Supporting Information

Shalev et al. 10.1073/pnas.1307365110

SI Materials and Methods

Materials. Geneticin (G418) and Apramycin were purchased from Apollo Scientific Limited as sulfate salts. Gentamicin sulfate was purchased from Molekula. All other chemicals and biochemicals, unless otherwise stated, were obtained from Merck or Sigma. RNA oligomers that were chemically synthesized and purified by denaturing polyacrylamide gel electrophoresis followed by desalting using solid phase extraction reverse-phase chromatography were purchased from Dharmacon and were used without further purification.

Structure Determination and Refinement. Data processing was performed with XDS (1) or Mosflm (2). Data collections and crystal data statistics are summarized in Table S1. Initial phases of both crystals were determined by molecular replacement using Phaser MR, CCP4 suite (3), using the coordinates of the bacterial A site in complex with Paromomycin (PDB ID code 1J7T) (4) or the human A site in complex with Apramycin (PDB ID code 2G5K) (5) as a search model. The molecular structures were constructed and manipulated with COOT (6). Data refinement has been performed using Refmac5, CCP4 suite, Phenix Refine (7), and CNS (8, 9). Structure refinement statistics are summarized in Table S1. Graphical representations were made using PyMOL (10).

Promastigote Viability Assay (LC_{50}). Compounds to be assayed were diluted in serial concentrations ranging from 0.7 to 2,000 μM (0.15–40 μM and 0.7–400 μM for G418 and Paromomycin, respectively) in a complete promastigote medium, and were aliquoted in triplicate (125 μL per well) into 96-well flat-bottom plates (Nunc). Promastigotes (2.0×10^6 cells per mL; 125 μL per well) were added to each well and incubated for 72 h at 26 °C. The alamarBlue viability indicator was added (25 μL per well) and the plates were incubated for an additional 5 h, at which time the fluorescence (λex = 544 nm; λem = 590 nm) was measured in a microplate reader (Fluoroskan Ascent FL). Complete medium was used as a negative control (0% inhibition of promastigote growth). Amphotericin B (1 μM, Sigma-Aldrich), a drug used to treat visceral leishmaniasis, was included as a positive control in each plate.

1. Kabsch W (2010) XDS. *Acta Crystallogr D Biol Crystallogr* 66(Pt 2):125–132.
2. Battye TG, Kontogiannis L, Johnson O, Powell HR, Leslie AG (2011) iMOSFLM: A new graphical interface for diffraction-image processing with MOSFLM. *Acta Crystallogr D Biol Crystallogr* 67(Pt 4):271–281.
3. Collaborative Computational Project, Number 4 (1994) The CCP4 suite: Programs for protein crystallography. *Acta Crystallogr D Biol Crystallogr* 50(Pt 5):760–763.
4. Vicens Q, Westhof E (2001) Crystal structure of paromomycin docked into the eubacterial ribosomal decoding A site. *Structure* 9(8):647–658.
5. Kondo J, François B, Urzhumtsev A, Westhof E (2006) Crystal structure of the Homo sapiens cytoplasmic ribosomal decoding site complexed with apramycin. *Angew Chem Int Ed Engl* 45(20):3310–3314.
6. Emsley P, Cowtan K (2004) Coot: Model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* 60(Pt 12 Pt 1):2126–2132.
7. Adams PD, et al. (2010) PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* 66(Pt 2): 213–221.
8. Brünger AT, et al. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr D Biol Crystallogr* 54(Pt 5): 905–921.
9. Brunger AT (2007) Version 1.2 of the Crystallography and NMR system. *Nat Protoc* 2(11):2728–2733.
10. The PyMOL Molecular Graphics System, Version 1.5.0.4.S.L. Available at www.pymol.org. Accessed July 1, 2013.

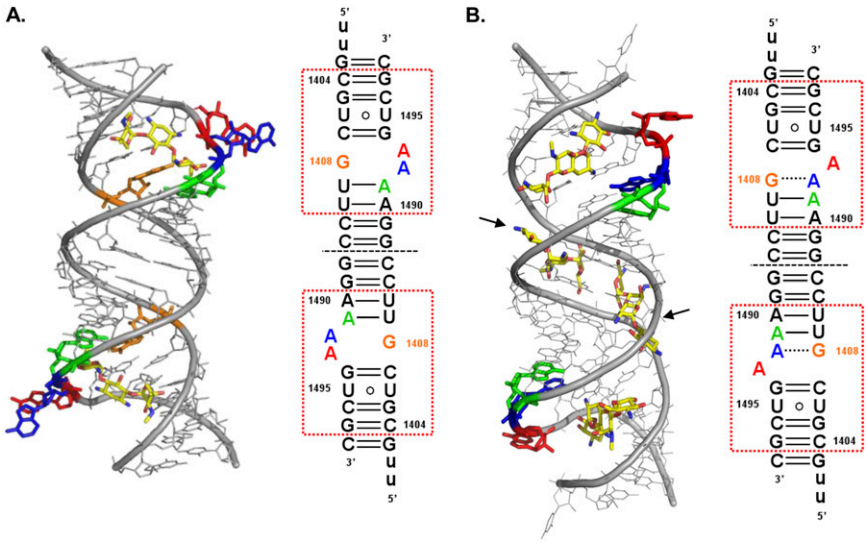

Fig. S1. Two-dimensional (*Right*) and 3D (*Left*) representations of the double A-site complexes as obtained in the X-ray crystal structures. (*A*) The double A-site construct in complex with G418 (PDB ID code 4K32): each A site binds one molecule of G418 (yellow). Both A sites are in the ON-state conformation where A1492 (blue) and A1493 (red) are bulged out from the helical core. (*B*) The double A-site construct in complex with apramycin (PDB ID code 4K31): each A site binds one molecule of apramycin (yellow). Two additional apramycin molecules can be observed in the G–C pairs region separating between the two A sites (marked in black arrows). Both A sites are in the OFF-state conformation where A1493 (red) is bulged out from the helical core and A1492 (blue) interacts with G1408 (orange) in a noncanonical manner. A1491 (green) forms a Watson–Crick pair with U1409 in both complexes.

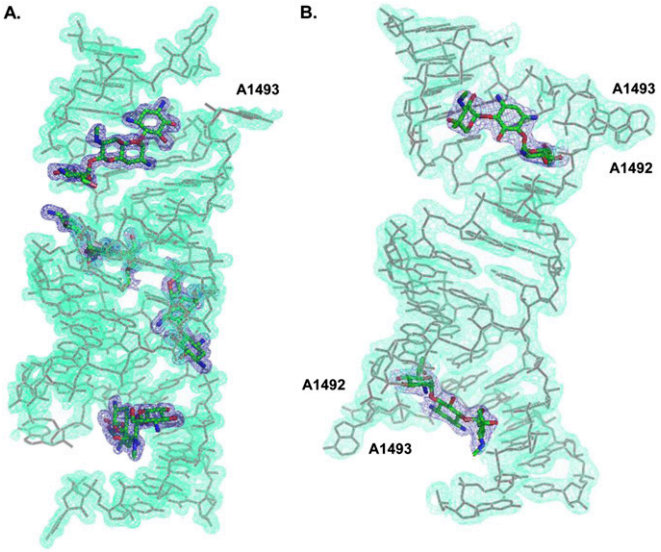

Fig. S2. Electron density maps of the Apra–Leish (*A*) and G418–Leish (*B*) structures. $2F_o - F_c$ maps are contoured at 1.0σ around rRNA molecules (cyan) and 1.5σ around ligand molecules (dark blue).

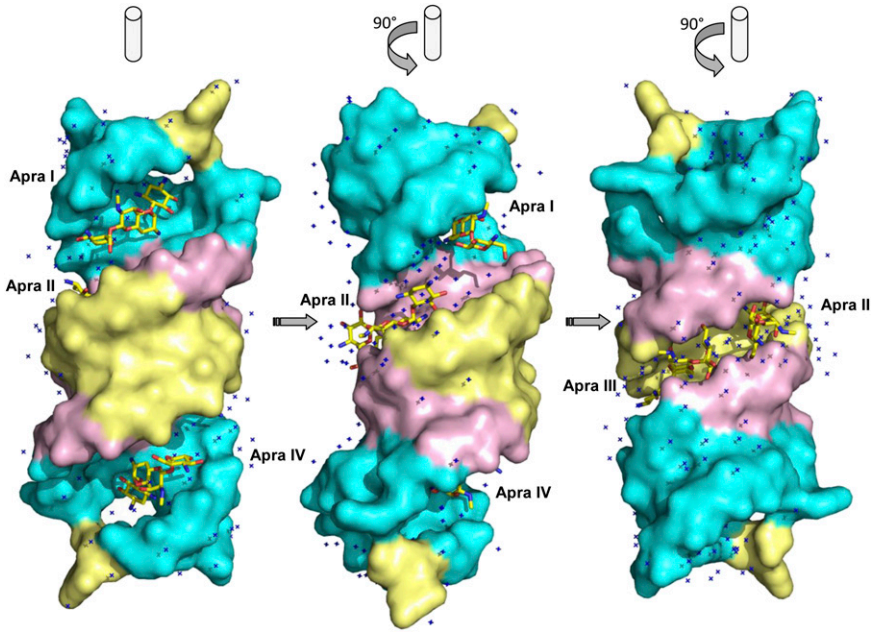

Fig. S3. Surface representation of apramycin bound to the double A-site rRNA construct corresponding to its putative binding site in leishmanial ribosomes. Apramycin molecules are represented as yellow sticks. The surface colors indicate residue conservation among prokaryotic and eukaryotic systems, where residues marked in cyan are highly conserved among all kingdoms, residues marked in light pink are rather diverse and residues marked in light yellow are non–A-site nucleotides that were used to stabilize the model. Double helices (left to right) are rotated by 90° around the helical core. Apramycin molecules are numbered I-IV (top to bottom).

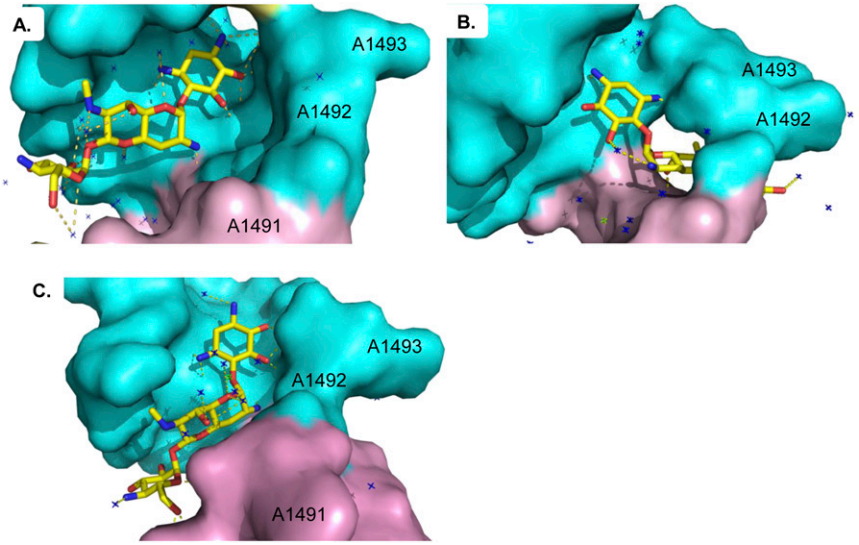

Fig. S4. Surface representation of apramycin bound to its binding site in *Leishmania* (as determined in this study) (*A*), bacterial (*B*), and human (*C*) ribosomal A sites. Apramycin molecules are represented as yellow sticks. The surface colors indicate residue conservation among prokaryotic and eukaryotic systems, where residues marked in cyan are highly conserved among all kingdoms and residues marked in light pink are rather diverse. Potential polar interactions (most likely H bonds) are marked by yellow dashed lines. Crystallographically identified water molecules and monovalent ions are shown using crosses colored in blue or green, respectively. PDB ID codes for apramycin/leshmanial, apramycin/bacterial and human A-site structures are 4K31, 1YRJ, and 2G5K, respectively.

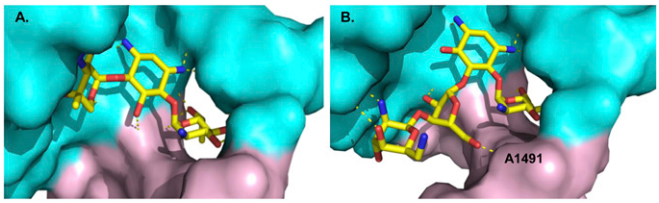

Fig. S5. Surface representations of aminoglycoside binding sites in leishmanial ribosomes. (*A* and *B*) The G418 position as crystallographically determined (*A*) and putative binding mode of Paromomycin as obtained by manual docking (*B*) based on this ligands binding to the bacterial ribosome. Aminoglycosides are represented in stick representation and highlighted in yellow. The coloring of the surface represent residue conservation among prokaryotic and eukaryotic systems, where residues marked in cyan are highly conserved among all kingdoms and residues marked in light pink are rather diverse. The Paromomycin position was obtained by superimposition of the A-site coordinates from the G418/leishmanial A-site structure (PDB ID code 4K32) and the paromomycin coordinates from the crystal structure of paromomycin bound to the bacterial A site (PDB ID code 1J7T). Figure was created with Pymol.

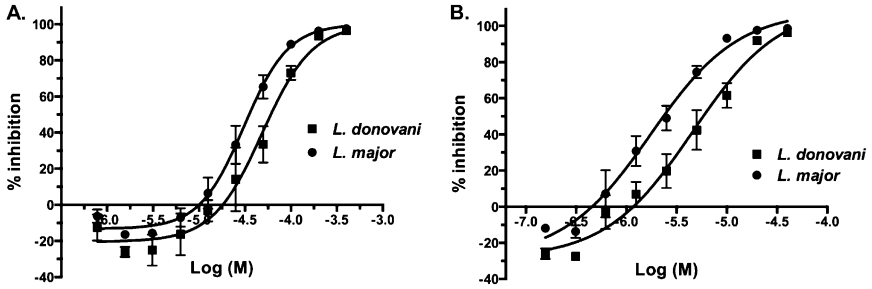

Fig. S6. Typical *L. major* and *L. donovani* growth inhibition curves used for the determination of LC_{50} for Paromomycin (*A*) and G418 (*B*).

Table S1. Crystal data, data collection, and data refinement statistics

	G418	Apramycin
PDB ID code	4K32	4K31
Crystal data		
Space group	$P2_12_12$	$P3_1$
Unit cell parameters, Å	a = 33.1 b = 90.8 c = 47.0	$a = b$ = 32.8 c = 107.4
Z*	1	1
Data collection		
Beamline	ID14-4 ESRF	ID14-4 ESRF
Wavelength, Å	0.9394	0.9394
Resolution, Å	23.5–2.65	35.8–1.4
Observed reflections	18,136	67,545
Unique reflections	3,615	23,324
Completeness, %	80.9	94.5
in outer shall	84.5	98.0
R_{merge}, %[†]	4.8	4.8
in outer shall	30.8	13.7
Mean, (I)/sd(I)	25.1	14.1
in outer shall	5.2	5.7
Multiplicity, %	5	2.9
in outer shell	3.2	2.6
Structure Refinement		
Resolution range, Å	23.5–2.65	35.8–1.4
Used Reflections	3,614	23,280
R-factor[‡]/R_{free}[§], %	21.4/24.7	22.9/25.1
Number of RNfA atoms	900	940
Number of amnoglycosides	2	4
Number of ions	—	1 K^+
Number of water molecules	11	250
rmsd		
Bond lengths, Å	0.006	0.007
Bond angles, °	0.9	0.9

*Number of RNA molecules in the asymmetric unit.

[†]R_{merge} = 100 x $\Sigma_{hklj}|I_{hklj} - \langle I_{hklj}\rangle| / \Sigma_{hklj} \langle I_{hklj}\rangle$.

[‡]R-factor = 100 x $\Sigma||F_o| - |F_c||/\Sigma|F_o|$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively.

[§]Calculated using a random set containing 10% of observations that were not included in refinement.