Award Number: W81XWH-14-2-0135

TITLE: Arylimidamide-Azole Combinations against Leishmaniasis

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REPORT DATE: September 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| September 2017 | Annual | 28 AUG 2016 – 27 AUG 2017 |

**4. TITLE AND SUBTITLE**

Arylimidamide-Azole Combinations against Leishmaniasis

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-14-2-0135

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)**

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**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release; distribution is unlimited.

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Arylimidamide compounds DB2342, DB2332, DB2336, and AA2-160 which had previously demonstrated *in vitro* potency as sole agents against the amastigote-macrophage form of *Leishmania* parasites known to cause cutaneous and visceral leishmaniasis (*L. major and L. donovoni*) were tested for *in vivo* toxicity with the purpose of determining the maximum tolerated dose. Compound DB2342 but not DB2332, DB2336, and AA2-160 caused mild toxicity in BALB/c mice when dosed respectively at 140, 140, 140, and 160 mg/kg. *In vivo* testing of these compounds using an assay that determines inhibition of cutaneous infection induced by *L. major* showed that D2336, DB2342, DB2332, and AA2-160 had no *in vivo* efficacy in the Mouse Leishmania Lesion Suppression test (MLS). As a result none of these compounds was progressed for efficacy in the *in vivo* Mouse Lesion Cure model. Arylimidamide compounds AA3-26, AA3-20, AA2-185, AA3-34, and AA3-29 demonstrated high *in vitro* efficacies as sole agents against *L. major* and *L donovani* yielding $IC_{50}$ values ranging from 134.6 ÷ 1134 ng/ml. Some of these compounds are scheduled for *in vivo* testing later this year. Due to the fact that none of the Arylimidamide compounds demonstrated *in vivo* efficacy, no Arylimidamide-Azole combinations were tested in the *in vivo* models of CL during the past year. PI on this project applied and was granted a one year NCE.

**15. SUBJECT TERMS**

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
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## 1. INTRODUCTION:

Existing oral treatments of visceral and cutaneous leishmaniasis (VL and CL) have significant drawbacks to include serious side effects, variable efficacy, and expense. Intravenous treatment with liposomal amphotericin B (AmBisome) is expensive, lengthy, and impractical for deployed soldiers (treatment requires 21 days of intermittent IV therapy in a hospital setting). An inexpensive oral treatment for both VL and CL that provides consistent efficacy against all species of *Leishmania* that infect man is a clear unmet need. This proposal is focused on a group of arylimidamide compounds which showed initial potency against visceral leishmaniasis *in vitro* and efficacy against visceral leishmaniasis *in vivo*. These compounds also showed interesting synergy with azoles which enhanced the efficacy of the arylimidamide compounds. The element of work performed at WRAIR encompasses the testing of these arylimidamide analogues against species of *Leishmania* that cause CL.

## 2. KEYWORDS:

leishmaniasis, cutaneous, visceral, arylimidamide

## 3. OVERALL PROJECT SUMMARY:

1) Evaluation of the antileishmanial efficacy and pharmacokinetics of Arilymidamide-Azole combinations

2) Characterization of the biochemical effects of Arilymidamide-Azole combinations on Leishmania.

## 4. KEY RESEARCH ACCOMPLISHMENTS:

Key Research Accomplishments

*In vitro* potency of A1A compounds as sole agents was demonstrated in an amastigote macrophage assay showing very promising $IC_{50}$s ranging from 190-1134 ng/ml against *L. major and L. donovani.* Several of these compounds will be tested for *in vivo* efficacy beginning in late 2017.

Accomplishments in Support of the Statement of Work

1a. Assess of *in vitro* efficacy of newly synthetized A1A compounds against intracellular *Leishmania.*

*In vitro* potency testing[2,4] (see Appendix Table 1 for detailed data) of A1A compounds demonstrated strong potency of these compounds as sole agents against *L. major* and *L. donovani*. In collaboration with Dr. Karl Werbovetz several of these compounds will be chosen to test for *in vivo* efficacy during the first 6 months of the NCE period.

2a. *In Vivo* Compound Evaluations

*In vivo* testing was conducted in accordance with the WRAIR Cutaneous Leishmaniasis Drug Discovery Algorithm[1,5] (this is also described in the grant application) which involves testing of compounds with demonstrated potency and metabolic stability *in vitro* followed by *in vivo* testing of intraperitoneally dosed compounds for efficacy in a leishmania lesion suppression assay in immune-permissive BALBC/c mice (BALB/c mice are immune-permissive for Leishmania infection due to an imbalanced Th1/Th2 ratio) against *L. major* followed by testing of IP-dosed compounds for lesion cure against *L. major* in BALB/c mice. Compounds that have shown curative efficacy in Tier 1 are then progressed to a higher tier of study for lesion cure using oral dosing in BALB/c (Tier 2). Orally dosed compounds capable of curing lesions in BALB/c mice that pass Tier 2 testing are then assessed further through testing in immunocompetent Syrian Golden Hamsters (Tier 3). Lead compounds are also tested for efficacy against New World *Leishmania spp*. in a newly validated footpad model in BALB/c mice infected with *L. guyanensis*. Compounds that have progressed to Tier 3 are then assessed for preclinical studies using Ames testing, hERG assays, in vitro micronucleus assays, drug-drug interaction studies, liver enzyme induction assays, etc. Candidate drugs that survive this testing battery are deemed suitable for consideration for clinical testing in man.

Up to 500 mg compound DB2342, DB2332, DB2336, and AA2-160 were synthetized and assessed for *in vivo* toxicity and efficacy against Old World leishmanial parasites. Arylimidamide compounds DB2342, DB2332, DB2336, and AA2-160 had been previously tested and had shown to have good *in vitro* efficacy against *Leishmania spp.* Based on the CL testing stratedy and in the base animal protocol, compounds that have not been previously tested in humans are thoroughly tested for *in vivo* toxicity. Initial *in vivo* toxicity testing conducted with the purpose of determining the maximum tolerated dose showed that compound DB2342 but not DB2332 and DB2336 caused mild toxicity in BALB/c mice when dosed at 140 mg/kg. As a result a decision was made to test DB2342 at 80 mg/kg DB2332 and DB2336 at 140 mg/kg in the MLS screen. Subsequent *in vivo* testing was conducted to initially assess the potential of these compounds to inhibit *L. major* infection and lesion suppression in the MLS screen (method described in detail in the grant application). As shown in Figure 1, DB2342, DB2332, and DB2336 did not demonstrate efficacy in the lesion suppression model. Parasite load at the infection site remained high and comparable to the Vehicle Control group during the 10 day drug administration period as well as after treatment ended. Their efficacy was not different from the vehicle control group. Positive control Ambisome on the other side reduced the parasite load at the infection site below the limit of detection. In the 5 mice belonging to the AmBisome group the parasite load remained below the limit of detection until the last day of the study.

In a separate study, compound AA2-160 was also tested at 160 mg/kg for *in vivo* toxicity before the efficacy testing took place. This compound did not show *in vivo* efficacy against *L. major* in the MLS either (Figure 2).

## 5. IMPACT:

The assessment of A1A compounds showed *in vitro* but not *in vivo* efficacy. New A1A compounds that have shown *in vitro* efficacy will be tested for *in vivo* efficacy in BALB/c mice infected with *L. major* parasites in early 2018

## 6. CHANGES/PROBLEMS:

Because of the failure to demonstrate *in vivo* efficacy with Arylimidamide compounds as sole agents against the Old World *L. major* parasite in the MLS screen we did not conduct Arylimidamide-Azole Combinations efficacy testing during the past year. CPT Vesely has applied and granted a one year No-Cost Extension on the grant.

## 7. PRODUCTS:

No product developed yet.

## 8. PARTICIPANTS:

Collaborations under this grant include investigators at Ohio State University, Georgia State University, the University of South Florida, and the University of Kansas.

## 9. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

1- Caridha D, Parriot S, Hudson TH, Lang T, Ngundam F, Leed S, Sena J, Harris M, O'Neil M, Sciotti R, Read L, Lecoeur H, Hickman M, Grogl M. 2017. Use of Optical Imaging Technology in the Validation of a New, Rapid, Cost-Effective Drug Screen as Part of a Tiered In Vivo Screening Paradigm for Development of Drugs To Treat Cutaneous Leishmaniasis. Antimicrobial Agents and Chemotherapy 61.

## 10. INVENTIONS, PATENTS AND LICENSES

Nothing to report.

## 11. REFERENCES:


1. Grogl, M., Hickman, M. Ellis, W. Hudson, T. Lazo, J., Sharlow, E., Johnson, J., Berman, J., and Sciotti, R. Review: Drug Discovery Algorithm for Cutaneous Leishmaniasis. Am J Trop Hyg 88(2), pp. 216-221, 2013.

2. Khraiwesh, Mozna, Leed, Susan, Roncal, Norma, Johnson, Jacob, Sciotti, Richard, Smith, Philip, Read, Lisa, Paris, Robert, Hickman, Mark and Grogl, Max. Antileishmanial Activity of Compounds Derived from the Medicines for Malaria Venture Open Access Box Against Intracellular Leishmania major Amastigotes. American Journal of Tropical Medicine and Hygiene, published online 26 October 2015.

3. Canfield, C.J., Pudney, M., and Gutteridge, W.E. Interactions of Atovaquone and other antimalarial drugs against P. falciparum in vitro. Experimental Parasitology 80, 373-381, 1995.

4. Sharlow E, Leimgruber S, Murray S, Lira A, Sciotti R, Hickman M, Hudson T, Leed S, Caridha D, Barrios A, Close D, Grogl, M Lazo J. Auranofin Is an Apoptosis-Simulating Agent with in Vitro and in Vivo Anti-leishmanial Activity. ACS Chemical Biology, Dec 2013.

5. Caridha D, Parriot S, Hudson TH, Lang T, Ngundam F, Leed S, Sena J, Harris M, O'Neil M, Sciotti R, Read L, Lecoeur H, Hickman M, Grogl M. 2017. Use of Optical Imaging Technology in the Validation of a New, Rapid, Cost-Effective Drug Screen as Part of a Tiered In Vivo Screening Paradigm for Development of Drugs To Treat Cutaneous Leishmaniasis. Antimicrobial agents and chemotherapy 61.

## 12. APPENDICES:

In vitro potency of AA compounds against amastigote-macrophage forms of *L. donovoni* and *L. major.* The $IC_{50}$ values are shown along with the $R^2$ value demonstrating the goodness of fit of the IC50 values calculated.

| Lot Number | Molecular Weight | Amastigote L. major IC50 (ng/mL) | Amastigote L. major $R^2$ | Amastigote L. donovani IC50 (ng/mL) | Amastigote L. donovani $R^2$ |
|---|---|---|---|---|---|
| AA3-26 | 421.54 | 460.8 | 0.8874 | 734.6 | 0.8828 |
| AA3-30 | 449.59 | 134.6 | 0.9343 | 173.9 | 0.9503 |
| AA2-185 | 435.56 | 520.5 | 0.8832 | 165.5 | 0.9083 |
| AA3-34 | 435.56 | 190.2 | 0.9412 | 274 | 0.9529 |
| AA3-29 | 421.54 | 1134 | 0.9338 | 456.5 | 0.9631 |

Table 1: In vitro efficacies of A1A compounds against *L. major* and *L. donovani*

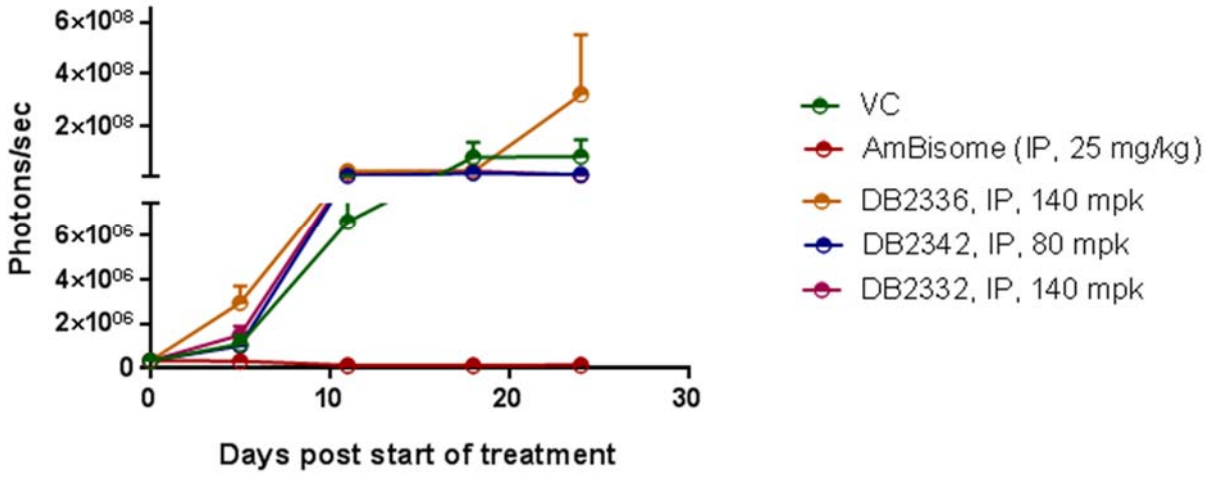


Figure 1: Progression of luminescence signal in BALB/c mice infected with $1X10^7$ stationary phase *L. major* promastigotes and treated IP with 140 mg/kg DB2336 and DB2332 and 80 mg/kg DB2342. Bars represent means ± SEMs for a total of 5 BALB/c mice. *In vivo* imaging results for leishmania suppression screen (MLS assay) were obtained using luciferase expressing *Leishmania major* parasites. Endpoints for this *in vivo* test include measurement of bioluminescence signal (in photons/second) in the vehicle and treated groups (which reflects the parasite burden over time) and the size of

the lesions in the vehicle and treated groups. The positive control (liposomal amphotericin B or AmBisome) was administered IP at 25 mg/kg.

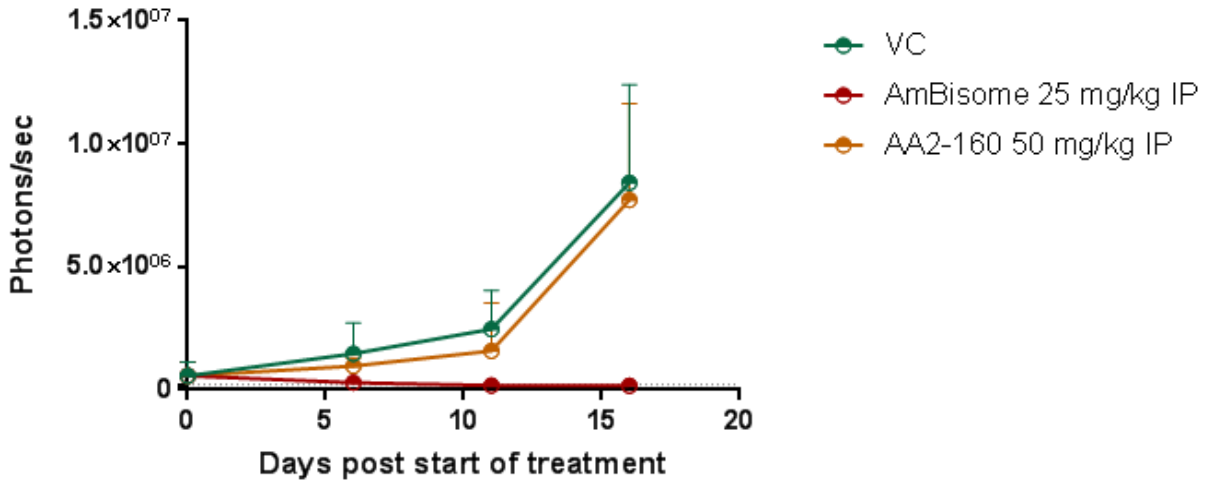


Figure 2: Progression of bioluminescence signal in BALB/c mice infected with $1X10^7$ stationary phase L. major promastigotes at the base of the tail and treated IP with 50 mg/kg AA2-160 compound. Bars represent means ± SEMs for a total of 5 BALB/c mice. In vivo imaging results for leishmania suppression screen (MLS assay) were obtained using luciferase expressing Leishmania major parasites. Endpoints for this in vivo test include measurement of bioluminescence signal (in photons/second) in the vehicle and treated groups (which reflects the parasite burden over time) and the size of the lesions in the vehicle and treated groups. The positive control (liposomal amphotericin B or AmBisome) was administered IP at 25 mg/kg.