQAMSLRVEKVLVDAPTKFQHLTIFESDPKGPWGTVMALD 60
          MPG L+ GWFREE+ WPGQA L+VEKVLVD PT+FQHLT+FESDP GPWGTVM LD
Sbjct: 1  MPGPGLLPDGWFREESTMWPQQAQGLKVEKVLVDQPTFQHLTVFESDPSPGWGTVMTLD 60

Query: 61  GCIQVTDYDEFVYHEVLGHTSLCSHPKXXXXXXXXXXXXXXXXXXXXH-----GTVEHCD 115
          G IQ+TDYDEFVYHE+L + SL H KP H G V+ +
Sbjct: 61  GAIQLTDYDEFVYHEMLANLSLACHHKPERVLVIGGGGGVVREVLRHKSEKDGVVQVSVE 120

Query: 116  LVDIDGVEVMEQSKQHFQISRSLDPRATVRVDDGLAFVRQTPDNTYDVIIDTTDPAGP 175
          LVDIDG VM+QSK+HFPQ++ +P T V DG AFVR PD+ YDV+IIDTTDP GP
Sbjct: 121  LVDIDGAVMQSKKHFPQVACGFANPCVTATVGDGAAFVRNVPDVSVDVVIIDTTDPKGP 180

Query: 176  ASELFGAEFYKHLRILKPDGICCNQGESIWLNLELIEKMSRFI-RETGFASVQYALMHV 234
          ASELFG FY +VLRIL+P G+ CNQGES+WL+ LIEKM F+ ++ GFA+V+YA++++
Sbjct: 181  ASELFGADFYTNVLRILRPGGVVNCQGESVWLRPLIEKMMGFLKKDIFATVKYAMIYI 240

Query: 235  PTYPCGSIGTLCISKAGVDVTKPLRPVEDMPFAKDLKYYDSEMHKASFALPRFARHIN 293
          PTYPCGSIGTL+C+K A DVT P+RPVE + FA LKYY S+MHKA+F LPRFA H+N
Sbjct: 241  PTYPCGSIGTLVCAKSADTDVTPMRPVESLGFADQLKYYSSDMHKAFAVLPFRFAAHLN 299
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c

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>gi|63253298|ref|NP_003123.2| spermidine synthase [Homo sapiens]
Length = 302

Score = 224 bits (571), Expect = 5e-72, Method: Compositional matrix adjust.
Identities = 114/293 (38%), Positives = 172/293 (58%), Gaps = 6/293 (2%)

Query: 3  GSELISGGWFREENDQWPGQAMSLRVEKVLVDAPTKFQHLTIFESDPKGPWGTVMALDGC 62
          G I GWFRE WPGQA+SL+VE++L+ +++Q + +F S +G V+ LDG
Sbjct: 12  GPAAIREGWFRCTSLWPGQALSLQVEQLLHRRSRVQDILVFRSKT---YGNVLLVDGV 68

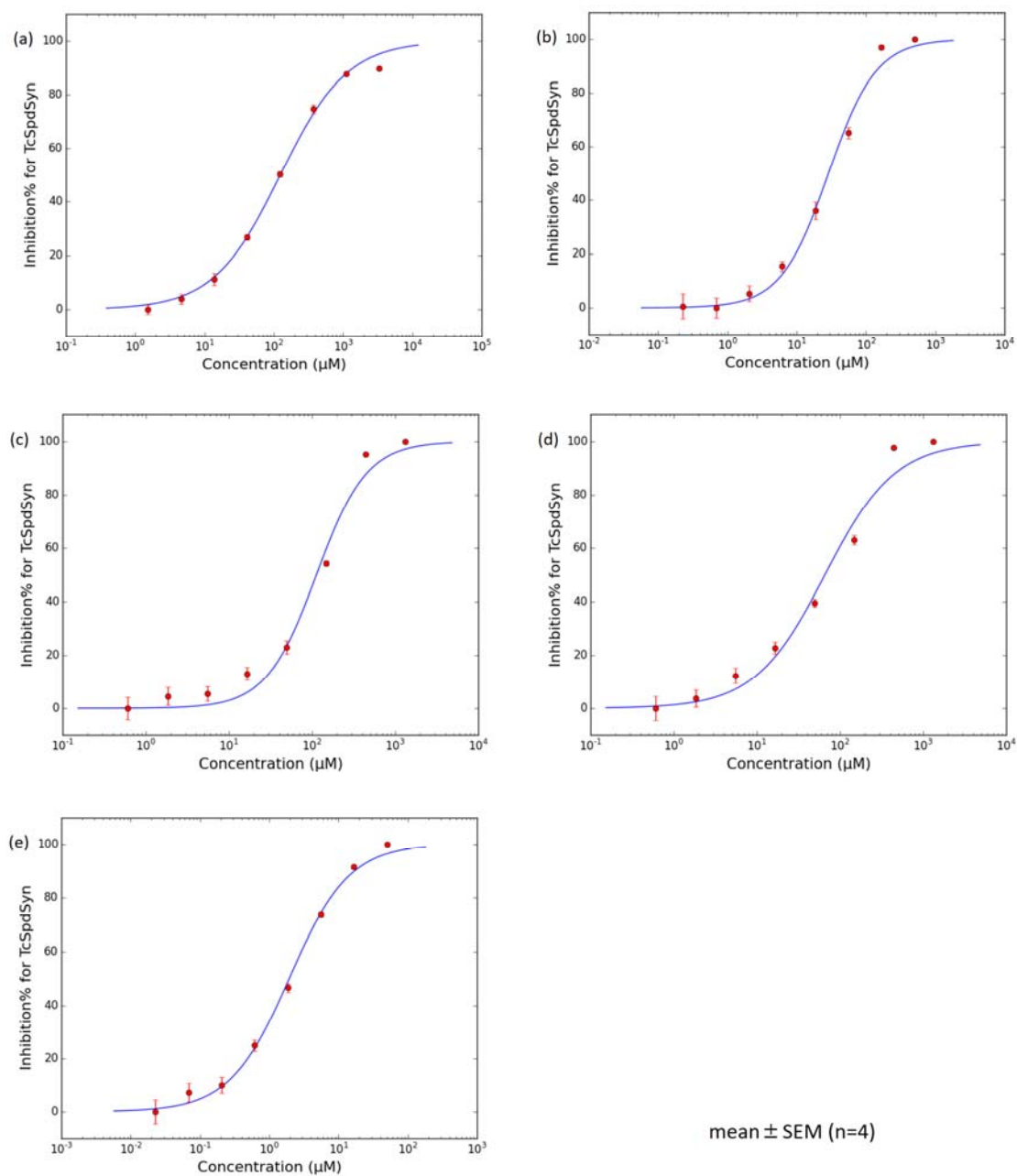
Query: 63  IQVTDYDEFVYHEVLGHTSLCSHPKXXXXXXXXXXXXXXXXXXXXHGTVEHCDLVDIDG 122
          IQ T+ DEF Y E++ + LCSHP P H +VE +ID +
Sbjct: 69  IQCTERDEFSYQEMIANLPLCSHPNPKVLIIIGGGGGVLEVVKHPSVESVQCEIDED 128

Query: 123  VMEQSKQHFQISRSLDPRATVRVDDGLAFVRQTPDNTYDVIIDTTDPAGPASELFGE 182
          V++ SK+ P ++ + + T+ V DG F++Q D +DV+I D++DP GPA LF E
Sbjct: 129  VIQVSKKFLPGMAIGYSSSKLTLHVGDGFEFMKQNQD-AFDVVIIDSSDPMGPAESLFKE 187

Query: 183  AFYKHLRILKPDGICCNQGESIWLNLELIEKMSRFIRETGFASVQYALMHVPTYPCGSI 242
          ++Y+ + LK DG+ C QGE WL+L+LI++M +F + F V YA +PTYP G I
Sbjct: 188  SYYQLMKTALKEDGLCCQGEQWLHLDLIKEMRQFCQSL-FPVVAYAYCTIPTYPGSI 246

Query: 243  GTLCSKAGVDVTKPLRPVEDMPFAK-DLKYYDSEMHKASFALPRFARHINN 294
          G ++CSK + +P++P+ A+ LKYY+S+++H+A+F LP FAR N
Sbjct: 247  GFMLCSKNPSTNFQEPVQPLTQQQVAQMQLKYYNSDVHRAAFVLPFARKALN 299
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Supplementary Figure 2. Aligned protein sequence of spermidine synthase between *Trypanosoma cruzi* (CL Brener strain) and A: *T. brucei*, B: *Leishmania major*, C: *Homo sapiens*. Aligned sequences were obtained from iNTRODB.



Supplementary Figure 3. The fitting curve for IC₅₀ calculation of the compounds on Table 1 (Compounds 1, 2, 3, 4, and 4MCHA correspond to (a), (b), (c), (d) and (e), respectively). The vertical line and horizontal axis's represent inhibition% for TcSpdSyn and compound concentration. The closed circles show the inhibition% measured at each dose. The error bars indicate the standard error of the inhibition% measured at each dose. For curve fitting, the following four parameter logistic (4PL) model¹ is used. The parameter values of the equation of each compound are shown in Supplementary Table 3.

$$y = \min + \frac{\max - \min}{1 + 10^{(\text{Hill}(\text{Log}_{10}(X_{50}) - x))}}$$

Supplementary Table 1. Supplier ID and Namiki ID of the active compounds by *in vitro* assay listed in Table 1.

Compound ID	Supplier Name	Supplier ID	Namiki ID
1	Bepharm	B21234	NS-00034421
2	Vitas-M	STK107845	NS-00694195
3	Pharmeks	P2001S-338404	NS-03964486
4	Enamine	EN300-115921	NS-00027256

Supplementary Table 2. Supplier ID and Namiki ID of inactive compounds by *in vitro* assay.

Namiki ID	Supplier ID	Supplier Name
NS-01988866	IBS-L0127228	Alinda
NS-04133406	AC-20091	APIChem
NS-00084264	OR13234	Apollo
NS-08456239	ADM 12409198	Asinex
NS-09711136	ART 09584052	Asinex
NS-09686125	ART 12883679	Asinex
NS-09723909	ART 15417152	Asinex
NS-00738895	ASN 03204271	Asinex
NS-00553327	BAS 00112672	Asinex
NS-00568801	BAS 00132206	Asinex
NS-00557872	BAS 00162617	Asinex
NS-00375233	BAS 00485302	Asinex
NS-00456710	BAS 01052336	Asinex
NS-00683031	BAS 03673624	Asinex
NS-06088261	B10057	Bepharm
NS-01984066	B110683	Bepharm
NS-01984075	B11935	Bepharm
NS-00242318	B126389	Bepharm
NS-00216529	B185962	Bepharm
NS-00242327	B19373	Bepharm
NS-08726051	B225643	Bepharm

NS-00502269	B226825	Bepharm
NS-00084817	B237070	Bepharm
NS-00520067	B34480	Bepharm
NS-01447165	B53509	Bepharm
NS-00038373	B55162	Bepharm
NS-00027436	B6027	Bepharm
NS-00026629	B7480	Bepharm
NS-00086239	B7776	Bepharm
NS-00106974	B83970	Bepharm
NS-00240797	B86318	Bepharm
NS-00042374	B94867	Bepharm
NS-04691291	2F-088	Bionet
NS-00897223	1C-097	Bionet(Fragment)
NS-01971130	IN-0425	CombiBlocks
NS-00045707	QA-1027	CombiBlocks
NS-00210693	QA-3805	CombiBlocks
NS-02726054	QC-6510	CombiBlocks
NS-03104067	QE-9891	CombiBlocks
NS-00025515	QY-1767	CombiBlocks
NS-00096534	SS-4524	CombiBlocks
NS-00422542	EN300-112596	Enamine
NS-03042830	EN300-113084	Enamine
NS-00024529	EN300-122589	Enamine
NS-10494236	EN300-127485	Enamine
NS-00135682	EN300-13617	Enamine
NS-00021451	EN300-13896	Enamine
NS-03065527	EN300-139143	Enamine
NS-00025508	EN300-15718	Enamine
NS-00044917	EN300-160189	Enamine
NS-00034115	EN300-175002	Enamine
NS-00025550	EN300-18624	Enamine
NS-00096048	EN300-20404	Enamine

NS-00095940	EN300-215558	Enamine
NS-00044424	EN300-31275	Enamine
NS-05620599	EN300-33359	Enamine
NS-00208253	EN300-34367	Enamine
NS-00040634	EN300-36560	Enamine
NS-00039012	EN300-38966	Enamine
NS-00083514	EN300-56240	Enamine
NS-00127946	EN300-61777	Enamine
NS-07300875	EN300-64804	Enamine
NS-04286829	EN300-68454	Enamine
NS-01820579	EN300-69071	Enamine
NS-05682291	EN300-82463	Enamine
NS-00000321	Z104507050	Enamine
NS-09460347	Z1123821842	Enamine
NS-09851924	Z1130245470	Enamine
NS-05677846	Z1198335676	Enamine
NS-02654486	Z1255356155	Enamine
NS-10120169	Z169504308	Enamine
NS-03448452	Z237732596	Enamine
NS-00652324	Z238921002	Enamine
NS-01767178	Z31375831	Enamine
NS-01639043	Z31380603	Enamine
NS-02048933	Z370706740	Enamine
NS-08389397	Z384973502	Enamine
NS-00036560	Z415740552	Enamine
NS-04286824	Z432090862	Enamine
NS-08436212	Z445132884	Enamine
NS-01248894	Z45415551	Enamine
NS-00250557	Z56780034	Enamine
NS-06342424	Z600584194	Enamine
NS-07238675	Z649635088	Enamine
NS-00206553	Z90660992	Enamine

NS-00029324	Z944939438	Enamine
NS-03050150	12R0026	JWPharmlab
NS-03052261	20R0212	JWPharmlab
NS-01958208	LT00007983	Labotest
NS-05078226	LT00030844	Labotest
NS-01963106	LT00050116	Labotest
NS-01971832	LT00118654	Labotest
NS-01976901	LT00154482	Labotest
NS-01981402	LT-IV X 478	Labotest
NS-02049344	F1278-0327	LifeChemicals
NS-04370463	F3366-5220	LifeChemicals
NS-00021546	9899	Matrix
NS-00024028	10868	Matrix
NS-00022663	18523	Matrix
NS-03120259	21508	Matrix
NS-00096533	33847	Matrix
NS-06850867	41077	Matrix
NS-00375649	BTB12148	Maybridge
NS-00625817	CD07798	Maybridge
NS-00041234	KM01833	Maybridge
NS-02168278	KM09877	Maybridge
NS-02171378	RDP00177	Maybridge
NS-02172479	RF00498	Maybridge
NS-02172984	RF02876	Maybridge
NS-00042069	RJC00810	Maybridge
NS-02181735	SEW03911	Maybridge
NS-00101613	SPB07361	Maybridge
NS-04428093	CC75719	Maybridge(Fragment)
NS-02142145	13321	MolMall
NS-02142989	15379	MolMall
NS-02143192	15633	MolMall
NS-02143187	102931	MolMall

NS-04364531	1049802	OTAVA
NS-05686762	1192847	OTAVA
NS-04409108	7020692941	OTAVA
NS-00601376	P2000S-19366	Pharmeks
NS-00616224	P2000S-23760	Pharmeks
NS-00599907	P2000S-26470	Pharmeks
NS-00629207	P2000S-27173	Pharmeks
NS-02281209	P2001S-052746	Pharmeks
NS-00638619	P2001S-063604	Pharmeks
NS-02293313	P2001S-113312	Pharmeks
NS-01448839	P2001S-160661	Pharmeks
NS-00694795	P2001S-168762	Pharmeks
NS-02320185	P2001S-186681	Pharmeks
NS-02320927	P2001S-187781	Pharmeks
NS-00881867	P2001S-238236	Pharmeks
NS-01061606	P2001S-264815	Pharmeks
NS-02234815	P2000N-10331	Pharmeks(Natural)
NS-02235337	P2000N-11786	Pharmeks(Natural)
NS-02250802	P2000N-37233	Pharmeks(Natural)
NS-02503810	OSSK_485183	Princeton
NS-09668660	OSSL_477426	Princeton
NS-01484266	OSSL_822359	Princeton
NS-02648504	PH012669	SALOR
NS-00475129	R295736	SALOR
NS-02628888	R436860	SALOR
NS-02642429	S136107	SALOR
NS-04101213	S914789	SALOR
NS-00242430	1-0026	ScientificExchange
NS-03323092	Z-007027	ScientificExchange
NS-02719860	Z-025192	ScientificExchange
NS-02683904	AJ-333/15478078	SPECS
NS-02721148	ST002006	TimTec

NS-00690438	STK016799	Vitas-M
NS-02525824	STK163122	Vitas-M
NS-02596772	STK220715	Vitas-M
NS-03444130	STK280472	Vitas-M
NS-05176117	STK298937	Vitas-M
NS-05615458	STK338050	Vitas-M
NS-01447216	STK368001	Vitas-M
NS-03667421	STK387017	Vitas-M
NS-06400628	STK521785	Vitas-M
NS-00472084	STK734312	Vitas-M
NS-00873275	STL032034	Vitas-M
NS-01963305	STL033042	Vitas-M
NS-00671753	STL327502	Vitas-M
NS-00469312	UZI/2599943	Zelinsky
NS-00505849	UZI/2625541	Zelinsky
NS-02729450	UZI/2647801	Zelinsky
NS-03081113	UZI/2655098	Zelinsky
NS-00354396	UZI/6095081	Zelinsky
NS-01451287	UZI/6188274	Zelinsky
NS-00464302	UZI/7131862	Zelinsky
NS-01036208	UZI/8190366	Zelinsky
NS-00240683	UZI/9019794	Zelinsky
NS-10533350	UZI/9062663	Zelinsky

Supplementary Table 3. The parameter values of the equation used for the curve fitting.

Compound ID	min	Max	Hill	LoggedX50
1	0.00	100.00	0.90	2.09
2	0.00	100.00	1.27	1.46
3	0.00	100.00	1.44	2.05
4	0.00	100.00	1.03	1.82
4MCHA	0.00	100.00	1.01	0.29

References

1. Fomenko, I, Durst, M & Balaban, D. Robust regression for high throughput drug screening. *Comput Meth Programs Biomed* **82**, 31-37 (2006).