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Molecular Docking Studies on Streptomycin Antileishmanial Activity

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Abstract

Resistance to pentavalent antimonial drugs and the lack of vaccines make it urgent to find novel therapeutic options to treat Leishmaniasis, a tropical disease caused by the *Leishmania* protozoan parasite. The study reported here is to investigate if Streptomycin, an aminoglycoside, and Amphotericin B, the second-line treatment drug, exhibit antileishmanial activity through a similar mechanism. By using MOE (Molecular Operating Environment), we performed molecular docking studies on these drugs binding to a range of targets including ribosome targets in *Leishmania* and *H. sapiens.* Our study shows that the two drugs do not bind to the same pockets in *Leishmania* targets but to the same pockets in the human ribosome, with some differences in interactions. Moreover, our 2D maps indicated that Amphotericin B binds to the A-site in the human cytoplasmic ribosome, whereas streptomycin does not.

Keywords

Leishmaniasis, Streptomycin, Amphotericin B, Molecular Docking, Aminoglycosides, Antileishmanial

1. Background

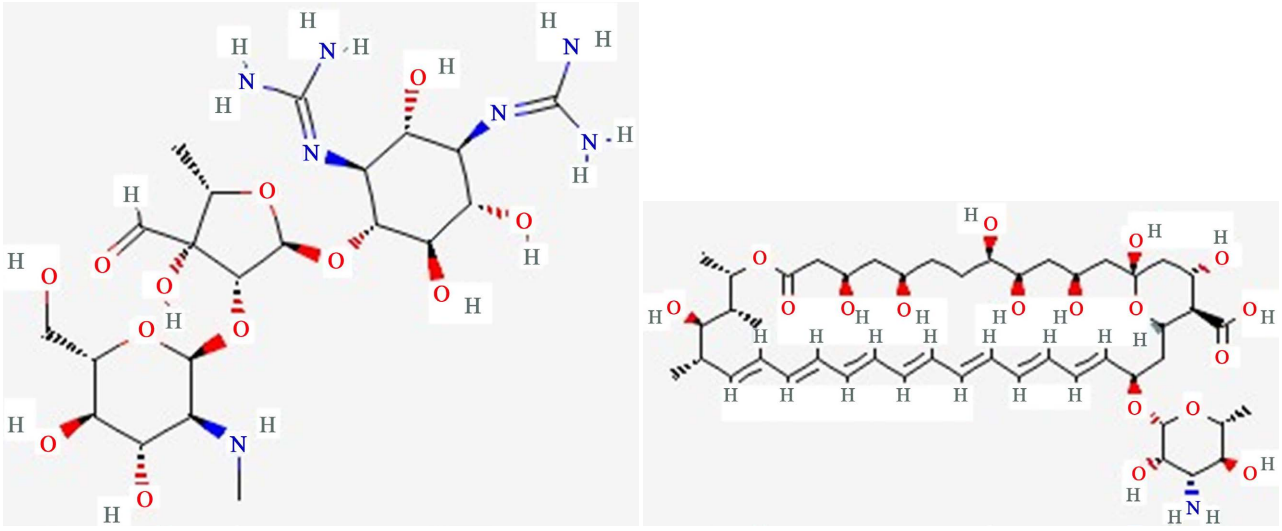
Leishmaniasis is a neglected tropical disease that puts at least 350 million people at risk and is one of the most important worldwide [1] [2] [3] [4]. The disease is caused by over 20 species of *Leishmania*, a protozoan parasite transmitted by the *Phlebotomus* sand fly [2] [3]. Cutaneous (CL), Mucocutaneous (MCL), and a fatal form, Visceral (VL) are the three types of leishmaniasis [4]. Their common clinical symptoms are skin lesions, as well as the increased size of lymph nodes,

liver, and spleen. The flagellated promastigote, which is the mobile form of the parasite, lives in the sand fly and transforms into an immobile amastigote once it is phagocytized in the host immune cell (particularly macrophages) [3] [4] [5]. Essentially, *Leishmania* resides in the lysosome where they survive by switching off immune functions of the cell [6]. Amastigotes multiply, eventually rupturing the cell, and then spread to new cells [6].

Amphotericin B, Miltefosine, and Sodium Stibogluconate have been used as standard treatment for leishmaniasis [1] [7] [8] [9]. In addition, Paromomycin as an antibiotic became a new recommended drug for treating leishmaniasis [3] [9] [10] [11]. More specifically, the antileishmanial activity of paromomycin is thought to be primarily because of the -OH group in the 6' position in Ring I [10]. Paromomycin binds directly at helix 44 in the decoding site, causing a flipped-out conformation of residues. This conformation interferes with translation (tRNA is not recognized properly) and causes misreading of the [10] [11]. Those current chemotherapeutic drugs target the amastigote form of the parasite, however there is evidence showing that the susceptibility of different drug may vary with *Leishmania* spp. For example, the resistance to pentavalent antimonial drugs and immunosuppression in co-infections with HIV, have lowered treatment efficacy [12] [13] [14]. Amphotericin B has been used to treat VL caused by *L. donovani* as well as cases of MCL; however, it is administered slowly because of toxicity. The Food and Drug Administration approved lipid formulations have reduced toxicity but are costly [12] [15]. The aminoglycoside antibiotic Paromomycin has been shown to exhibit antileishmanial activity and Phase 2 trials have shown 90% of VL patients cured [12] [16]. Its mechanism of action is inhibition of protein synthesis by binding it to the ribosome [10] [11] [12]. Formulations of paromomycin are in development for CL [12]. In sum, treatment using Amphotericin B, antimonial drugs, and paromomycin are limited by resistance, high cost, and toxicity.

In brief, the reality is that none of those drugs can completely cure the illness. In addition, there are no effective vaccines for the illness either [12] [17] [18]. There is first, second, and third generation vaccines that have been developed but issues such as the broad scope of the problem, identification of appropriate candidate antigens and animal models, as well as cost have limited their effectiveness [17] [18] [19]. Even non-drug strategies such as vector management with nets have been only mildly successful [20]. There is also increasing concern regarding potential increases of infections due to climate change and increased urbanization [20] [21] [22]. This is especially concerning in poor, urban endemic areas [12]. More research is needed regarding leishmaniasis for not only a standard vaccine but new treatment strategies, which involves the discovery of new targets and new effective and low toxic drugs [12].

In this study, we propose streptomycin (molecular weight 581.6 g/mol), an aminoglycoside, as a potential drug candidate for leishmaniasis (**Figure 1(a)**). Krasner [23] observed that the drug inhibited the growth of *L. tarentolae* in culture and Katoof [24] showed that 20% streptomycin solution cured patients of



(a) (b)

Figure 1. Chemical structures of Streptomycin (left) [29] and Amphotericin B (right) [30].

cutaneous leishmaniasis. Streptomycin is well known as an antibiotic, but studies on its effects in eukaryotes such as protozoa are limited. Streptomycin consists of streptidine, a streptose sugar, and L-glucosamine. The streptidine component has a 6' -OH in the aminocyclitol similarly to paromomycin. Cole and Danielli [25] found that streptomycin-sensitive amoebae had ribosomes that showed a high affinity for streptomycin, thus the sensitivity to streptomycin was related to how well the compound was absorbed by the ribosomes [23]. There is some dispute as to where the primary action of aminoglycosides occurs in the parasite, namely the cytoplasmic ribosome or the mitochondrial ribosome. Maarouf *et al.* [26] concluded that paromomycin binds with the mitochondrial ribosome. Other studies determined that itis the cytoplasmic ribosome [10] [27] [28].

Amphotericin B (Amp B) (molecular weight 924.19/mol) is considered the best drug treatment for visceral Leishmaniasis as well as severe mycotic infections (**Figure 1(b)**) [8] [30] [31]. It is known that Amphotericin B binds to sterols in the lipid-bilayer; in this study, we evaluate its affinity to cytoplasmic and mitochondrial sites. Amp B exerts antileishmanial activity by binding to sterols such as ergosterol in the parasite cell membrane. The Amp B complex is hydrophobic and is stabilized by sterol; conversely, it is hydrophilic on the inside, consisting of multiple hydroxyl groups [31]. This results in pores forming in the membrane, thus killing the cell [8] [31] [32].

Recently, crystallographic studies have allowed the reconstruction of molecular targets for drug design [33] [34]. In addition, molecular docking can be used to determine the lowest energy conformation of the ligand-target complex, which indicates the most possible active binding conformation. Moreover, molecular docking software has helped to visualize ligand-receptor interactions through modeling in *Leishmania* [10] [33] [34]. Parasite targets such as Leishmanolysin zinc-metalloprotease (GP63), a major virulent factor for Leishmania-

sis and Kinetoplastid Specific Ribosome Protein (KSRP), a scaffold for *Trypanosome* ribosomes, have been investigated using cryo-electron microscopy modeling [34] [35].

It is somewhat unclear whether streptomycin (and other aminoglycosides) exert antileishmanial activity at the ribosome of the cytoplasm or the ribosome of the mitochondria. Both streptomycin and Amphotericin B are good probes for determining precisely where aminoglycosides exert their activity. We hypothesized that both drugs exhibit antileishmanial activity through a similar mechanism, meaning that they prefer to bind in the same binding site when interacting with their target. In this study, the two ligands streptomycin and Amphotericin B were docked into a group of targets, including the cytoplasmic ribosome, the mitochondrial ribosome, GP63 and KSRP to investigate if there are targets with which the streptomycin and Amphotericin B bind to the same binding site with similar interactions.

2. Materials and Methods

In this theoretical study, the two compounds streptomycinand Amphotericin B were docked into a group of targets, including the cytoplasmic ribosome, the mitochondrial ribosome, GP63 and KSRP.

All molecular docking studies were conducted using Molecular Operating Environment (MOE) 2019.01; Chemical Computing Group ULC, 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2019 [36]. The software was operated using Microsoft Windows 7 Professional operating system on an Intel Xeon 3.40 GHz dual processor with 64.0 GB memory.

The two compounds (Streptomycin and Amphotericin B) were built using the Builder module, with the energy minimized, and stored in the software.

Seven target molecules were selected for the study and their names and PDB IDs were listed in **Table 1**.

Bacterial, Human and *Leishmania* targets were selected for comparison. We selected *Leishmania* and *H. sapiens targets* (cytoplasmic and mitochondrial ribosome) because of their homology (In fact drug interaction may explain ototoxic effects in humans) [37] [38]. Because it is known that streptomycin targets the bacterial ribosome, and its structure is not like eukaryotes, we used it in our study as a control (Mycobacteria) [28] [38]. GP63 is expressed on the surface of *Leishmania* and plays a critical role in virulence. We selected it along with the novel *Leishmania* ribosome protein, Kinetoplastid Specific Ribosome Protein [34] [35].

In our computational model, the oxygen atoms were colored red, the nitrogen atoms were colored blue, and the carbon atoms were colored grey. The carbon atoms in the drugs were highlighted as green to distinguish binding.

Water molecules were removed in the Sequence Editor and the active site of each target was determined using Site Finder module. Dummy atoms were created from alpha spheres and the top five atom/residue sizes were selected for ligand-target interaction calculations. The lowest free energy was calculated by

Table 1. Names and PDB IDs of the selected targets.

Name	PDB ID
Leishmania Cytoplasmic rRNA	4K31
Homo-sapiens Cytoplasmic rRNA	2G5K
Leishmania Mitochondrial rRNA	3JCS
Homo-sapiens Mitochondrial rRNA	3BNN
Mycobacterium ribosome	5XYU
GP63	1LML
Kinetoplastid Ribosome Protein	5OSG

the Triangle Method and Rescoring 1: London dG [34] [39] [40]. The five lowest energy poses were kept and the interactions between the ligand and target were further analyzed.

3. Results and Discussion

Tables 2-8 list the lowest energy docking complex of the two ligands binding in the different targets. We compared the binding affinity of streptomycin and Amphotericin B to the selected cytoplasmic and mitochondrial targets. We found that streptomycin and amphotericin only bind in the same pockets preferentially on human targets (2G5K, 3BNN) but not the *Leishmania* targets (4K31, 3JCS) (Tables 2-5).

Moreover, the two compounds did not bind at the same pockets preferentially in the *Mycobacterium* ribosome (5XYU), GP63 (1LML) or the Kinetoplastid Specific Ribosome (5OSG) (Tables 6-8). Figure 2 shows the 3D diagrams and the closeup views illustrating the streptomycin and Amphotericin B bound in Pocket 1 of *Homo-sapiens* cytoplasmic rRNA (2G5K). Both drugs bind to the similar location on the target. Interestingly, 2D maps indicated that both streptomycin and Amphotericin B bind at the A-site in *Homo-sapiens* cytoplasmic rRNA (2G5K) preferentially in pocket 1 (Figure 3). Specifically, surrounding the A-site residue 39, Amphotericin B interacted with residues 4 - 10 and 32 - 40. Streptomycin interacted with residues 7 - 11, 35 - 38, and 40. Figure 4 shows the 3D ribbon diagrams and the closeup views illustrating the streptomycin and amphotericin B bound in Pocket 1 of *Homo-sapiens* mitochondria rRNA (3BNN). 2D maps also indicated that streptomycin and Amphotericin B bind in *Homo-sapiens* mitochondria rRNA (3BNN) preferentially in pocket 1 (Figure 5). Amphotericin B interacted with residues 4 - 12 on A chain and 6 - 15 on chain B, which covers the A-site residue 16 on chain B. Streptomycin interacted with residues 7 - 13 and 6 - 15, and basically away from the A-site on both chains.

Based on these screening results of the selected seven targets, we narrowed down the preferential targets of both streptomycin and Amphotericin B to *Homo-sapiens mitochondria rRNA* and *Homo-sapiens* cytoplasmic rRNA.

Table 2. Top Binding Scores of Amp B and Strep bind in *Leishmania* Cytoplasmic rRNA (4K31).

Amp B 4K31	Pocket	Size	Strep 4K31	Pocket	Size
−9.5150	1	287	−9.4493	2	85
−9.1718	2	85	−8.9408	1	287
−8.3044	4	7	−7.2215	7	16
−7.9928	3	11	−7.1268	3	11
−8.9086	5	8	−6.9539	4	7

Table 3. Top Binding Scores of Amp B and Strep bind in *Homo-sapiens* Cytoplasmic rRNA (2G5K).

Amp B 2G5K	Pocket	Size	Strep 2G5K	Pocket	Size
−9.4166	1	431	−9.5038	1	431
−9.2217	5	39	−9.3125	3	42
−8.7914	4	38	−9.1567	2	43
−8.3146	6	17	−7.9291	5	39
−8.0008	7	4	−7.5662	4	38

Table 4. Top Binding Scores of Amp B and Strep bind in *Leishmania* Mitochondria rRNA (3JCS).

Amp B 3JCS	Pocket	Size	Strep 3JCS	Pocket	Size
−5.1662	2	36	−3.9663	16	29
−5.1239	1	54	−3.8804	1	54
−5.0242	14	29	−3.8775	2	36
−4.6723	4	36	−3.6897	4	20
−3.7957	7	19	−3.6524	5	36

Table 5. Top Binding Scores of Amp B and Strep bind in *Homo-sapiens* Mitochondrial rRNA (3BNN).

Amp B BNN	Pocket	Size	Strep 3BNN	Pocket	Size
−9.8324	1	241	−9.3799	1	241
−8.6298	2	27	−9.1375	5	11
−8.6015	6	19	−8.5765	4	30
−8.4047	4	30	−8.2261	3	33
−8.1414	3	33	−8.0428	2	27

Table 6. Top Binding Scores of Amp B and Strep bind in *Mycobacterium* ribosome (5XYU).

Amp B XYU	Pocket	Size	Strep 5XYU	Pocket	Size
−11.4038	4	412	−9.2959	8	406

Continued

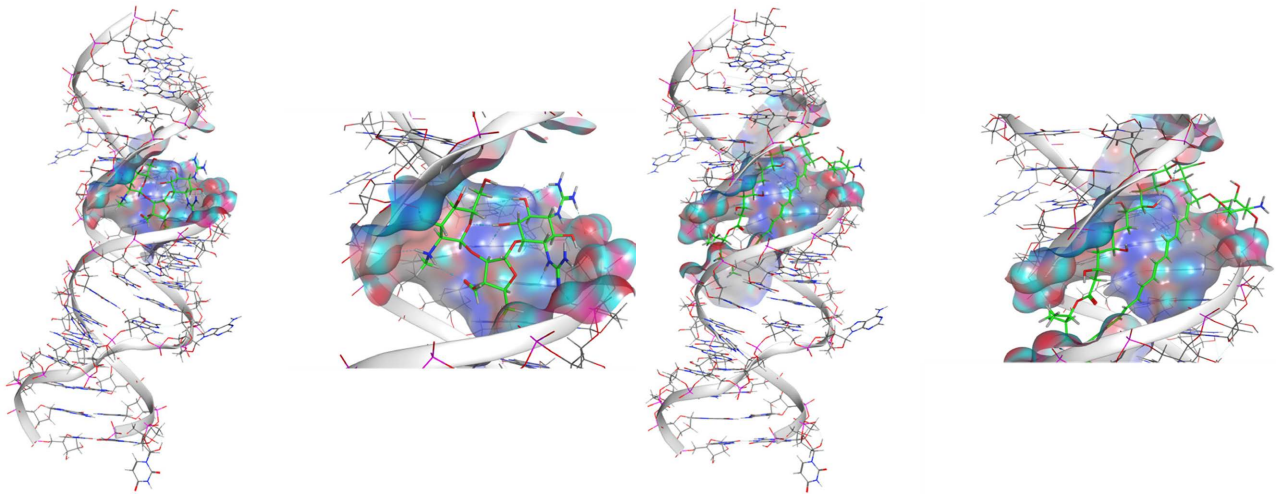
−11.4636	13	225	−9.1717	9	342
−11.4038	3	618	−8.8174	15	150
−11.2420	8	406	−8.7250	20	111
−10.9383	29	93	−8.6965	13	225

Table 7. Top Binding Scores of Amp B and Strep bind in Leishmanolyin GP63 (PDB id 1LML).

Amp B LML	Pocket	Size	Strep 1LML	Pocket	Size
−15.8880	12	20	−7.1208	8	22
−15.4921	1	214	−6.4338	7	17
−13.9027	24	30	−6.2473	1	214
−13.4397	2	22	−6.1527	9	46
−13.2043	17	14	−5.7204	23	18

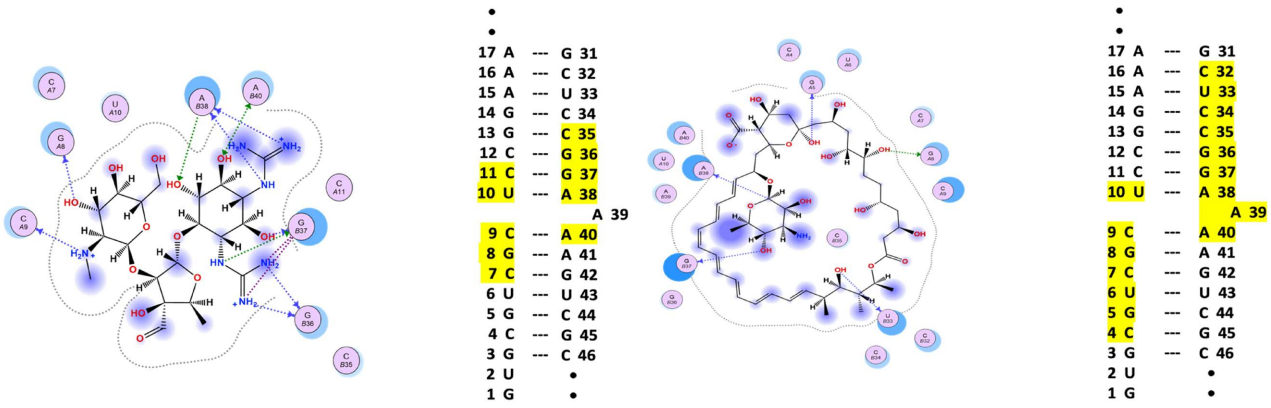
Table 8. Top Binding Scores of Amp B and Strep bind in Kinetoplastid Specific Ribosome Protein (5OSG).

Amp B 5OSG	Pocket	Size	Strep 5OSG	Pocket	Size
−9.5004	1	1176	−8.1102	2	109
−9.1523	4	58	−7.9978	5	81
−8.8866	5	81	−7.8831	1	1176
−7.9667	2	109	−6.9908	4	58
−7.8802	9	46	−6.8994	9	46



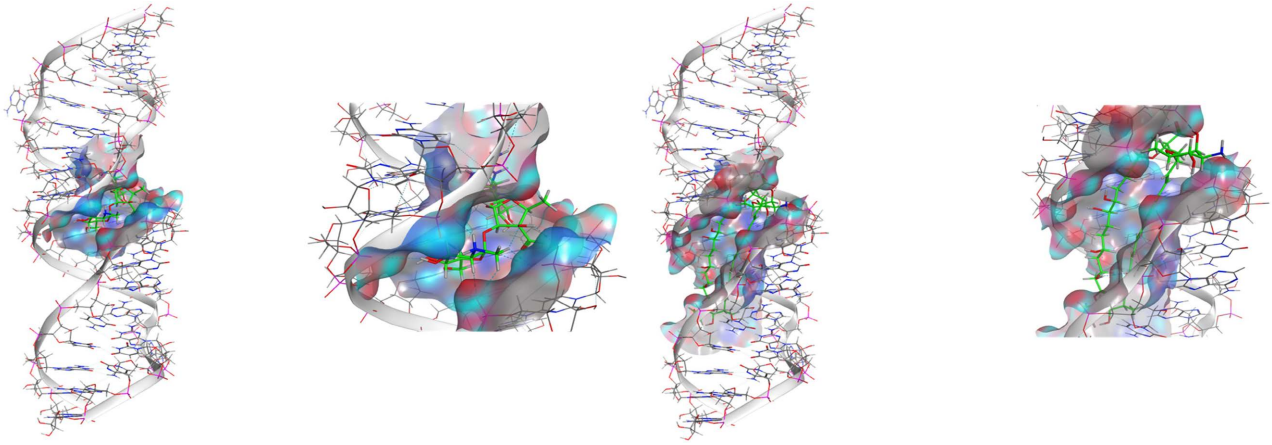
(a) (b)

Figure 2. Ribbon diagrams and the closeup views illustrating the streptomycin (a) and Amphotericin B (b) bound in Pocket 1 of *Homo-sapiens* cytoplasmic rRNA (2G5K). For emphasizing the relative binding location of drugs in target the pocket was presented with space filling model and all carbon atoms of both drugs are highlighted green.



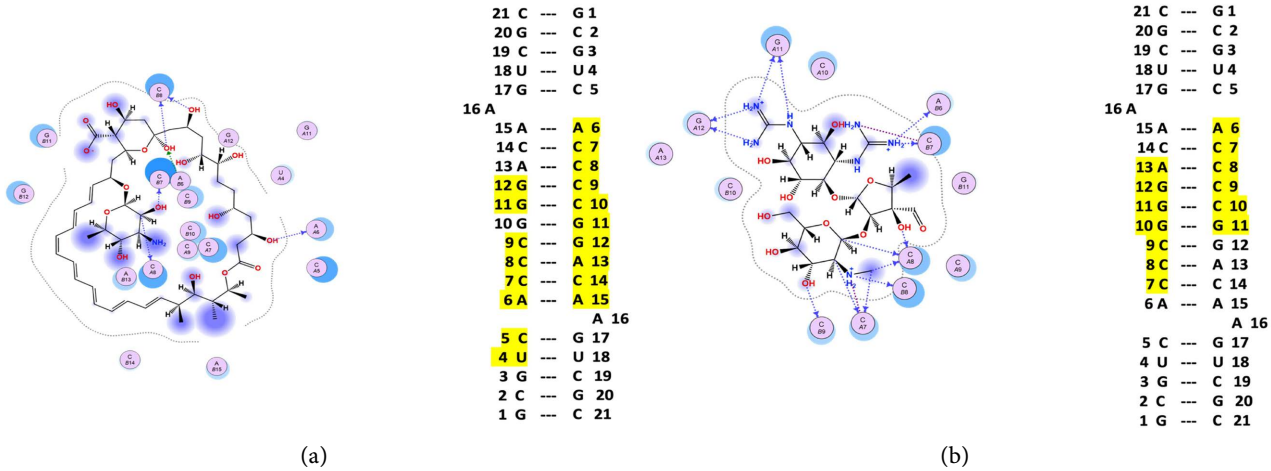
(a) (b)

Figure 3. 2D diagrams of interactions between streptomycin (a) and Amphotericin B (b) with *Homo-sapiens* cytoplasmic rRNA (2G5K) in Pocket 1.The sequence has been shown on the right with residues interacting with ligand highlighted yellow.



(a) (b)

Figure 4. Ribbon diagrams and the closeup views illustrating the streptomycin (a) and Amphotericin B (b) bound in Pocket 1 of *Homo-sapiens* mitochondria rRNA(3BNN). For emphasizing the relative binding location of drugs in target the pocket was presented with space filling model and all carbon atoms of both drugs are highlighted green.



(a) (b)

Figure 5. 2D diagrams of interactions between streptomycin (a) and Amphotericin B (b) with *Homo-sapiens* mitochondria rRNA (3BNN) in Pocket 1. The sequence has been shown on the right with residues interacting with ligand highlighted yellow.

Streptomycin and Amphotericin B both attack the same binding sites on these human targets. It is important to note that our molecular docking results indicate that streptomycin and Amphotericin B do not preferentially bind to the same pocket in *Leishmania.* This is plausible because the mechanism of action for both drugs is not the same. Amphotercin B targets ergosterol in the *Leishmania* plasma membrane [8] [31] [32]. Moreover, streptomycin and Amphotericin B do not bind to the same binding sites to the bacteria target in our study, as ergosterol is not found in the plasma membrane of bacteria [41]. However, streptomycin is a common antibiotic used against gram-negative bacteria [42]. In addition, streptomycin and Amphotericin B did not bind similarly to our other parasite targets, GP63 and the Kinotoplastid Specific Ribosome. We therefore conclude that both drugs dock similarly to both human targets, and not the other selected targets.

Streptomycin and Amphotericin B are current drugs used for treatment for antibacterial and antifungal infections, respectively. Our results show that streptomycin has less interaction with the A-site in human ribosomal RNA (**Figure 3** and **Figure 5**). Specifically, our 2D maps indicate that Amphotericin B binds to 6 more residues (covers/on A-site) to the RNA in the cytoplasm, however, streptomycin does not touch the A-site residue in the cytoplasmic RNA. To our knowledge, this is a novel finding using molecular docking analysis. Nephrotoxicity from Amphotericin B is dose-dependent and is absorbed poorly upon administration [43]. Lipid formulations of Amphotericin B are used to overcome toxicity, but it is relatively expensive and is not as accessible, particularly in endemic areas [43]. Streptomycin is a potential drug candidate along with paromomycin, which is now used in combination with Amphotericin B for the treatment of Leishmaniasis [43] [44] [45]. In fact, drug combinations using paromomycin loaded into nanoparticles have reduced parasite burden in both *in vitro* and *in vivo* studies that were more effective than the use of liposomal Amphotericin B and miltefosine alone [45] [46]. Thus, our group and others have given more attention to aminoglycosides and the use of novel drug-delivery methods to overcome toxicity and poor absorption in treatment.

The subtle difference in interactions to the human ribosome of both drugs should be studied further to investigate whether they have clinical implications such as absorption and toxicity.

4. Conclusion

Our results show that streptomycin and Amphotericin B bind to the same pockets in the ribosomes of the human mitochondria and cytoplasm. Future studies should further evaluate the differences in interactions at these pockets to assess if there are any clinical implications.

CRediT Author Statement

Todd A. Young: Conceptualization, Methodology, Investigation, Writing-Original

draft preparation. **Matthew George Jr.:** Conceptualization, Writing-Reviewing and Editing. **Ayele Gugssa:** Methodology. **William M. Southerland:** Funding acquisition. **Yayin Fang:** Conceptualization, Investigation, Visualization, Writing-Reviewing and Editing. **Clarence M. Lee:** Conceptualization, Supervision, Writing-Reviewing and Editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that there are not known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All data in the publication are available upon reasonable request.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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