Letter

The Identification of a Hit Series of Antileishmanial Compounds Through the Use of Mixture-Based Libraries

Marc A. Giulianotti, Brian Andrew Vesely, Ala Azhari, Ashley Souza, Travis LaVoi, Richard A. Houghten, Dennis E Kyle, and James W. Leahy

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.7b00045 • Publication Date (Web): 10 Jul 2017

The Identification of a Hit Series of Antileishmanial Compounds Through the Use of Mixture-Based Libraries

Marc A. Giulianotti†,‡, Brian A. Vesely§, Ala Azhari§, Ashley Souza§, Travis LaVoi‡, Richard A. Houghten‡, Dennis E. Kyle§* and James W. Leahy†,#,¶*

† Department of Chemistry, University of South Florida, CHE 205, 4202 E. Fowler Avenue, Tampa, FL 33620, United States.

‡ Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port St. Lucie, Florida 34987, United States.

§ Department of Global Health, College of Public Health, University of South Florida, 3720 Spectrum Boulevard, Suite 304, Tampa, Florida 33612, United States.

Florida Center of Excellence for Drug Discovery and Innovation, University of South Florida, 3720 Spectrum Boulevard, Suite 303, Tampa, FL 33612, United States.

¶ Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, MDC 7, 12901 Bruce B. Downs Boulevard, Tampa, FL 33612, United States.

KEYWORDS Leishmaniasis, Screening, Mixture-Based Libraries, Lead Optimization

ABSTRACT: From a screening campaign that included mixture-based libraries containing more than 6 million compounds, a lead series of bis-cyclic guanidines was identified as the most promising. Lead optimization resulted in the identification of potent (IC_{50} < 500 nM) and selective compounds within this series as well as potent and selective monoguanidines.

Leishmaniasis is a disease caused by infection with *Leishmania* parasites, which are spread by the bite of phlebotomine sand flies. The most severe form of the disease is visceral leishmaniasis caused by the protozoan parasites *Leishmania donovani* and *Leishmania infantum*.[1,2] People suffering from visceral leishmaniasis experience cachexia, cytopenia, fever, hepatosplenomegaly and death if not treated.[2] It is estimated that there are 200,000 to 400,000 new cases of visceral leishmaniasis each year and >20,000 deaths a year due to the disease.[3] Current therapeutics for patients with visceral leishmaniasis include liposomal amphotericin B (**1**), sodium stibogluconate (**2**), miltefosine (**3**), pentamidine (**4**) and paromomycin (**5**, Figure 1). All of these therapeutics are less than ideal through some combination of ease-of-use, efficacy, cost, safety, and drug resistance.[4] While much effort is underway on the development of new therapeutics, there is a pressing need to continue to identify novel compounds for the treatment of this disease.

We have previously reported on the use of mixture-based libraries to rapidly identify and prioritize active scaffolds.[5-7] Using this approach, we evaluated a scaffold-ranking library containing >6,000,000 compounds in a *L. donovani* axenic amastigote assay as well as a high-content imaging screen utilizing macrophages infected with *L. donovani* amastigotes. As previously described in detail, [5-7] a scaffold-ranking library is a collection of mixture samples where each mixture sample contains only compounds with the same core scaffold, and therefore all of the compounds in a given mixture are close structural analogs. Additionally, each compound in a specific mixture sample is represented in approximately equal molar concentration. From this initial screen, the bis-cyclic guanidine scaffold-ranking sample **Lib1** demonstrated potency in both of the aforementioned leishmaniasis assays with no sign of cytotoxicity in an uninfected macrophage counter screen. A positional scanning library was then screened in order to develop an SAR profile around the core bis-cyclic guanidine scaffold. From this analysis, 27 individual compounds were selected as good candidates to validate the profile.

Upon confirmation of the SAR trends with the individual compounds, a lead optimization campaign was undertaken to

Figure 1. Currently Used Antileishmaniasis Agents

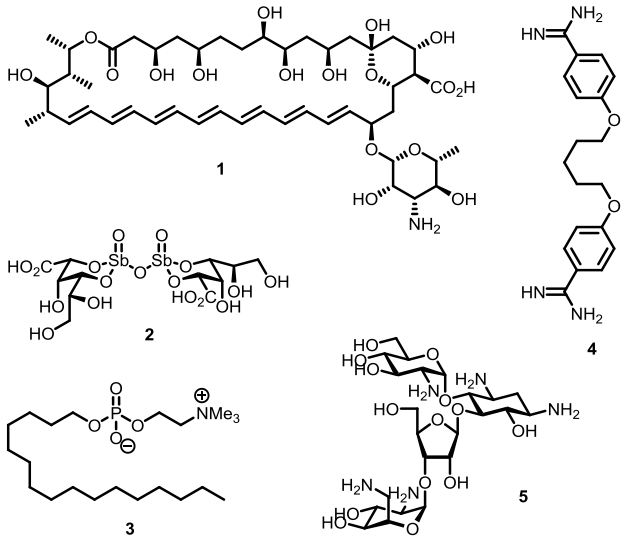
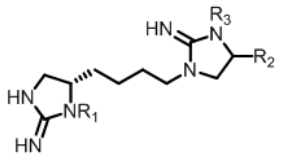
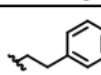
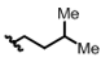
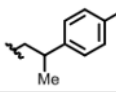
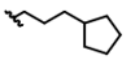
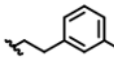
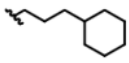
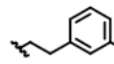
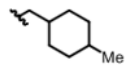
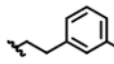
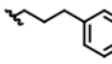
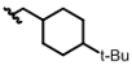
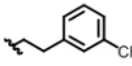
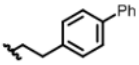
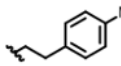
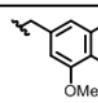
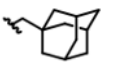
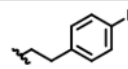
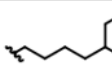
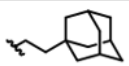
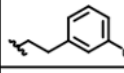
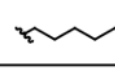
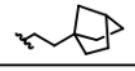


Table 1. Leishmaniasis Activity of R_1 Positional Scanning Study of Lib1



ID#	R_1	a.a.	i.m.	ID#	R_1	a.a.	i.m.	ID#	R_1	a.a.	i.m.	ID#	R_1	a.a.	i.m.
Lib1.001	H	0%	2.5	Lib1.012		0%	1.1	Lib1.023		10%	5.2	Lib1.033		0%	4.6
Lib1.002		69%	>10	Lib1.013		0%	0.9	Lib1.024		31%	>10	Lib1.034		61%	1.2
Lib1.003		94%	1.2	Lib1.014		99%	5.2	Lib1.025		91%	>10	Lib1.035		100%	1.2
Lib1.004		91%	>10	Lib1.015		66%	>10	Lib1.026		93%	0.9	Lib1.036		100%	>10
Lib1.005		42%	>10	Lib1.016		37%	1.3	Lib1.027		0%	2.5	Lib1.037		100%	2.4
Lib1.006		86%	3.7	Lib1.017		5%	>10	Lib1.028		14%	1.7	Lib1.038		100%	1.6
Lib1.007		81%	0.7	Lib1.018		23%	5.2	Lib1.029		61%	1.9	Lib1.039		98%	1.2
Lib1.008		87%	>10	Lib1.019		0%	5.1	Lib1.030		99%	3.6	Lib1.040		63%	>10
Lib1.009		14%	3.5	Lib1.020		4%	5.3	Lib1.031		100%	0.6	Lib1.041		99%	1.2
Lib1.010		0%	>10	Lib1.021		100%	1.3	Lib1.032		63%	2.9	Lib1.042		99%	0.5
Lib1.011		34%	>10	Lib1.022		0%	>10								

a.a. is the % inhibition at 5 μM in the axenic amastigotes assay. i.m. is the IC_{50} in μM in the infected macrophage assay. The structure for this table is the same for each of the subsequent tables.

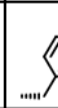
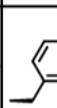
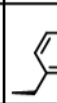
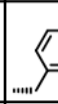
further explore the SAR profile around the scaffold. These efforts resulted in the discovery of several new compounds possessing sub-micromolar IC_{50}s in the *L. donovani* infected macrophage assay. Among the hits is a monocyclic guanidine compound, **64**.

We initiated our study by screening 28 of our scaffold-ranking libraries at 4 doses (50, 25, 5, and 2.5 μM) in an assay using axenic *L. donovani* amastigote strains, CR6 and CS1 as described in Wang et al.[8] Only scaffold-ranking sample **Lib1** showed substantial activity at any of the doses tested, producing >90% inhibition at the 25 and 50 μM doses.[9]

While the axenic amastigote assay allows for a quick assessment of a sample's intrinsic anti-leishmanial activity, it does have the weakness in that the effect of the host mammalian cell cannot be taken into account. In order to assess the anti-leishmanial efficacy of the scaffold-ranking samples against intracellular parasites, a high content screening (HCS) assay with *L. donovani* infected J774 macrophages was utilized.[10] In addition, a counter screen assay utilizing non-infected J774 macrophages was performed on the scaffold-ranking samples to assess their potential toxicity to the host mammalian cell. From these two assays, a selectivity index (SI) was calculated by dividing the IC_{50} obtained from the non-infected macrophage by the IC_{50} from the infected macrophage assay. From this infected macrophage screen, we found several scaffolds with attractive activity (sub-micromolar IC_{50}) and selectivity (> 50 SI) profiles, one of which was **Lib1**. We thus turned our attention to exploring a positional scanning study on this scaffold.

Using our previously described synthetic methods,[5-7] we screened a 110 sample array around **Lib1** (42 carboxylic acids x 42 carboxylic acids x 26 amino acids). In this manner, we were able to evaluate a total of 45,864 different bis-cyclic guanidine analogs, where each compound was replicated in 3 different samples. The results of these screens are shown in Tables 1 – 3. Although the libraries were evaluated at several concentrations, the best range of results was apparent at 5 μM, and the percent inhibition at this concentration is shown.[11] This data allowed us to rank order the relative likelihood that active individual compounds contain certain functionalities at a given po-

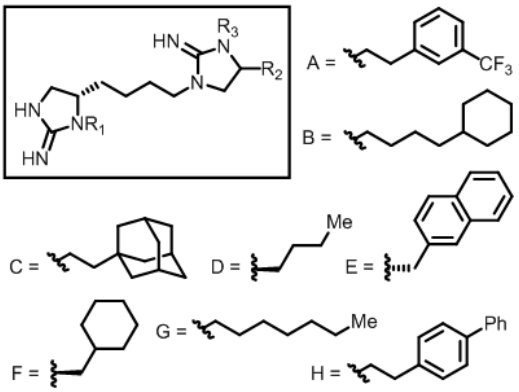
Table 2. R_2 Positional Scanning Study of Lib1

ID#	R_2	a.a.	i.m.	ID#	R_2	a.a.	i.m.
Lib1.043		91%	>10	Lib1.056		0%	>10
Lib1.044		99%	>10	Lib1.057		3%	>10
Lib1.045	H	84%	>10	Lib1.058		23%	5.3
Lib1.046		100%	1.2	Lib1.059		0%	5.4
Lib1.047		100%	5.1	Lib1.060		29%	>10
Lib1.048		0%	5.8	Lib1.061		56%	5.2
Lib1.049		7%	>10	Lib1.062		98%	5.3
Lib1.050		65%	>10	Lib1.063		99%	4.5
Lib1.051		0%	>10	Lib1.064		100%	5.1
Lib1.052		79%	>10	Lib1.065		95%	>10
Lib1.053		96%	>10	Lib1.066		26%	3.8
Lib1.054		99%	>10	Lib1.067		99%	>10
Lib1.055		97%	>10	Lib1.068		100%	0.6

a.a. is the % inhibition at 5 μM in the axenic amastigotes assay. i.m. is the IC_{50} in μM in the infected macrophage assay.

sition and to begin to augur certain trends. For example, based on the 5 μM data, it is clear that the mixture defined with cyclohexylbutyl at the R_1 position (Table 1 sample **Lib1.031**), is more active (100%) than the one defined with isobutyl (0%) at the same R_1 position (Table 1 sample **Lib1.022**). This implies that it is more likely to find active individual compounds with cyclohexylbutyl defined at R_1 than with isobutyl at R_1. Across all three of these positions, oxygenated substituents dramatically reduced activity at the 5 μM dose. Also, there appears to be little preference for stereochemical specificity at the R_2 position (Table 2), where diastereomeric mixtures (e.g. **Lib1.046**/**Lib1.054** and **Lib1.049**/**Lib1.057**) demonstrated nearly identical activities. In addition to the axenic amastigote data in Tables 1 to 3, the IC_{50} data from the HCS infected macrophage assay is also provided. The infected macrophage data does not correlate perfectly with the axenic amastigote data, which is consistent with what we observed in our initial scaffold-ranking studies. While both assays measure the ability of a sample to inhibit the growth of the amastigotes at a given dose, the infected macrophage assay requires the samples to penetrate the macrophage first in order to get to the amastigote. Regardless, a number of the samples that produced sub 2.5 μM IC_{50} in the infected macrophage assay also produced a >80% response

Table 4. Individually Synthesized Compound Activity



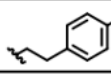
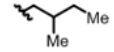
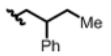
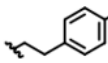
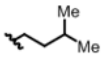
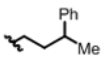
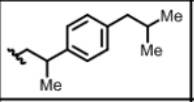
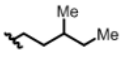
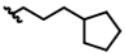
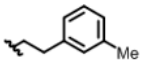
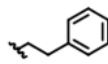
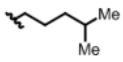
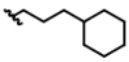
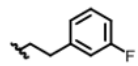
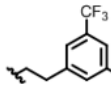
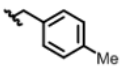
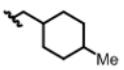
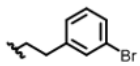
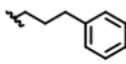
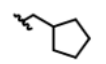
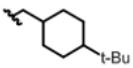
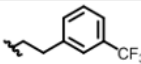
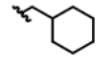
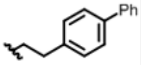
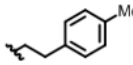
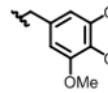
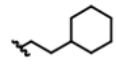
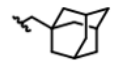
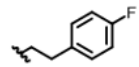
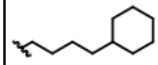
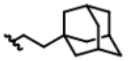
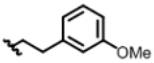
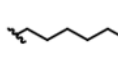
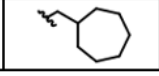
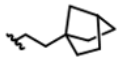
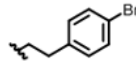
#	R_1	R_2	R_3	a.a. (5 μM)	a.a. (2.5 μM)	i.m. IC_{50} (μM)	cytotox IC_{50} (μM)	SI
6	A	D	G	26%	9%	0.6	48	81
7	A	D	B	43%	0%	>5	50	10
8	A	D	H	100%	47%	0.3	28	89
9	A	E	G	100%	37%	3.5	50	14
10	A	E	B	75%	6%	1.8	50	28
11	A	E	H	0%	0%	1.3	50	39
12	A	F	G	6%	0%	>5	50	10
13	A	F	B	94%	0%	1.8	26	14
14	A	F	H	97%	35%	>5	46	9
15	B	D	G	98%	68%	1.3	16	12
16	B	D	B	99%	71%	>5	15	3
17	B	D	H	98%	78%	5	12	2
18	B	E	G	97%	6%	1.3	50	38
19	B	E	B	0%	0%	0.9	50	56
20	B	E	H	0%	15%	>5	34	7
21	B	F	G	95%	97%	0.7	25	36
22	B	F	B	95%	77%	>5	24	5
23	B	F	H	95%	70%	0.3	20	69
24	C	D	G	94%	96%	>5	32	6
25	C	D	B	8%	11%	0.6	27	41
26	C	D	H	0%	0%	0.9	22	25
27	C	E	G	0%	0%	0.6	36	57
28	C	E	B	99%	99%	2.6	32	13
29	C	E	H	0%	0%	1.7	41	25
30	C	F	G	85%	14%	2.5	26	11
31	C	F	B	28%	0%	>5	23	5
32	C	F	H	57%	14%	0.9	17	20

a.a. is the % inhibition in the axenic amastigotes assay. i.m. is the infected macrophage assay. cytotox is the cytotoxicity in J774 macrophage cells. SI is the selectivity index (cytotox/i.m.).

at the 5 μM dose in the axenic amastigotes assay, demonstrating these samples' ability to inhibit the growth of the amastigotes in both assay formats.

In order to confirm some of the SAR trends that were observed in the positional scanning library data as well as to identify individual hit compounds with antileishmanial activity, a set of previously synthesized bis-cyclic guanidines were selected for testing.[12] The compounds were synthesized as previously described incorporating 3 different functionalities in each of the R_1, R_2 and R_3 positions resulting in 27 structural analogs (Table 4). These different functionalities, when incorporated into the positional scanning library, demonstrated a range of activities in the anti-*Leishmania* proliferation assays (Tables 1 to 3) therefore making them a good set to test the SAR, which is shown in Table 4. In general, when cyclohexylbutyl was fixed in the R_1 position (Compounds **15** to **23**), the compounds showed an increased response in the axenic amastigote assay (6

Table 3. R_3 Positional Scanning Study of Lib1

ID#	R_3	a.a.	i.m.	ID#	R_3	a.a.	i.m.	ID#	R_3	a.a.	i.m.	ID#	R_3	a.a.	i.m.
Lib1.069	H	0%	5.4	Lib1.080		13%	>10	Lib1.091		7%	0.9	Lib1.101		0%	1.4
Lib1.070		38%	>10	Lib1.081		21%	1.3	Lib1.092		7%	5.1	Lib1.102		9%	1.2
Lib1.071		63%	3.6	Lib1.082		86%	>10	Lib1.093		58%	1.3	Lib1.103		91%	1.3
Lib1.072		88%	0.6	Lib1.083		3%	1.2	Lib1.094		79%	1.8	Lib1.104		94%	1.3
Lib1.073		11%	1.3	Lib1.084		19%	1.3	Lib1.095		10%	2.7	Lib1.105		11%	>10
Lib1.074		11%	1.3	Lib1.085		1%	1.8	Lib1.096		21%	1.7	Lib1.106		97%	3.5
Lib1.075		25%	1.4	Lib1.086		13%	4.8	Lib1.097		15%	1.4	Lib1.107		42%	>10
Lib1.076		57%	0.4	Lib1.087		2%	1.3	Lib1.098		92%	>10	Lib1.108		11%	>10
Lib1.077		11%	6.1	Lib1.088		12%	0.7	Lib1.099		100%	4.9	Lib1.109		90%	0.7
Lib1.078		9%	7	Lib1.089		99%	5.1	Lib1.100		15%	1.2	Lib1.110		62%	2.7
Lib1.079		12%	>10	Lib1.090		0%	0.7								

a.a. is the % inhibition at 5 μM in the axenic amastigotes assay. i.m. is the IC_{50} in μM in the infected macrophage assay.

out of 9 showed > 65% inhibition at 2.5 μM) relative to the other compounds (2 out of 18). These results are consistent with the data generated from the positional scanning library data (Table 1) where **Lib1.031** showed greater activity than **Lib1.041** or **Lib1.007**. Of those 9 individual compounds (**15-23**), the compounds where R_2 was R-2-naphthylmethyl were the inactive analogs (**18-20**). Again, this is consistent with the positional scanning data (Table 2) showing that **Lib1.066** was much less active than **Lib1.063** or **Lib1.067**. Using these trends as well as the range of activity in the infected macrophage and cytotoxicity assays, we initiated lead optimization activities.[13]

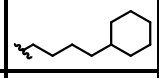
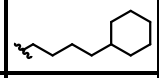
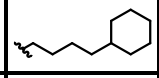
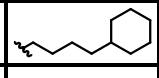
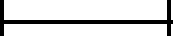
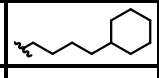
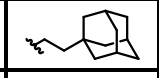
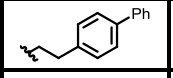
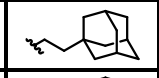
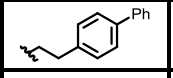
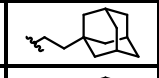
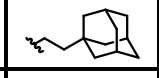
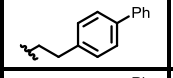
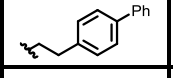
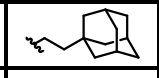
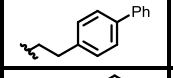
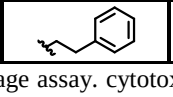
The first two candidates we selected for further exploration were **21** and **26**, both of which showed submicromolar activity in the infected macrophage assay with dramatically different activities in the axenic amastigote assay. In both cases, we prepared a series of deletion analogs (in hopes of decreasing their lipophilicity) as well as inverting the stereocenter built into the initial scaffold (**33** and **40**, Table 5). In both cases, this scaffold change resulted in a significant decrease in the activity. For the series around **21**, most of the deletion compounds resulted in a significant deleterious effect on the infected macrophage activ ity, with the sole exception being **34**. However, the good activity and SI profiles for **42** and **43** suggested that we might be able to focus our attention on monosubstituted analogs. Toward this end, while biphenyl compound **46** was not particularly active, the truncated phenethyl compound **47** showed excellent activity and selectivity.

We also pursued additional studies around compounds **6** and **8**, which differed only about the R_3 position, but showed considerable activity in the infected macrophage assay (Table 6). While we again observed some compounds with acceptable activity and SI profiles, we were pleased to note that monosubstituted analog **50** was quite potent. We therefore synthesized and analyzed some analogs around this compound. Desfluoro analog **55** gave an excellent profile and indicated a preference for meta-substitution (see **54**, **56** and **57**). Saturation of the ring revealed that **59** was another analog with an excellent profile.

Encouraged by the identification of multiple monosubstituted analogs with submicromolar activity and low cytotoxicity, we wanted to determine the necessity of having two guanidine units. We were further struck by the prospect that the butyl substitution at R_2 could serve as a surrogate for the scaffold linker (Figure 2). In this regard, we could consider the R_1 and R_3 substituents together. Toward this end, we synthesized a series of monoguanidine analogs with and without the butyl substituent (Table 7), which clearly showed that the butyl group improved

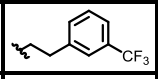
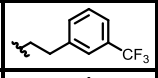
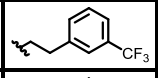
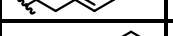
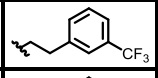
Figure 2. Similarity of R_1 and R_3

Table 5. Analogs of **21** and **26**

ID	*	R_1	R_2	R_3	i.m. IC_{50} (μM)	cytotox IC_{50} (μM)	SI
21	S				0.7	25	36
33	R				8.9	50	6
34	S			H	1.8	27	16
35	S		H		3.5	6	2
36	S	H			2.5	50	20
37	S		H	H	5.2	24	5
38	S	H	H		10	13	1
39	S	H	H	H	4.8	50	10
26	S				0.9	22	25
40	R				3.8	21	6
41	S			H	4.2	13	3
42	S		H		0.7	23	33
43	S	H			1.2	50	43
44	S		H	H	3.5	50	14
45	S	H		H	10	50	5
46	S	H	H		4.9	50	10
47	S	H	H		0.6	20	36

i.m. is the infected macrophage assay. cytotox is the cytotoxicity in J774 macrophage cells. SI is the selectivity index (cytotox/i.m.).

Table 6. Analogs of **6** - **8**

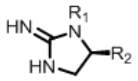
ID	*	R_1	R_2	R_3	i.m. IC_{50} (μM)	cytotox IC_{50} (μM)	SI
6	S				0.6	48	81
7	S				5.0	50	10
8	S				0.3	28	88
48	R				0.6	50	79
49	S		H		2.5	9	4
50	S		H	H	0.9	7	9
51	S			H	3.5	11	3
52	S	H			1.3	22	17
53	S	H		H	5.0	50	10
54	S		H	H	3.5	50	14
55	S		H	H	0.9	50	56
56	S		H	H	3.9	22	6
57	S		H	H	1.2	16	13
58	S		H	H	1.8	19	10
59	S		H	H	0.6	17	28
60	S		H	H	5.4	14	3
61	S		H	H	4.4	6	1

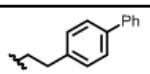
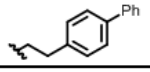
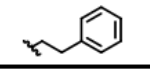
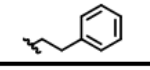
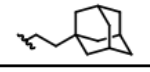
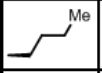
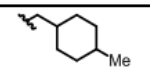
i.m. is the infected macrophage assay. cytotox is the cytotoxicity in J774 macrophage cells. SI is the selectivity index (cytotox/i.m.).

the activity. Furthermore, the optimal activity of **47** was closely reflected in analog **64**, which showed an excellent SI and was more potent in our infected macrophage assay as miltefosine (**3**), a clinically-used antileishmanial agent that is used as our standard.

In summary, using our scaffold ranking and positional scanning libraries, we were able to identify a series of guanidine-based compounds that possessed potent antileishmanial activity in multiple assays as well as selectivity over human macrophages, demonstrating that activity is unlikely to arise through simple promiscuous interactions. These compounds are structurally distinct from any of the currently used antileishmaniasis agents as shown in Figure 1, and thus represent a novel opportunity for lead optimization. We were subsequently able to use these libraries to focus in on compounds such as **64** that retain not only excellent activity (an IC_{50} of 1.13 μM in the infected macrophage assay when run in quadruplicate with a standard deviation of 0.81 μM) and selectivity (an SI of 23 over J774 macrophages) but a reasonable molecular weight (245 g/mol) and physicochemical properties (e.g. cLogP = 3.26, tPSA = 39). Work toward the further optimization of these compounds with optimal pharmacokinetic and pharmacodynamics properties as well as attempts to ascertain their target or mechanism of action is underway and will be reported in due course.

Table 7. Monoguanidines



ID	R_1	R_2	i.m. IC_{50} (μM)	cytotox IC_{50} (μM)	SI
62			2.4	15	6
63		H	4.8	15	3
64			1.1	25	23
65		H	10	8	1
66			4.7	12	3
67			0.6	6	10

i.m. is the infected macrophage assay. cytotox is the cytotoxicity in J774 macrophage cells. SI is the selectivity index (cytotox/i.m.).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental details for the preparation and characterization of all compounds and how the assays were performed (PDF)

AUTHOR INFORMATION

Corresponding Author

Corresponding Author

*Phone: (813) 974-4642. Email jwleahy@usf.edu.

Funding Sources

This work was supported in part by the Florida Drug Discovery Acceleration Program, State of Florida Department of Department of Health. We also gratefully acknowledge support for this research from the University of South Florida Office of Research and Innovation and the Florida Center of Excellence for Drug Discovery and Innovation.

REFERENCES

1. Organization, W. H., Control of the leishmaniases. World Health Organ Tech Rep Ser 2010, (949), xii-xiii, 1-186, back cover.

2. Palatnik-de-Sousa, C. B.; Day, M. J., One Health: the global challenge of epidemic and endemic leishmaniasis. *Parasites Vectors* **2011**, *4*, 197.

3. WHO. Leishmaniasis. World Health Organ. Fact Sheet 2014, 375.

4. Mowbray, C. E.; Braillard, S.; Speed, W.; Glossop, P. A.; Whitlock, G. A.; Gibson, K. R.; Mills, J. E.; Brown, A. D.; Gardner, J. M.; Cao, Y.; Hua, W.; Morgans, G. L.; Feijens, P. B.; Matheeussen, A.; Maes, L. J., Novel Amino-pyrazole Ureas with Potent In Vitro and In Vivo Antileishmanial Activity. *J. Med. Chem.* **2015**, *58*, 9615-24.

5. Wu, J.; Zhang, Y.; Maida, L. E.; Santos, R. G.; Welmaker, G. S.; LaVoi, T. M.; Nefzi, A.; Yu, Y.; Houghten, R. A.; Toll, L.; Giulianotti, M. A., Scaffold Ranking and Positional Scanning Utilized in the Discovery of nAChR-Selective Compounds Suitable for Optimization Studies. *J. Med. Chem.* **2013**, *56*, 10103-10117.

6. Houghten, R. A.; Pinilla, C.; Giulianotti, M. A.; Appel, J. R.; Dooley, C. T.; Nefzi, A.; Ostresh, J. M.; Yu, Y.; Maggiora, G. M.; Medina-Franco, J. L.; Brunner, D.; Schneider, J. Strategies for the use of mixture-based synthetic combinatorial libraries: scaffold ranking, direct testing in vivo, and enhanced deconvolution by computational methods. *J. Comb. Chem*. **2008**, 10, 3−19.

7. Rideout, M. C.; Boldt, J. L.; Vahi-Ferguson, G.; Salamon, P.; Nefzi, A.; Ostresh, J. M.; Giulianotti, M.; Pinilla, C.; Segall, A. M. Potent antimicrobial small molecules screened as inhibitors of tyrosine recombinases and Holliday junction-resolving enzymes. *Mol. Diversity* **2011**, *15*, 989−1005.

8. Wang, M. Z.; Zhu, X.; Srivastava, A.; Liu, Q.; Sweat, J. M.; Pandharkar, T.; Stephens, C. E.; Riccio, E.; Parman, T.; Munde, M.; Mandal, S.; Madhubala, R.; Tidwell, R. R.; Wilson, W. D.; Boykin, D. W.; Hall, J. E.; Kyle, D. E.; Werbovetz, K. A., Novel arylimidamides for treatment of visceral leishmaniasis. *Antimicrob. Agents Chemother.* **2010**, *54*, 2507-16.

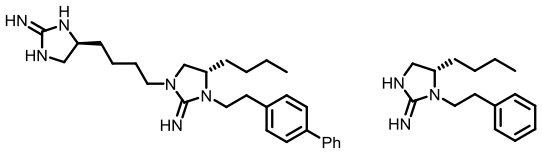
9. Screening was conducted on 28 different scaffold libraries that contained > 6 million compounds. These data are provided in the Supplementary Information. In order to show concentrations of mixtures in terms of molarity, we used an average molecular weight of 500 g/mol for each compound in the mixture. For example, we considered a 5 μg/mL to equate to a 10 μM solution. Actual concentrations were used for the evaluation of single compounds.

10. Siqueira-Neto, J. L.; Moon, S.; Jang, J.; Yang, G.; Lee, C.; Moon, H. K.; Chatelain, E.; Genovesio, A.; Cechetto, J.; Freitas-Jr, L. H., An image-based high-content screening assay for compounds targeting intracellular Leishmania donovani amastigotes in human macrophages. *PLoS Neglected Trop. Dis.* **2012**, *6*, e1671.

11. Santos, R. G.; Appel, J. R.; Giulianotti, M. A.; Edwards, B. S.; Sklar, L. A.; Houghten, R. A.; Pinilla, C., The mathematics of a successful deconvolution: a quantitative assessment of mixture-based combinatorial libraries screened against two formylpeptide receptors. *Molecules* **2013**, *18*, 6408-24.

12. Fleeman, R.; LaVoi, T. M.; Santos, R. G.; Morales, A.; Nefzi, A.; Welmaker, G. S.; Medina-Franco, J. L.; Giulianotti, M. A.; Houghten, R. A.; Shaw, L. N., Combinatorial Libraries As a Tool for the Discovery of Novel, Broad-Spectrum Antibacterial Agents Targeting the ESKAPE Pathogens. *J. Med. Chem.* **2015**, *58*, 3340-3355.

13. To facilitate screening, each sample was run as single data points, with miltefosine (**3**) as a control on each plate. The IC_{50} for this standard is 1.43 ± 0.20 μM, and no plates contained significant outliers for the control.



Insert Table of Contents artwork here