Synthesis and Activity of a New Series of Antileishmanial Agents

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S Supporting Information

ABSTRACT: We have determined that tetrahydroindazoles such as **1** show potent activity against *Leishmania donovani*, the causative agent of leishmaniasis. While the Hsp90 activity and anticancer properties of **1** have previously been explored, we present here our efforts to optimize their activity against *L. donovani* via the synthesis of novel analogues designed to probe the hydrophobic pocket of the protozoan Hsp90 orthologue, specifically through the auspices of functionalization of an amine embedded into the scaffold.

KEYWORDS: Antiinfectives, leishmaniasis, Hsp90, chaperone

Neglected tropical diseases (NTDs) represent an undeniable threat to human health.^{1,2} The World Health Organization has prioritized 17 diseases that affect more than 1.5 billion people around the world, the vast majority of whom are impoverished.³ One of these diseases is leishmaniasis, a zoonotic parasitic infection caused by protozoa of the genus *Leishmania*. This parasite is transmitted to humans through the bite of phlebotomine sandflies, and there are estimated to be more than 12 million people currently infected.⁴ The disease is manifested in three primary forms: cutaneous, mucocutaneous, and visceral leishmaniasis. Visceral leishmaniasis (VL), which is also known as kala-azar, represents the most lethal form of the disease and is fatal if left untreated.⁵ The most common method for treating VL is through the injection of pentavalent antimonials such as meglumine antimoniate or sodium stibogluconate, though resistance is reaching alarming levels.⁶ Other treatment options have been explored, including miltefosine (Figure 1), liposomal amphotericin B, paromomycin, and pentamidine, but each is accompanied by its own set of challenges.⁷ Therefore, there remains a significant need for the

Figure 1. Structures of miltefosine and radicicol.

discovery and development of new potential therapeutic agents for the treatment of leishmaniasis.

We have been actively engaged in an ongoing screening effort for the identification of potential new antiprotozoal agents. As part of these efforts, we recently found that SNX2112 (**1**, Figure 2)^{8,9} showed potent activity in a high throughput axenic *L. donovani* amastigote assay.¹⁰ This compound was previously known to have potent antitumor

Figure 2. Interactions of **1** with Hsp90.

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activity, and a prodrug analogue (SNX5422, **2**) is currently in multiple clinical trials for oncology indications.^{11,12} The biochemical target of **1** is Hsp90, a ubiquitous chaperone protein known to facilitate protein folding and one that is often overexpressed in stressed cells.¹³ It is the putative target of known bioactive natural products such as radicicol¹⁴ and has proven to be a viable focus for oncology drug discovery. A number of Hsp90 inhibitors have been investigated in human clinical trials such as breast cancer, colorectal cancer, and hepatic cancer.¹⁵ Given that Hsp90 or related orthologues have been identified not just in mammals but in organisms from bacteria to yeast, it seemed reasonable that it could therefore serve as a viable target for the development of antiprotozoal agents. Indeed, there have been a number of reports of Hsp90 inhibitors that also possess activity against protozoan parasites,¹⁶ including *Trypanosoma brucei* (African sleeping sickness),^{17,18} *Plasmodium falciparum* (malaria),^{18,19} and *Leishmania* spp. (leishmaniasis).²⁰ We therefore sought to explore the prospects of optimizing this initial hit as a potential antileishmanial agent. Critical to these efforts would be to ensure that any analogues would demonstrate this activity without demonstrating significant cytotoxicity, which would mitigate the potential usefulness of these agents as selective antileishmanial agents.

The initial discovery of **1** focused primarily on modifications to the anthranilamide portion in an attempt to optimize for pharmacokinetic parameters.²¹ This decision was based in part on docking studies that suggested the anthranilamide contributed to two of the three critical hydrogen bonds with the target and the apparent solvent exposure of the amine substituent. Notably, little attention was paid to the geminal methyl substituents other than to note that their presence was essential for activity. Given that their presence was primarily a result of the use of dimedone as the starting material for the synthesis of the initial lead series, we felt that this portion warranted additional consideration.

In order to confirm the initial hit compound, we prepared compounds **9–11** (Scheme 1). We eschewed the CF₃ substituent in the hopes of restricting the cytotoxicity in this series.²¹ As expected, analogue **9** displayed potent and selective activity in the axenic amastigote assay, while the desmethylated **10** showed no activity at 25 μ M. We also prepared quinazoline **11**, which restricted the majority of the rotatable bonds to facilitate docking studies. Since this compound also displayed activity in our initial assay, we felt that it was an excellent candidate for docking into the active site of Hsp90. Using publicly available coordinates (3D0B)²² for a cocrystal in AutoDock 1.5.6²³ suggested that **11** would fit well into the active site and that the geminal methyl substituents would access a hydrophobic pocket that might be exploited for additional activity or selectivity (Figure 3). We therefore set out to prepare analogues that would allow us to introduce diversity late in the synthesis in order to evaluate their activity. We also considered the typical Lipinski/physicochemical parameters for each of these analogues in order to maximize the likelihood that any active compounds would have a reasonable chance at achieving acceptable oral bioavailability.²⁴

We envisioned that an appropriately adorned pyrazolo[3,4-*c*]pyridine core would serve as a suitable replacement for the initial scaffold such that the amine would provide a versatile handle for the installation of functionality that could probe this pocket. Toward that end, alkylation of the ethyl ester of *N*-benzylglycine with chloroacetone followed by *tert*-butoxide

Scheme 1^a

^aReagents and conditions: (a) Ac₂O, iPr₂NEt, DMAP, CH₂Cl₂ (89%*); (b) 2-bromo-4-hydrazinylbenzonitrile, AcOH, EtOH (59%*); (c) (i) 3-methoxypropan-1-amine, Pd(OAc)₂ (10 mol %), dppf (10 mol %), NaOtBu, PhMe, 120 °C, microwave (64%*); (ii) H₂O₂, NaOH, EtOH, DMSO, 100 °C, microwave (72%*); (d) 7-hydrazinylquinazolin-4-amine, AcOH, EtOH (54%). *Yields given are for R = Me. Comparable yields were obtained for R = H.

promoted cyclization gave cyclic β -diketone **13** (Scheme 2).^{25,26} C-Acylation of this material proved more difficult than we anticipated, but the initial mixture of C- and O-acylated compounds could be converted to the desired **14** by cyanide equilibration.²⁷ Condensation of this material with the arylhydrazine gave the requisite bromonitrile (**15**), which was able to undergo Buchwald–Hartwig amination and nitrile hydration to afford tertiary amine **16**. The benzyl group could be reductively removed under acidic conditions to give **17**, which served as a viable intermediate for the generation of substituted analogues. Acylation of **17** was able to be conducted with a series of readily available acid chlorides (Scheme 3). Similarly, sulfonylation was easily achieved. Urea formation was conducted through the auspices of a benzyl carbamate, which could be debenzylated to give the parent **26**.

In addition to evaluating these analogues in the initial high throughput axenic amastigote assay, we also investigated their activity in a high content screening (HCS) infected macrophage assay using transformed J774 murine macrophages that also allows us to evaluate cytotoxicity and ensure that the activity we see is not a general cytotoxic phenomenon.²⁸ The results of these evaluations are displayed in Table 1. Clearly, the ostensible hydrophobic pocket accommodates a fair amount of substitution at this position. The HCS data indicates that the secondary and tertiary amines (**17** and **16**) are not particularly active, though it is noteworthy that the more substituted benzyl amine is the more active of the two, which suggests a preference for greater substitution. This diminished activity presumably arises from the physiological protonation of the amine under the assay conditions. More significantly, all of the amides showed reasonable HCS activity, and acetamide **18** was nearly equipotent with the parent (**9**). By contrast, sulfonamide

Figure 3. Minimized poses of **11** in Hsp90 showing the geminal methyl groups pointing toward hydrophobic pockets. Compound **11** docked into Hsp90. The ball and stick (left) and surface (center) models show how the compound accesses the active site. The right panel shows a channel that exposes the geminal methyl groups that should accommodate modifications at this site.

Scheme 2^a

^aReagents and conditions: (a) chloroacetone, NaHCO₃, THF/H₂O (82%); (b) KOtBu, Et₂O (79%); (c) Ac₂O, NEt₃, CH₂Cl₂; (d) NaCN, K₂CO₃, CH₂Cl₂ (46% over 2 steps); (e) 2-bromo-4-hydrazinylbenzonitrile, AcOH, MeOH (55%); (f) (i) 3-methoxypropan-1-amine, Pd(OAc)₂ (10 mol %), dppf (10 mol %), NaOtBu, PhMe, 120 °C, microwave (61%); (ii) K₂CO₃, MeOH/H₂O, 125 °C, microwave (34%); (g) H₂, 10% Pd/C EtOH, HCl (72%).

24 was considerably less active, and neither of the ureas showed any activity. It should also be noted that none of the compounds prepared demonstrated any significant cytotoxicity, suggesting that the activity was specific to the *Leishmania* amastigotes. Notably, some analogues such as **18** and **24** show considerably more activity in the infected macrophage assay than in our initial axenic amastigote assay. This type of behavior has previously been observed with opioid receptor antagonists,²⁹ suggesting more than one target could be involved in the observed macrophage activity.

Since the ultimate goal of this work is to identify a compound that not only demonstrates antileishmanial activity but also has the potential to be orally bioavailable, we considered the typical Lipinski “rule of 5” parameters for each of the compounds evaluated (Table 1). Furthermore, we also considered other factors such as the number of rotatable bonds and the total polar surface area (tPSA).²⁴ These evaluations were completed

Scheme 3^a

^aReagents and conditions: (a) RCOCl, pyridine (60–80%); (b) MeSO₂Cl, pyridine (57%); (c) 4-nitrophenyl benzylcarbamate, NEt₃, CH₂Cl₂ (35%); (d) H₂, 10% Pd/C, AcOH/HCl (34%).

using the DruLiTo drug likeness tool open source calculator.³⁰ With the exception of the number of rotatable bonds for **25**, each of the compounds fell within acceptable parameters for oral bioavailability. We tested our most active N-linked analogue **18** in a kinetic solubility assay to confirm that these modifications did not result in a deleterious effect on the physicochemical properties of the original lead, and were pleased to see that it demonstrates excellent solubility (>490 μM) in PBS buffer (pH 7.4).

We also sought to determine whether the antileishmanial activity could be tied to Hsp90 inhibition. However, we were surprised to find that none of the new analogues prepared displayed significant inhibitory activity against human Hsp90.³¹ In fact, unsubstituted analogue **10** was more potent against Hsp90 than any of the compounds that were active in the antileishmanial assays. Among the possible explanations for this are that these compounds are selective for the protozoan orthologue for Hsp90 (Hsp83) over human Hsp90 or that they are exerting their antileishmanial activity through a different mechanism.

Table 1. Biological and Physicochemical Data For All Analogs

compd	IC ₅₀ (μM)			Lipinski/physicochemical properties (DruLiTo)					
	axenic amastigote	infected macrophage	J774 cytotoxicity	molecular weight	cLogP	H-bond acceptors	H-bond donors	tPSA	rotatable bonds
miltefosine	3.26	1.43	>50	408	8.7	4	0	55.43	20
radicicol	0.62	0.29	2.35	365	2.4	6	2	96.36	0
1	0.42	0.61	<0.06	437	2.9	7	4	49.74	5
9	0.65	0.88	3.52	356	1.8	7	3	58.97	7
10	>25	>10	>50	332	0.7	7	3	58.97	7
11	2.42	1.33	>50	302	0.9	6	2	57.39	1
16	2.29	6.33	>50	418	0.9	8	3	62.21	9
17	3.90	>10	>50	334	0.0	8	4	58.97	7
18	13.9	1.06	>50	374	−0.4	9	3	79.28	8
19	6.43	3.54	>50	386	−0.1	9	3	79.28	9
20	1.90	3.54	37.3	398	0.2	9	3	79.28	9
21	4.48	3.58	>50	402	−0.9	10	3	88.51	10
22	2.99	1.52	>50	422	1.0	9	3	79.28	9
23	2.60	1.75	26.3	446	1.1	9	3	79.28	9
24	>20	5.38	25.5	410	−0.6	10	3	104.73	8
25	>20	>10	>50	460	0.4	10	4	79.28	11
26	>20	>10	>50	376	−1.2	10	5	79.28	8

In summary, we have found a series of novel pyrazolo[3,4-*c*]pyrimidines based on the known Hsp90 agent SNX-2112 that shows activity against *Leishmania donovani* in multiple assays. Significantly, several of these new compounds demonstrate more potent activity than the clinically used miltefosine in both of these assays and excellent selectivity over any observed cytotoxicity against human J774 macrophage cells. Work toward a better understanding of this activity, including the biochemical target, is currently underway. Although the starting point for this project was a compound that demonstrated an excellent ADME/PK profile, this is an early drug discovery project, and we will continue to assess these parameters as we seek a lead compound with optimal properties.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00039.

Experimental details for the preparation of all compounds, how the assays were performed, and the Hsp90α activity and the kinetic solubility data for 18 (PDF)

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Notes

The authors declare no competing financial interest.

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