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Antiparasitic lethality of sulfonamidebenzamides in kinetoplastids

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ABSTRACT

A sulfonamidebenzamide series was assessed for anti-kinetoplastid parasite activity based on structural similarity to the antiparasitic drug, nifurtimox. Through structure-activity optimization, derivatives with limited mammalian cell toxicity and increased potency toward African trypanosomes and *Leishmania* promastigotes were developed. Compound **22** had the best potency against the trypanosome (EC_{50} = 0.010 μM) while several compounds showed ~10-fold less potency against *Leishmania* promastigotes without impacting mammalian cells (EC_{50} > 25 μM). While the chemotype originated from an unrelated optimization program aimed at selectively activating an apoptotic pathway in mammalian cancer cells, our preliminary results suggest that a distinct mechanism of action from that observed in mammalian cells is responsible for the promising activity observed in parasites.

The Trypanosomatida order of flagellated protists, including the African trypanosome *Trypanosoma brucei* and several *Leishmania* spp., are responsible for widespread human diseases that include African sleeping sickness[1] and leishmaniasis,[2] respectively. Together, these diseases infect over 2 million people and place a tremendous burden on the local medical infrastructure where they occur. Therapeutic options for these parasitic diseases are limited by toxicity and efficacy, with the rising specter of emerging resistance increasing the possibility that the suboptimal therapeutics currently in use will soon be obsolete. Consequently, finding novel, safe and effective antiparasitic agents is critical to bridge the chasm between available options and none at all. The recent disclosure of GNF6702, a selective inhibitor of the kinetoplastid proteasome that imparts broad spectrum activity,[3] shows progress on this front. For our own efforts, we investigated the possibility of targeting multiple parasites to reveal a distinct, sulfonamide-based compound series that potently impacts both *T. brucei* and *Leishmania* trypanosomatid parasites.

Our sulfonamidebenzamide-based compound library originated from an orthogonal medicinal chemistry program aimed at detecting agents that selectively upregulated the expression of the C/EBP-homologous protein (CHOP) in cancerous mammalian cells.[4] Increased expression of *CHOP*, which occurs in normal cells in response to unresolved, misfolded protein accumulation, ultimately leads to programmed cell death (PCD) in mammals if cellular homeostasis is not otherwise achieved.[5,6] While there is evidence that singled-celled protists can respond to stimuli and initiate a programmed cellular response similar to metazoan apoptosis, the noted absence of clear homologs to the caspases involved suggest alternative mechanisms may be engaged or that the phenotypes that are similar to the PCD-based program are due to "incidental death".[7–12] Our interest in these compounds as potential antiparasitic agents was piqued by the realization that the series, represented by compound **1**, shared some common skeletal features with anti-trypanosomal drug, nifurtimox (Lampit®, **2**, Fig. 1). Both compounds bear a nitrofuryl group that is conjugated to an adjacent *pi*-system (blue shaded region) which is tethered to a sulfone or sulfonamide (green shaded space) through a nitrogen-carbon framework (beige shaded area).

The exact mechanism of action of nifurtimox has not been elucidated, though studies propose at least two alternative pathways

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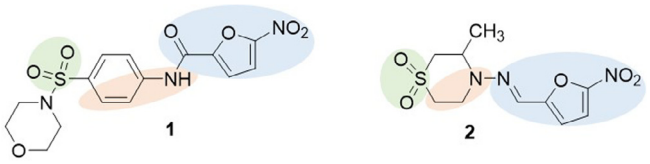
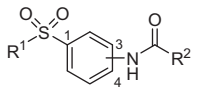


Fig. 1. Similarity between scaffold **1** and nifurtimox **2**.

involving the induction of oxidative stress leading to apoptosis[13–16] or reductive activation by a eukaryotic type I nitroreductase[17–19] – both of which implicate the involvement of the nitrofuryl moiety as a warhead. Given the structural congruency between these templates and the ambiguity of mechanisms involved with each, we decided to investigate if these compounds harbored antiparasitic activity.

Sulfonamidebenzamides were synthesized as previously described.[4] Structural integrity was confirmed by ^{1}H and ^{13}C NMR, and purity was validated to be >95% by UV/LCMS. Compounds were assessed for their impact on parasite viability in 96- or 384-well plate format against *T. brucei* bloodstream form (BSF)[20–22] and *L. amazonensis* promastigote form parasites,[23] respectively (Table 1). Immortalized cell lines which are commonly used to assess general mammalian cytotoxicity are hypersensitive to apoptotic pathway modulators.[4,24] As many of these compounds were originally developed as inducers of the mammalian cell apoptotic CHOP pathway, immortalized cell lines were not useful indicators of general cytotoxicity. As a result, we utilized a

Table 1
Sulfonamidebenzamide structure-activity data against *T. brucei* and *L. amazonensis*.[a,b]



Compound	Central ring substitution	R^1	R^2	Cytotoxicity EC_{50} (μM)[c]	*T. brucei* EC_{50} (μM)[d]	*T. brucei* selectivity index, SI[e]	*L. amazonensis* EC_{50} (μM)[f]	*L. amazonensis* selectivity index, SI[e]
1	1,4	*N*-morpholine	5-NO_2-furyl	>25	0.46 ± 0.05	>54	3.0 ± 0.105	>8
2	1,4	*N*-piperidine	5-NO_2-furyl	>25	0.22 ± 0.06	>114	13.7 ± 7.0	>2
3	1,4	4-CH_3-*N*-piperidine	5-NO_2-furyl	>25	0.12 ± 0.02	>208	7.7 ± 5.0	>3
4	1,4	4,4-dimethyl-*N*-piperidine	5-NO_2-furyl	>25	0.09 ± 0.01	>278	0.28 ± 0.1	>89
5	1,4	4-*t*-butyl piperidine	5-NO_2-furyl	>25	0.03 ± 0.02	>833	1.4 ± 0.3	>18
6	1,4	*N*-3-azaspiro[5.5] undecane	5-NO_2-furyl	>25	0.03 ± 0.00	>833	1.5 ± 0.9	>17
7	1,4	4-OH-*N*-piperidine	5-NO_2-furyl	>25	0.31 ± 0.07	>81	4.4 ± 1.8	>6
8	1,4	4-Cl-*N*-piperidine	5-NO_2-furyl	>25	0.08 ± 0.04	>313	7.9 ± 2.8	>3
9	1,4	4-F-*N*-piperidine	5-NO_2-furyl	>25	0.42 ± 0.03	>60	7.4 ± 2.5	>3
10	1,4	4-*NH*-piperazine	5-NO_2-furyl	>25	0.73 ± 0.03	>34	10.5 ± 4.3	>2
11	1,4	cyclohexyl	5-NO_2-furyl	>25	0.49 ± 0.05	>51	1.6 ± 0.7	>16
12	1,4	4-pyran	5-NO_2-furyl	>25	0.53 ± 0.04	>47	0.3 ± 0.05	>83
13	1,4	phenyl	5-NO_2-furyl	>25	0.11 ± 0.02	>227	4.3 ± 1.8	>6
14	1,4	*N*-morpholine	2-furyl	>25	>10	NA	>25	NA
15	1,4	*N*-morpholine	2-thiophene	>25	>10	NA	>25	NA
16	1,4	*N*-morpholine	phenyl	>25	>10	NA	>25	NA
17	1,4	*N*-morpholine	5-CH_3-furyl	>25	>10	NA	>25	NA
18	1,4	*N*-morpholine	4-NO_2-phenyl	>25	>10	NA	>25	NA
19	1,4	4,4-dimethyl-*N*-piperidine	3-NO_2-phenyl	>25	6.6 ± 0.15	>4	13.3 ± 6.2	>2
20	1,4	4,4-dimethyl-*N*-piperidine	5-CF_3-furyl	>25	7.55 ± 1.46	>3	10.7 ± 1.0	>2
21	1,4	*N*-morpholine	5-NO_2-thiophene	>25	0.060 ± 0.00	>417	1.3 ± 0.03	>19
22	1,4	4,4-dimethyl-*N*-piperidine	5-NO_2-thiophene	>25	0.010 ± 0.001	>2500	0.1 ± 0.01	>250
23	1,4	4-*t*-butyl piperidine	5-NO_2-thiophene	>25	0.030 ± 0.001	>833	0.1 ± 0.01	>250
24	1,4	*N*-3-azaspiro[5.5] undecane	5-NO_2-thiophene	>25	0.020 ± 0.000	>1250	0.1 ± 0.01	>250
25	1,3	4,4-dimethyl-*N*-piperidine	5-NO_2-thiophene	>25	0.045 ± 0.001	>556	1.3 ± 0.1	>19
26	1,3	4,4-dimethyl-*N*-piperidine	5-NO_2-furyl	>25	0.32 ± 0.001	>78	0.1 ± 0.02	>250
27	1,3	4,4-dimethyl-*N*-piperidine	2-furyl	>25	5.11 ± 0.86	>5	1.2 ± 0.1	>21
28	1,3	4,4-dimethyl-*N*-piperidine	3-NO_2-phenyl	>25	10.39 ± 1.06	>2	3.0 ± 0.03	>8
29	1,3	*N*-morpholine	5-NO_2-furyl	>25	1.47 ± 0.19	>17	0.9 ± 0.1	>28

NA = not applicable.
[a] Experimental data is an average of at least 3 runs ($n \geqslant 3$).
[b] Nifurtimox as a control afforded *T. brucei* EC_{50} = 7.5 ± 0.05 μM, *L. amazonensis* EC_{50} = 0.8 ± 0.1 μM, IMR90 EC_{50} > 25 μM.
[c] Mammalian cytotoxicity was determined using IMR90 cells.
[d] Bloodstream form parasites.
[e] SI = IMR90 EC_{50}/parasite EC_{50}.
[f] Promastigote stage parasites.