Natural Antiparasitic Coumarins: An *In Silico* Study

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Question

What natural coumarins show the best antiparasitic activity in Leishmania and Trypanosoma parasites?

Introduction

Parasitic infections, namely leishmaniasis and trypanosomiasis, affect millions of people worldwide (WHO, 2012b). These two diseases hit the poverty-stricken the hardest. Leishmaniasis is caused by the leishmania parasite, and is found mainly in South America, the Middle East, and Africa, where poverty is widespread (WHO, 2012a). Epidemics of this disease have seriously impeded socioeconomic development in the Amazon basin, Andean countries, Morocco, and Saudi Arabia (WHO, 2012a). American trypanosomiasis, also known as Chagas disease, is a disease caused by the parasite *Trypanosoma cruzi*, and affects approximately 10 million people, mainly in Latin America (WHO, 2012b). These diseases are both cured easily if treatment is administered soon after the infection begins, but treatment can be expensive, and therefore unavailable to most of the infected population. Coumarins are a class of compounds which have been found, in many scholarly studies, to have antiparasitic activities. Those antiparasitic coumarins which are found in nature are especially important, as they would help reduce the cost of treatment, thus allowing more people to be cured.

In order to find which coumarins would be best to use for the treatment of these infections, the coumarins (ligands) are docked into various protein targets and the Rerank Scores, among other data, are reported by the Molegro Virtual Docker (MVD). The Rerank Score is a unitless measurement which is an estimate of the strength of the interaction between a ligand and a protein (Molegro, 2011). The larger the negative value of the Rerank Score, the better the strength, or binding affinity. With a larger binding affinity, a ligand will reside for a longer time within the protein, and cause a biological response within the protein, which in this case, will hopefully be to cure the infected person of the infection. Therefore, during this research, the ligands, or coumarins, with the highest Rerank Scores will be those most closely examined, as they will most likely be the best compounds for curing parasitic infections.

Materials and Methods

To begin this research, first I began by looking up scholarly articles online using the keywords "coumarins and Leishmania" or "coumarins and Trypanosoma." After skimming through many articles, several were downloaded and examined more thoroughly for the antiparasitic information detailed within. Only coumarins which came from natural sources and which had experimental antiparasitic activities, whether IC_{50}, LD_{50}, EC_{50}, or MC_{100} values with respect to each parasite, were documented in a Microsoft Excel spreadsheet. Once all articles were completely examined, 34 coumarins in total were documented in the spreadsheet.

After the spreadsheet was created, each coumarin was modeled in the SPARTAN computational chemistry software using the tools in it. Care was taken to ensure that the correct stereochemistry was modeled, as incorrect stereochemistry could lead to inaccurate results.

Materials and Methods (cont.)

When each coumarin had been modeled, the same several tests were run on each coumarin, computationally, using SPARTAN. The first test was to find the lowest energy conformation of each coumarin. To find this, I used the "Equilibrium Conformer" calculation, as well as using the "Molecular Mechanics" and "MMFF" settings, then run the test. The second test was to find the optimized geometry for each coumarin. To find this, I used the "Equilibrium Geometry" calculation, as well as using the "Semi-Empirical" and "AM1" settings, then run the test while having the "QSAR," "Orbitals & Energies," and "Charges & Bond Orders" settings all checked.

Once each coumarin had been modeled and tested in SPARTAN, I moved on to docking each coumarin with several relevant protein targets, each a different protein in one of the two types of parasites being studied. This docking process took place in a program called Molegro Virtual Docker (MVD). First, each coumarin was uploaded to MVD and converted to a ligand and prepared, which could then be placed in the cavities in the proteins. Next, the protein target was loaded into MVD, and the docking process began. Each of the 34 coumarins was docked with each cavity of the protein target, using 30 runs per coumarin and using the "energy minimization" setting. Once the docking for each cavity had been completed, MVD produced many results, the most relevant of which for us was the "Rerank Score." The highest Rerank Score for each coumarin for each cavity was then documented in a separate spreadsheet.

The final part in the research was to be a QSAR analysis, which correlates electronic structures with the activities of the molecule. Unfortunately I was not able to complete this step in the research by this time.

Results

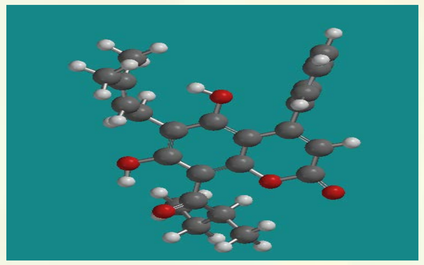

Figure 1. Image of (-) mammea A/BB, one of the coumarins examined, modeled using the SPARTAN software. Black spheres indicate carbon atoms, red spheres indicate oxygen atoms, and white spheres indicate hydrogen atoms. All coumarins were modeled similarly.

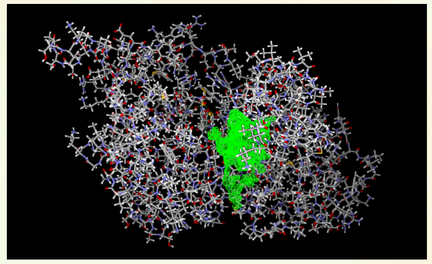

Figure 2. Image of 1a7k protein, found in *L. mexicana*, as shown in MVD. The green area indicates a cavity where ligands, i.e. coumarins, would be docked. Proteins had anywhere from one to three cavities.

Figures 1 and 2 show images of what a typical modeled coumarin and a protein target would look like in SPARTAN and MVD, respectively.

Of the 34 coumarins and their respective antiparasitic activities found in the scholarly articles, (-) mammea A/BB was the most effective, with an IC_{50} value of 0.88 µg/mL for the *L. amazonensis* amastigote. Auraptene, umbelliprenin, and cycloisobrachycoumarinone epoxide were also very effective against various parasites, with IC_{50} values of 5.10, 4.90, and 4.59 µg/mL, respectively. Among the least effective of these was coumarin, with an IC_{50} value of 100.11 µg/mL against the *T. cruzi* trypomastigote.

Results (cont.)

When I looked at the Rerank Scores compiled in the spreadsheet, several coumarins stood out to me. Of the 74 cavities total, umbelliprenin had the highest Rerank Score for 36 of them. Mammea A/AA had seven of the highest Rerank Scores, galbanic acid had six of the highest, and auraptene had four of the highest. Mammea A/AA had the highest score overall, with a score of -137.694 for cavity two of the 3kfl protein found in *L. major*, and umbelliprenin had the second and third highest scores of -131.239 and -124.377, for cavity one of the 1e7w protein found in *L. major* and cavity one of the 20eg protein also found in *L. major*, respectively.

Conclusion

The results show that the *in silico* tests back up the scholarly experiments in many cases, such as umbelliprenin and auraptene. Umbelliprenin had the highest Rerank Score for almost half of the protein cavities, which means that its binding affinity is high for many proteins. Due to its high binding affinity, it has a longer residence time in the protein, which can produce a biological response that causes the disease caused by the parasite to be cured. By using the coumarins that produced the greatest Rerank Scores for each respective protein, antidotes can be created to fight parasitic infections. Since all of the coumarins found in the scholarly articles originated from natural sources, not in a laboratory, these antidotes can be created at a lower cost, and can benefit those who need it most, the poor, those without proper nutrition, and those without well-built homes. With further research, including the QSAR analysis, hopefully more can be learned about curing these deadly diseases.

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