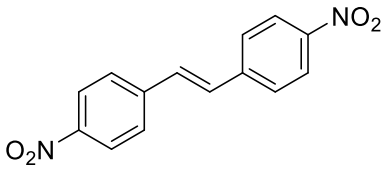
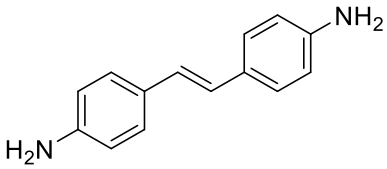
SUPPLEMENTAL MATERIAL

Chemistry General Experimental. Uncorrected melting points were measured on a Thomas-Hoover capillary melting point apparatus. H-NMR spectra were recorded on a Varian Gemini 2000 spectrometer (300 MHz) in dimethyl-d_6 sulfoxide (1). Anhydrous EtOH was distilled over Mg/I_2 immediately prior to use. Other anhydrous solvents (in Sure/Seal® containers) and organic starting materials and were purchased from Sigma-Aldrich Chemical, Milwaukee, WI, and were used without further purification. Reaction mixtures were monitored by thin-layer chromatography (TLC) on silica gel or by reverse-phase high-performance liquid chromatography (HPLC). Organic layers of extraction mixtures were neutralized as necessary with acidic or basic washes, washed with saturated NaCl solution, and dried over Na_2SO_4 or $MgSO_4$ before being evaporated under reduced pressure. Gravity and flash column chromatography were performed using Davisil grade 633, type 60A silica gel (200-425 mesh). Analytical HPLC chromatograms were recorded on an Agilent 1200 Series chromatograph using a Zorbax Rx C8 column (4.6 mm × 75 mm, 3.5 μm) and UV photodiode array detection at 230, 254, 265, 290, and 320 nm. Wavelengths reported are those at which the strongest signals of the major products were observed. Mobile phases consisted of mixtures of CH_3CN (0–75%) or CH_3OH (0–95%) in water containing formic acid (80 mM), ammonium formate (20 mM), and triethylamine (15 mM) using appropriate gradients. Flow rates were maintained at 1.5 mL/min at a column temperature of 40 °C. Preparative reverse-phase HPLC was performed on a Varian ProStar chromatography workstation configured with two PS-215 pumps fitted with 50 mL pump heads, a Dynamax Microsorb C18 (60 Å) column (41.4 mm × 25 cm, 8 μm), PS-320 variable wavelength UV-vis detector, and a PS-701 fraction collector. Mobile phases consisted of mixtures of CH_3CN (0-75%) or CH_3OH (0–95%) in water containing formic acid (40

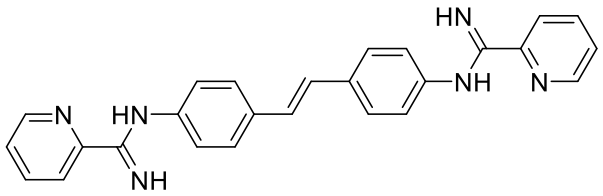
mM) and ammonium formate (10 mM). Flow rates were maintained at 40 mL/min. Detector wavelengths and mobile-phase gradients were optimized for the individual compounds. Select fractions were analyzed for purity using a Zorbax Rx C8 column (4.6 mm × 75 mm, 3.5 μm) and the latter mobile phases on an Agilent Technologies 1100 chromatograph. Pooled purified fractions were evaporated under reduced pressure, reconstituted in water, and lyophilized (3 cycles) on a VirTis BenchTop 2K or 6K lyophilizer. Low-resolution electrospray ionization (ESI) mass spectra were recorded on an Agilent Technologies 1100 Series LC/MSD trap mass spectrometer. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, and, unless stated otherwise, were within (0.4% of calculated values.



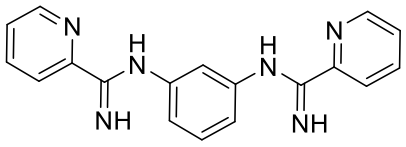
***trans*-4,4'-Dinitroostilbene** was prepared from 4-nitrobenzaldehyde and diethyl (4-nitrobenzyl)phosphonate following the procedure of Wu et al (1).



***trans*-4,4'-Diaminostilbene** was prepared from *trans*-4,4'-dinitroostilbene and tin(II) chloride dihydrate following the procedure of Wu et al (1).

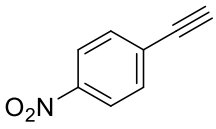


(*E*)-*N*,*N*''-[Ethene-1,2-diylbis(4,1-phenylene)]dipicolinimidamide dihydrochloride trihydrate (**27SMB 078**). A solution of *trans*-4,4'-diaminostilbene (1.50 g, 7.13 mmol) in dry THF (20 mL) was added dropwise to a solution of lithium bis(trimethylsilyl)amide in (1 M solution in THF, 15 mL, 15 mmol) The mixture was stirred for 20 min at ambient temperature before the addition of a solution of 2-cyanopyridine (1.56 g, 15.0 mmol) in THF (20 mL). The mixture was stirred overnight, diluted with water, and extracted into EtOAc. Combined extracts were washed with brine, dried (Na_2SO_4), and evaporated. The product was purified by preparative HPLC followed by conversion to the HCl salt to give a solid (1.10 g, 31%): %): mp 310-311 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.83, (s, 1H), 10.08 (s, 1H), 9.33 (s, 1H), 8.91(d, J = 4.4 Hz, 2H), 8.45 (d, J = 7.9 Hz), 8.23 (dt, J = 7.8, 1.5 Hz, 2H), 7.89–7.83 (m, 6H), 7.53–4.78 (m, 6H); EIMS *m/z* 419 $(M + 1)^+$; HPLC 100 area % (254 nm). Anal. Calcd for $C_{26}H_{22}N_6 \cdot 2HCl \cdot 3H_2O$: C, 57.25; H, 5.54; N, 15.41; Cl, 13.00. Found: C, 57.09; H, 5.53; N, 15.21; Cl, 12.91.

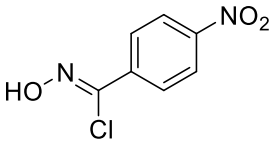


***N*,*N*''-(1,3-Phenylene)dipicolinimidamide dihydrochloride dihydrate** (**28SMB032**) was prepared analogously to 27SMB078 from 1,3-phenylenediamine (2.00 g, 18.5 mmol) and 2-cyanopyridine (4.00 g, 38.0 mmol) but using sodium bis(trimethylsilyl)amide in place of the lithium salt. Purification by preparative HPLC followed by conversion to the HCl salt gave a solid (2.80 g, 78%): mp 189-193 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.07, (s, 1H), 10.40 (s, 2H), 9.62 (s, 2H), 8.91(d, J = 4.2 Hz, 2H), 8.62 (d, J = 7.5 Hz), 8.24 (t, J = 7.6 Hz, 2H), 7.89–7.85 (m, 2H), 7.79–7.75 (m, 2H), 7.56 (d, J = 8.0 Hz, 2H); EIMS *m/z* 317 $(M + 1)^+$; HPLC 100 area % (230 nm).

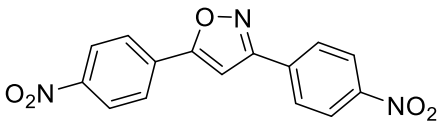
Anal. Calcd for $C_{18}H_{18}N_6 \cdot 2.3HCl \cdot 2H_2O$: C, 49.56; H, 5.15; N, 19.26; Cl, 18.69. Found: C, 49.53; H, 4.91; N, 19.08; Cl, 18.37.



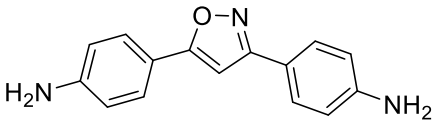
1-Ethynyl-4-nitrobenzene (2) was prepared from 4-bromonitrobenzene using Sonogashira–deprotection strategy following the procedure of Zhao et al (3). but the deprotection step was affected using sodium hydride (4).



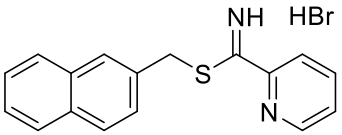
N-Hydroxy-4-nitrobenzimidoyl chloride (5) was prepared in two steps beginning with the oximation of 4-nitrobenzaldehye using hydroxylamine hydrochloride followed by chlorination using *N*-chlorosuccinimide (4).



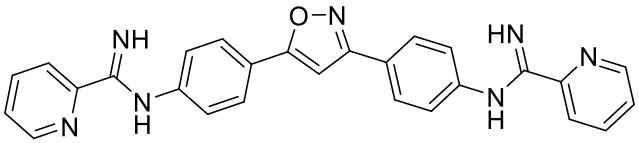
3,5-Bis(4-nitrophenyl)isoxazole (6) was prepared from 1-ethynyl-4-nitrobenzene and *N*-hydroxy-4-nitrobenzimidoyl chloride in the presence of bis(tributyltin) oxide (4).



3,5-Bis(4-aminophenyl)isoxazole (7) was prepared by the by the reduction of 3,5-bis(4-nitrophenyl)isoxazole with tin(II) chloride dihydrate in ethanol.



Naphthalen-2-ylmethyl pyridine-2-carbimidothioate hydrobromide was prepared by an established method (8).



***N*,*N*''-[Isoxazole-3,5-diylbis(4,1-phenylene)]dipicolinimidamide trihydrochloride monohydratehydrate** (**31DAP 069**). Crude naphthalen-2-ylmethyl pyridine-2-carbimidothioate hydrobromide (3.88 g, 10.8 mmol) was added to a mixture of of 3,5-bis(4-aminophenyl)isoxazole (1.26 g, 4.99 mmol) in dry acetonitrile (50 mL) and freshly distilled ethanol (50 mL) that was cooled in an ice–salt bath. The mixture was stirred at ambient temperature for 4 days before being evaporated to near dryness. The residue was stirred in hot water (ca. 300 mL) and the mixture was gravity filtered. Two crops of precipitate were recovered directly from the filtrate, and a third crop was obtained after basification of the filtrate (pH 9) using NaOH solution. Combined solids were purified by preparative HPLC. The product was converted to the HCl salt using ethanolic HCl and ether to give a pale yellow solid (1.06 g, 40%): mp 280 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.03, (s, 1H), 10.28 (s, 1H), 10.25 (s, 1H), 9.54 (s, 1H), 9.50 (s, 1H), 8.92(d, J = 4.1 Hz, 2H), 8.52 (dd, J = 8.3 and 1.2 Hz 2H), 8.25 (dt, J = 8.1 and 1.1 Hz, 2H), 8.14 (d, J = 8.0 Hz, 4 H), 7.89–7.86 (m, 3H), 7.74–7.69 (m, 4H); EIMS *m/z* 460 (M + 1)$^+$; HPLC 100 area % (290 nm). Anal. Calcd for $C_{27}H_{21}N_7O{\cdot}2.8HCl{\cdot}1.2H_2O$: C, 55.60; H, 4.53; N, 16.81; Cl, 17.02. Found: C, 55.39; H, 4.36; N, 16.70; Cl, 17.29.

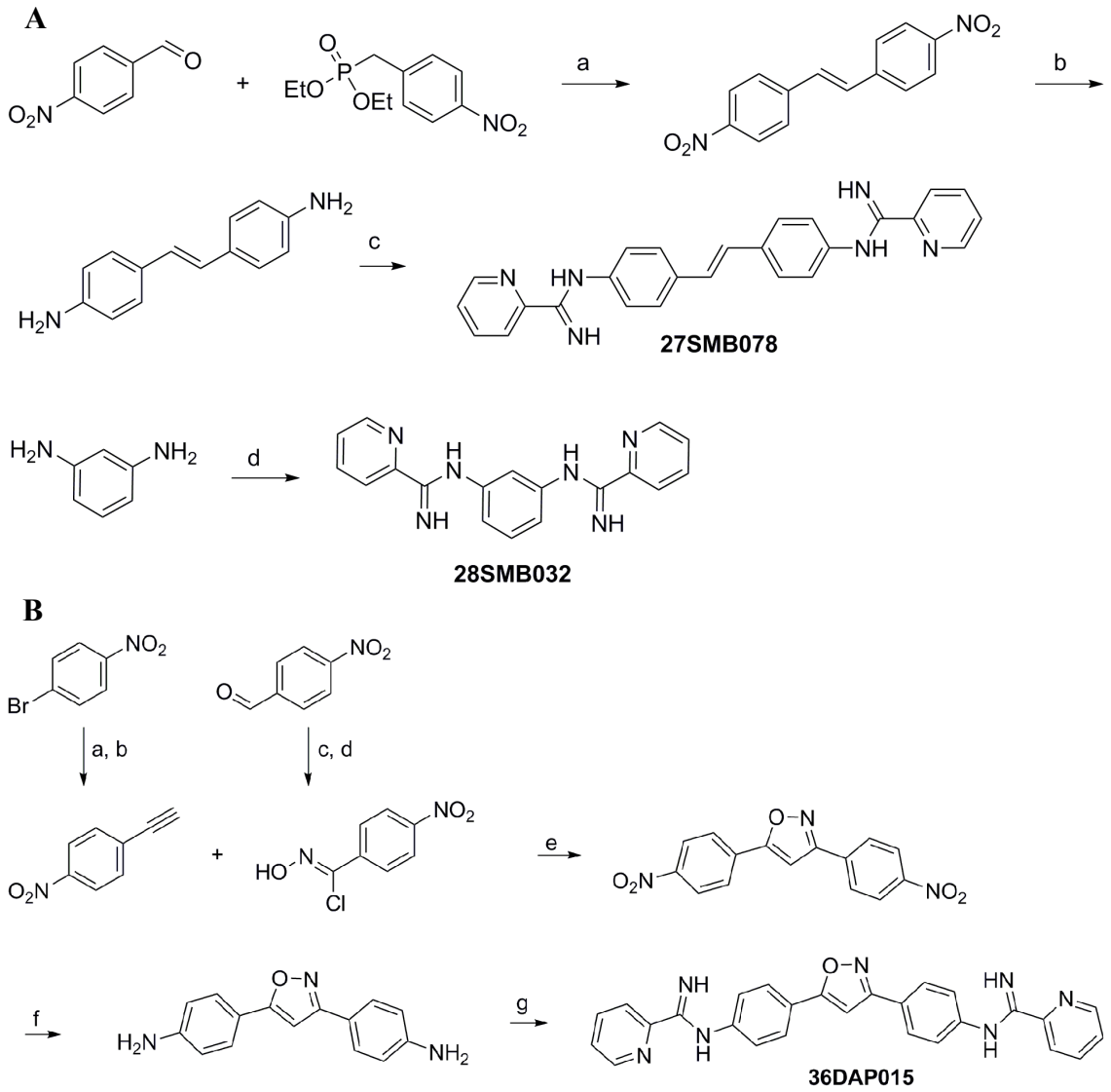


Fig S1 **A** Synthesis of 27SMB078, and 28SMB032. Reagents and conditions: (a) NaOMe, DMF; (b) SnCl2·2H2O, EtOH; (c) 2-cyanopyridine, LiN[Si(CH3)3]2, THF; (d) 2-cyanopyridine, NaN[Si(CH3)3]2, THF. **B** Synthesis of 31DAP069. Reagents and conditions: (a) 2-methyl-3-butyn-2-ol, PdCl2(PPh3)2, CuI, Et3N; (b) NaH, toluene; (c) NH2OH.H2O, EtOH, H2O; (d) N-chlorosuccinimide, DMF; (e) bis(tributylin) oxide, CH2Cl2; (f) SnCl2.2H2O, EtOH; (g) naphthalen-2-ylmethyl pyridine-2-carbimidothioate hydrobromide, CH3CN, EtOH.

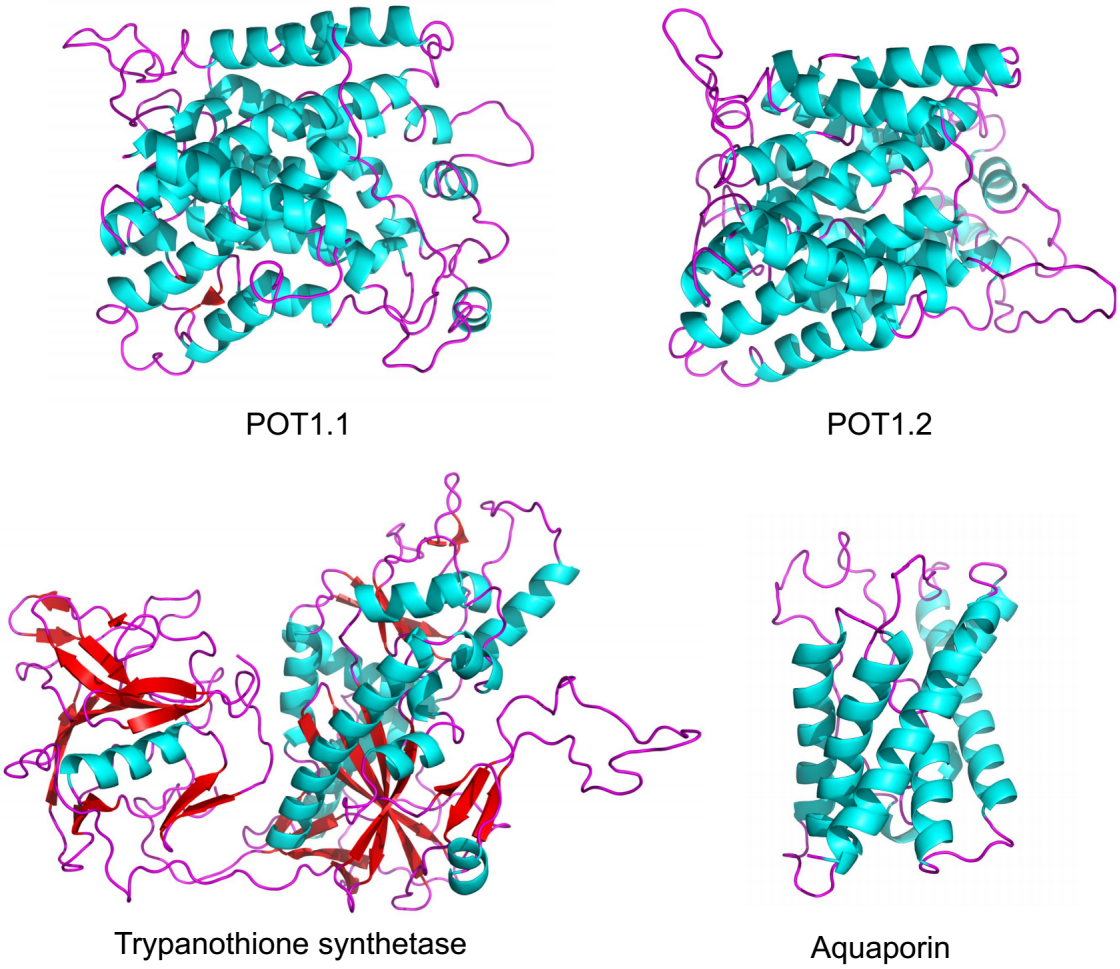


Fig S2 3D structures of selected homology models (minimized proteins) developed with SWISS-MODEL server.

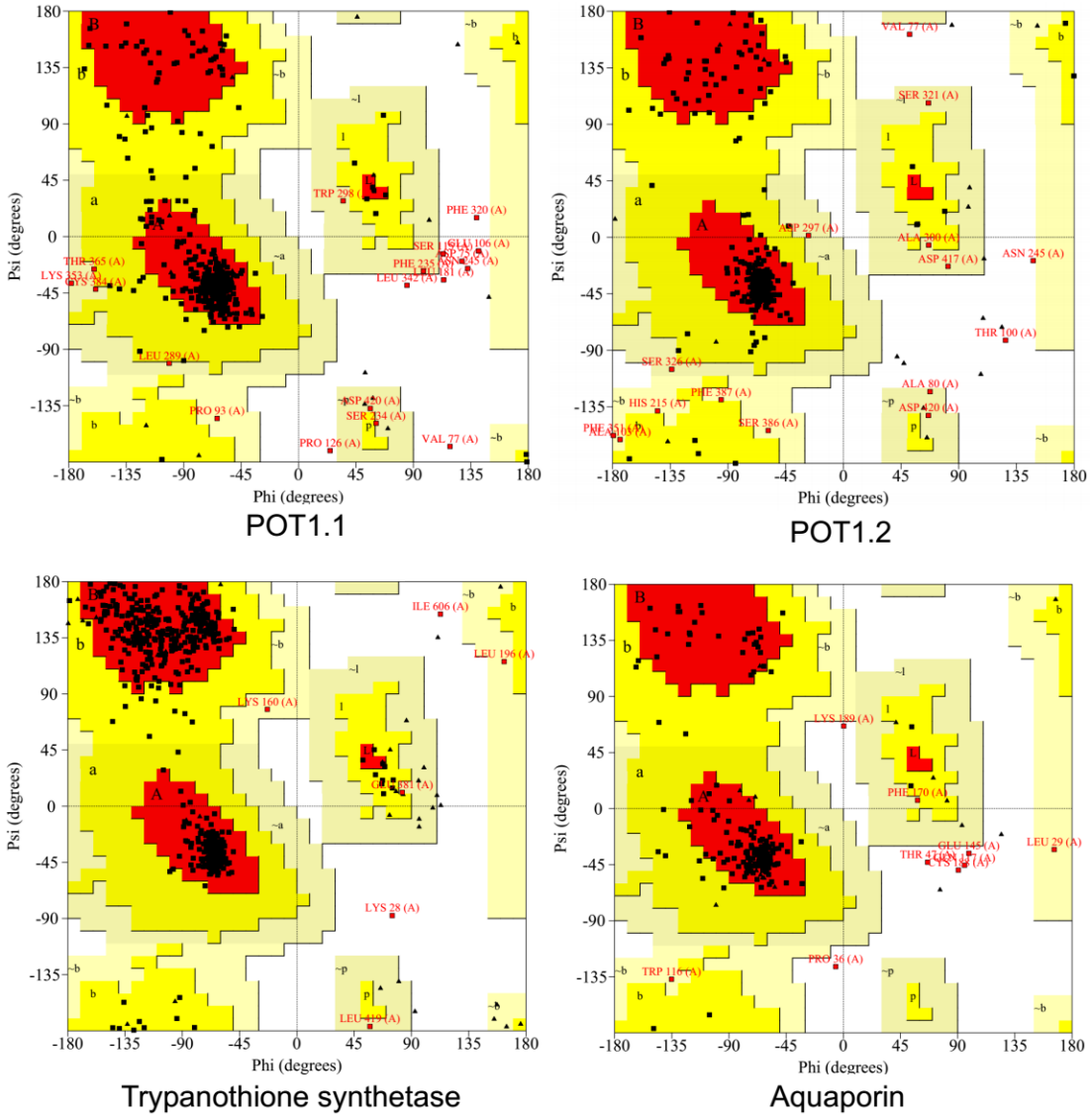


Fig S3 Ramachandran plots for some *T. cruzi* candidates obtained by PROCHECK, showing the dihedral angles Psi and Phi of amino acid residues. Red represents most favored regions; yellow represents additional allowed regions; beige represents generously allowed regions; and white areas are disallowed regions.

Table S1 EC_{50} and FICI of 28SMB032/Bz combinations against intracellular forms from Tulahuen β-galactosidase strain of *T.cruzi*

Drug ratio of 28SMB032:Bz	EC_{50}[a] (μM)		FICI		ΣFICI	xΣFICI
	28SMB032	Bz	28SMB032	Bz		
Intracellular forms						
5:0	0.17(0.14-0.21)					
4:1	0.18(0.15-0.21)	0.07(0.06-0.08)	1.08	0.02	1.10	0.95
3:2	0.19(0.16-0.23)	0.20(0.17-0.24)	1.13	0.05	1.18	
2:3	0.12(0.09-0.18)	0.30(0.21-0.43)	0.74	0.07	0.81	
1:4	0.10(0.08-0.14)	0.64(0.47-0.89)	0.62	0.15	0.77	
0:5		4.36(3.12-6.10)				

[a] Standard Error of the Mean (SEM): 95%CI

Table S2 *In silico* predicted ADMET properties for studied compounds

Properties	Compounds				
	2EVK008	27SMB078	28SMB032	31DAP069	36DAP015
Human Intestinal absortion[a]	>30%	>30%	>30%	>30%	>30%
Volume of distribution[b]	6.97 L/kg	4.09 L/kg	2.48 L/kg	2.84 L/kg	8.66 L/kg
CYP3A4 inhibiton[a]	Non-inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor	Non-inhibitor
CYP2D6 inhibiton[a]	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor
CYP2C19 inhibition[a]	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor
CYP2C9 inhibition[a]	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP1A2 inhibition[a]	Inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor	Non-inhibitor
Acute Oral toxicity[c]	500 mg/Kg	195 mg/Kg	195 mg/Kg	1012 mg/Kg	375 mg/Kg
AMES toxicity[a]	Non-mutagenic	Mutagenic	Mutagenic	Mutagenic	Mutagenic
Carcinogenicity[a]	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic
Genotoxicity[c]	Non-genotoxic	Non-genotoxic	Non-genotoxic	Non-genotoxic	Non-genotoxic
hERG blockage[d]	Non-blocker	Non-blocker	Non-blocker	Non-blocker	Non-blocker

Predictions based on: [a]admetSAR (27), [b]ACD/I-Lab (https://ilab.acdlabs.com/iLab2/), [c]PROTOX (34), and [d]Pred-hERG (32).

Table S3 Summary of statistic results of *T. cruzi* homology model proteins

Target (UniProt ID)	Template information			PROCHECK analysis			
	Coverage	Sequence Identity	Template	Most favored regions	Additional allowed regions	Generously allowed region	Disallowed regions
Aquaporin (Q8MXA3)	87%	38%	2NE2	84.7%	10.6%	1.8%	2.9%
Trypanothione synthetase (Q9GT49)	97%	60%	2VOB	92.7%	6.2%	0.7%	0.4%
POT1.1 (B2CQQ7)	64%	27%	3OB6	83.4%	12.2%	2.2%	2.2%
POT1.2 (Q4D143)	63%	26%	3L1L	86.1%	9.7%	3.3%	0.8%

Table S4 Details of *T. cruzi* target candidates and size of the grid used in target fishing study.

Target candidate (UniProt ID)	Source	PDB ID (Resolution)	Grid information	
			Dimensions (x, y, z)	Dock ligands with length ≤
Acidocalcisomal pyrophosphatase (Q4JH30)	PDB	5CUU (2.96 Å)	-16.69 x 37.22 x -12.42	22 Å
Aquaporin **(**Q8MXA3**)**	Bibliography search	Homology model	1.17 x 32.49 x -2.86	20 Å
Arginine kinase (O96507)	PDB	2J1Q (1.90 Å)	58.88 x 32.00 x 62.50	20 Å
Cruzain (P25779)	PDB	3KKU (1.28 Å)	-4.49 x -33.45 x 3.63	18 Å
Cyclophilin (Q4DPB9)	PDB	1XQ7 (2.07 Å)	46.46 x 40.68 x 9.16	20 Å
Deoxyribonucleic acid	Bibliography search	1PRP (2.10 Å)	10.11 x 22.79 x 8.68	24 Å
Dihydrofolate reductase-Thymidylate synthase (Q8T5T8)	PDB	3KJS (2.50 Å)	17.06 x -31.51 x 42.85	19 Å
Dihydroorotate dehydrogenase (Q4D3W2)	PDB	3W88 (1.40 Å)	3.94 x -14.72 x -11.54	17 Å
Farnesyl diphosphate synthase (Q95WL3)	PDB	4DWG (2.01 Å)	11.80 x 19.87 x 174.00	18 Å
Farnesyltransferase (Q4CWB4)	PDB	3WSB (2.40 Å)	-33.58 x 48.96 x -43.49	20 Å
Glucokinase (Q4E4E1)	PDB	5BRH (1.90 Å)	19.81 x -10.75 x 61.82	20 Å
Glyceraldehyde-3-phosphate dehydrogenase (P22513)	PDB	1K3T (1.95 Å)	26.92 x 10.15 x 8.24	22 Å
Glucose-6-phosphate isomerase (Q4E5N1)	PDB	4QFH (1.80 Å)	24.82 x 57.35 x 12.14	20 Å
Histidyl-tRNA synthetase (Q4DA54)	PDB	4YRC (2.10 Å)	64.39 x 8.09 x 19.17	20 Å
Hypoxanthine phosphoribosyltransferase (Q4DRC4)	PDB	1P19 (2.30 Å)	72.55 x 40.24 x 32.19	17 Å
Phosphodiesterase (Q53I60)	PDB	3V94 (2.33 Å)	-37.77 x -26.95 x -18.36	20 Å
POT1.1 (B2CQQ7)	Bibliography search	Homology model	39.81 x -3.53 x 31.66	24 Å

POT1.2 (Q4D143)	Bibliography search	Homology model	2.10 x 7.97 x 19.14	24 Å
Prostaglandin F2a synthase (Q8I6L9)	PDB	3ATZ (2.04 Å)	26.43 x 84.18 x 140.19	18 Å
Pteridine reductase 2 (Q8I814)	PDB	1MXF (2.30 Å)	17.36 x 52.13 x 24.71	22 Å
Pyruvate kinase (Q4D9Z4)	PDB	4KS0 (2.80 Å)	16.59 x 8.55 x 30.06	22 Å
Ribose 5-phosphate isomerase type B (Q4CQE2)	PDB	3K7S (1.90 Å)	35.27 x 23.46 x 11.47	20 Å
Spermidine synthase (Q4DA73)	Bibliography search	4YUY (1.58 Å)	3.33 x 9.23 x 45.33	22 Å
Sterol 14-alpha demethylase (Q7Z1V1)	PDB	4C27 (1.95 Å)	-7.02 x 11.01 x -18.21	17 Å
Superoxide dismutase (Q4DCQ3)	PDB	4DVH (2.23 Å)	18.80 x -4.57 x 19.54	20 Å
Trans-sialidase (Q26964)	PDB	3B69 (1.67 Å)	7.02 x -18.82 x -6.13	18 Å
Triosephosphate isomerase (P52270)	PDB	1SUX (2.00 Å)	28.50 x 96.56 x 62.12	19 Å
Trypanothione reductase (K4E0T9)	Bibliography search	4NEW (2.80 Å)	93.11 x -19.35 x -118.80	17 Å
Trypanothione synthetase (Q9GT49)	Bibliography search	Homology model	-35.24 x -1.01 x 11.46	20 Å
UDP-Galactopyranose mutase (Q4E1W2)	PDB	4DSG (2.25 Å)	70.18 x -15.83 x -29.06	20 Å

REFERENCES

1. Wu C, Wei J, Tian D Feng Y, Miller RH, Wang Y. 2008. Molecular Probes for Imaging Myelinated White Matter in CNS. J Med Chem 51:6682-6688.

2. Gilbert JC, Weerasooriya U. 1982. Diazoethenes: their attempted synthesis from aldehydes and aromatic ketones by way of the Horner-Emmons modification of the Wittig reaction. A facile synthesis of alkynes. J Org Chem 47:1837-45.

3. Zhao W, Tong B, Pan Y, Shen J, Zhi J, Shi J, Dong Y. 2009. Fabrication, Electrochemical, and Optoelectronic Properties of Layer-by-Layer Films Based on (Phthalocyaninato)ruthenium(II) and Triruthenium Dodecacarbonyl Bridged by 4,4'-Bipyridine as Ligand. Langmuir 25:11796-11801.

4. Patrick DA, Bakunov SA, Bakunova SM, Kumar EVKS, Lombardy RJ, Jones SK, Bridges AS, Zhirnov O, Hall JE, Wenzler T, Brun R, Tidwell RR. 2007. Synthesis and in Vitro Antiprotozoal Activities of Dicationic 3,5-Diphenylisoxazoles. J Med Chem 50:2468-2485.

5. Hussein AQ, El-Abadelah MM, Sabri WS. 1983. Heterocycles from nitrile oxides. I. 5-Imino-Δ2-1,4,2-oxathiazolines. J Heterocycl Chem 20:301-4.

6. Wei X, Fang J, Hu Y, Hu H. 1992. A convenient preparation of 3,5-diarylisoxazoles. Synthesis 1205-6.

7. Musante C. 1951. Some derivatives of pyrazole and isoxazole having antituberculous action. Farmaco (1946-1952) 6:32-8.

8. Stephens CE, Tanious F, Kim S, Wilson WD, Schell WA, Perfect JR, Franzblau SG, Boykin DW. 2001. Diguanidino and "Reversed" Diamidino 2,5-Diarylfurans as Antimicrobial Agents. J Med Chem 44:1741-1748.