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Targeting the HSP60/10 chaperonin systems of *Trypanosoma brucei* as a strategy for treating African sleeping sickness

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ABSTRACT

Trypanosoma brucei are protozoan parasites that cause African sleeping sickness in humans (also known as Human African Trypanosomiasis—HAT). Without treatment, *T. brucei* infections are fatal. There is an urgent need for new therapeutic strategies as current drugs are toxic, have complex treatment regimens, and are becoming less effective owing to rising antibiotic resistance in parasites. We hypothesize that targeting the HSP60/10 chaperonin systems in *T. brucei* is a viable anti-trypanosomal strategy as parasites rely on these stress response elements for their development and survival. We recently discovered several hundred inhibitors of the prototypical HSP60/10 chaperonin system from *Escherichia coli*, termed GroEL/ES. One of the most potent GroEL/ES inhibitors we discovered was compound **1**. While examining the PubChem database, we found that a related analog, **2e**-*p*, exhibited cytotoxicity to *Leishmania major* promastigotes, which are trypanosomatids highly related to *Trypanosoma brucei*. Through initial counter-screening, we found that compounds **1** and **2e**-*p* were also cytotoxic to *Trypanosoma brucei* parasites (EC_{50} = 7.9 and 3.1 μM, respectively). These encouraging initial results prompted us to develop a library of inhibitor analogs and examine their anti-parasitic potential in vitro. Of the 49 new chaperonin inhibitors developed, 39% exhibit greater cytotoxicity to *T. brucei* parasites than parent compound **1**. While many analogs exhibit moderate cytotoxicity to human liver and kidney cells, we identified molecular substructures to pursue for further medicinal chemistry optimization to increase the therapeutic windows of this novel class of chaperonin-targeting anti-parasitic candidates. An intriguing finding from this study is that suramin, the first-line drug for treating early stage *T. brucei* infections, is also a potent inhibitor of GroEL/ES and HSP60/10 chaperonin systems.

Parasitic infections, such as those that cause African sleeping sickness (also known as Human African Trypanosomiasis—HAT), Chagas disease, leishmaniasis, toxoplasmosis, and malaria, cause significant morbidity and mortality worldwide. While antibiotic resistance continues to mount, a pressing issue is that some of these diseases have never benefited from adequate antibiotic availability in the first place. Such is the case for HAT, which is caused by infection with the parasitic protozoa, *Trypanosoma brucei*. Transmission of *T. brucei* between mammalian hosts occurs through an insect vector, the tsetse fly (genus *Glossina*). HAT is endemic to the region between the Sahara and Kalahari deserts, where ~70 million people are at risk of contracting the disease.[1–4] Around 10,000 new cases of HAT are reported each year, although the actual number is likely much higher owing to insufficient reporting.[5] Two sub-species of parasites are responsible for HAT: *T. brucei gambiense* and *T. brucei rhodesiense*. While the general symptoms of HAT are similar, the speed of disease progression differs markedly between the two organisms: *T.b. gambiense* causes a more gradual onset of symptoms over the course of months to years, while *T.b. rhodesiense* causes acute disease that progresses within weeks to months.[5,6] Without treatment, both infections are fatal. Disease progression occurs in two stages. The first is

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termed the early, haemolymphatic stage, where parasites enter and spread in the bloodstream, lymph nodes, and systemic organs. Symptoms of this stage can include itching, fever, headaches, malaise, joint pains, and severe swelling of the lymph nodes. After a variable time period (weeks for *T.b. rhodesiense* and months for *T.b. gambiense*), parasites cross the blood–brain barrier and enter the central nervous system. Once this occurs, HAT is considered to be in the late, encephalitic stage, which is characterized by disruption of the sleep cycle and progressive mental deterioration leading to coma, systemic organ failure, and death.

None of the current drugs (Fig. 1) are ideal for treating HAT as they all suffer from varying pharmacological deficiencies. While first line treatments of suramin (*T.b. rhodesiense*) or pentamidine (*T.b. gambiense*) are often effective for the early systemic stage of disease, they are ineffective against the later CNS stage once parasites have crossed the blood–brain barrier.[5] Eflornithine, nifurtimox, and melarsoprol can treat CNS-stage *T.b. gambiense* infection, but only melarsoprol is effective against *T.b. rhodesiense*. Melarsoprol is itself toxic and leads to the death of ~5% of patients.[5,7,8] These drugs also have poor oral bioavailability, which necessitates frequent IV and/or IM injections. Unfortunately, no new drugs have been developed against *T. brucei* since the advent of eflornithine in the 1970s. Due to associated toxicities, the complexity of treatment regimens, and the rise of resistance to current HAT therapies, there is an urgent need to develop safe, effective, and easily administered treatments.[9] Towards this goal, we are investigating modulating the protein homeostasis pathways of *T. brucei* as a viable antibiotic strategy.

Molecular chaperones are key modulators of protein homeostasis as they are essential in helping many proteins fold into their functional forms and assist with their degradation.[10–12] HSP60/10 chaperonins are unique members of the molecular chaperone family that are generally found in eukaryotic mitochondria and bacterial cytosol (also known as GroEL/ES). Through a series of events driven by ATP binding and hydrolysis, unfolded substrate proteins are bound within the central cavity of the HSP60 ring and encapsulated by the HSP10 co-chaperonin lid structure, triggering protein folding in a sequestered chamber.[13–15] HSP60/10 chaperonins are viable antibiotic targets because cells rely on them to survive.[16–19] Notably, many organisms have multiple HSP60 isoforms that they modulate to adapt to their environments.[19–25] For instance, *T. brucei* have three HSP60 isoforms (Fig. 2A).[17,26] While studies have indicated that HSP60 is associated with the mitochondrial matrix, kinetoplast, and flagellar pocket of *T. brucei*, the distribution and function of each HSP60 isoform are not well characterized.[25,27–29] However, recent studies have identified that expression of the HSP60 isoforms vary depending on the life cycle stage of the parasite and that depletion of each single variant can result in decreased growth and/or survival (Fig. 2B).[17,26] The HSP60.1 isoform appears to be the canonical chaperonin system in *T. brucei* since it is essential, while the 60.2 and 60.3 isoforms are not.[17] This is further supported by the fact that only the HSP60.1 isoform contains the C-terminal GGM-repeat motif that is typically found in canonical chaperonin systems.[23,27,30,31] Collectively, these results suggest that *T. brucei* may be susceptible to HSP60-targeting antibiotics. Targeting the HSP60/10 chaperonins for antibiotic development would be a unique polypharmacological strategy as one drug could potentially inhibit the three chaperonin isoforms and have the cascading effect of modulating hundreds of downstream proteins. Thus, it may be difficult for *T. brucei* to develop resistance to such a broadly-acting class of antibiotics.

We previously performed high-throughput screening and discovered 235 small molecule inhibitors of the *Escherichia coli* GroEL/ES chaperonin system.[32] We have since found that several of our chaperonin inhibitors exhibit antibiotic effects against Gram-positive and Gram-negative bacteria.[34] One of the most potent GroEL/ES inhibitors that we discovered was compound **1** (Fig. 3), which inhibited both the substrate refolding and ATPase functions of the chaperonin system.[32,34] Unfortunately, compound **1** was inactive against the panel of bacteria we tested against, suggesting it may not be a good candidate for antibacterial development.[34] However, we found a related analog in the PubChem database where the benzimidazole core is replaced by a benzoxazole (Fig. 3, compound **2e**-*p*, PubChem CID #1098316).[33] While compound **2e**-*p* has been evaluated in 285 assays, it was reported to be active in only 8 bioassays, suggesting this scaffold may be inherently selective and thus a promising candidate to explore for further drug development. Notably, **2e**-*p* was reported as an active hit in a high-throughput screen for cytotoxic compounds against *Leishmania major* promastigotes. Because *Leishmania* are trypanosomatids highly related to *Trypanosoma brucei*, we postulated that compound **1** would also exhibit cytotoxicity to *Trypanosoma brucei*.

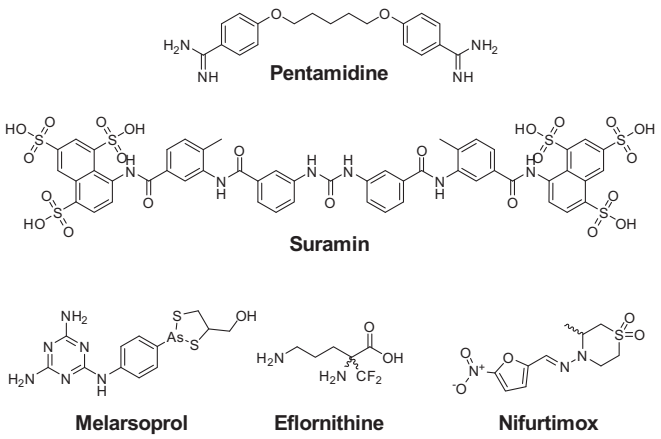


Figure 1. Structures of drugs currently used to treat African sleeping sickness.

A.

	% Identity (Similarity) Compared to:	
	E. coli GroEL	*T. brucei* HSP60.1
E. coli GroEL (548 AA)	100%	53% (69%)
T. brucei HSP60.1 (562 AA)	53% (69%)	100%
T. brucei HSP60.2 (541 AA)	43% (59%)	48% (66%)
T. brucei HSP60.3 (594 AA)	40% (55%)	55% (74%)
Human HSP60 (547 AA)	48% (61%)	53% (73%)

B.

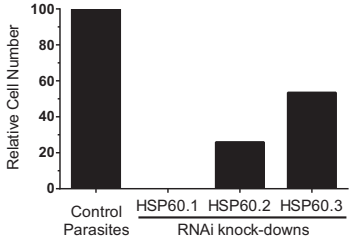


Figure 2. (A) Homology comparison of the three *T. brucei* HSP60 isoforms to *E. coli* GroEL (left) and the canonical *T. brucei* HSP60.1 isoform (right). Human mitochondrial HSP60 is also shown for comparison. AA = Amino Acids. (B) Previous studies report that genetic knock-down of any of the three HSP60 isoforms inhibit parasite growth (normalized to uninduced control parasites).[17]

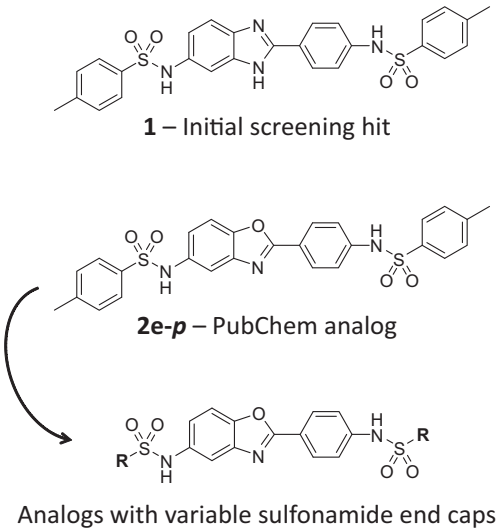


Figure 3. Compound **1** was an initial hit that emerged from our recent high-throughput screening for GroEL/ES inhibitors.[32] Compound **2e**-*p* is a related analog found in the PubChem database (CID #1098316) that has reported bioactivity in only 8 of the 285 assays it has been evaluated in.[33] One assay that **2e**-*p* is reported active in is against *Leishmania major* promastigotes, which are parasites closely related to *Trypanosoma brucei*. Analogs of compound **2e**-*p* under development herein retain the benzoxazole core, while exploring a variety of sulfonamide end-capping substructures (R). Notes on compound nomenclature: (i) the number corresponds to the alkyl or aryl group adjacent to the sulfonamide linker; (ii) the letter corresponds to the substituent present on the phenyl group for the compound **2** series of analogs; and (iii) *o*, *m*, and *p* correspond to *ortho*, *meta*, and *para*-positioning of the respective substituents on the phenyl rings.

When we tested compound **1** in a well-established, 72 h cell viability assay employing the *T. brucei brucei* subspecies, we found that it elicited anti-parasitic affects (EC_{50} 7.9 μM, Table 1).[35,36] As controls for cell viability testing, we included the four primary HAT therapeutics pentamidine (EC_{50} < 0.019 μM), suramin (EC_{50} = 0.12 μM), nifurtimox (EC_{50} = 2.8 μM), and eflornithine (EC_{50} > 42 μM). We note that for safety reasons, in these initial studies we tested hit-to-lead compounds against the *T. brucei brucei* subspecies, which infects animals but not humans, and not the *T. brucei gambiense* or *rhodesiense* strains that infect humans. However, we believe that the anti-parasitic effects of HSP60 inhibitors will likely translate to the human strains since this has been observed with other inhibitor classes.[37–39] In addition, sequence alignments of the *T. brucei brucei* and *T. brucei gambiense* HSP60 isoforms obtained from the NCBI database indicate the HSP60.1 isoforms are identical between the two subspecies (as are HSP10), while the HSP60.2, and HSP60.3 isoforms differ by only two conservative amino acid substitutions each (sequences for the *T. brucei rhodesiense* HSP60 isoforms were not available).[40] From our previous antibacterial testing, we found that compound **1** exhibited moderate cytotoxicity to human liver (THLE-3) and kidney (HEK 293) cell lines in an established cell culture assay that measures compound cytotoxicity over a 72 h time course.[34] Therefore, in the present study, we developed a set of analogs to try to enhance their anti-parasitic effects against *T. brucei* while reducing off-target cytotoxicity to human liver and kidney cells. We synthesized two series of compound **1** analogs through simple coupling of sulfonyl chlorides with the 5-amino-2-(4-aminophenyl)benzoxazole core (Scheme 1).[41,42] The first series was designed to probe the effects of a variety of substituents and substitution patterns on the sulfonamide end-capping phenyl group (Table 1). The second series was designed to probe what alkyl and aryl groups would be tolerated adjacent to the sulfonamide linkers (Table 2).

We found that the *T. brucei* HSP60 chaperonins could not be readily obtained from *E. coli* expression systems as they formed intractable inclusion bodies. Thus, we used *E. coli* GroEL/ES as a surrogate and tested compounds using our two primary biochemical assays that evaluate for inhibition of GroEL/ES-dMDH refolding and ATPase activity over time.[34] These assays employed 50 nM of GroEL oligomer (700 nM monomeric subunits) and physiological concentrations of ATP (1 mM), and thus low to sub-μM IC_{50} values indicate very potent inhibitors that are functioning at near stoichiometric concentrations. Of the 49 new analogs, 39% are more potent than the initial compound **1** hit (Tables 1 and 2). Aryl groups adjacent to the sulfonamide linkers generally provide the most potent GroEL/ES inhibitors. We believe the compounds directly interact with GroEL as they do not inhibit the native MDH reporter reaction (Fig. 4A). Furthermore, there is a strong correlation between inhibiting the refolding and ATPase functions of the chaperonin system (Fig. 4B), suggesting compounds may bind to the ATP sites of GroEL. Consistent with binding to the ATP pockets, series **2h** and **2j** are the most potent inhibitors as their -OH and $-NH_2$ groups putatively hydrogen bond with the catalytic D398 aspartate, while series **2m** inhibitors are the least effective putatively owing to charge-charge repulsion of their carboxylates with the D398 aspartate. Binding in such a mode would also position one of the sulfonamide linkers in proximity to mimic a phosphate group of ATP; however, the requirement of the sulfonamide linkers for potent inhibition remains to be determined. While we included the four primary HAT therapeutics pentamidine, suramin, nifurtimox, and eflornithine as putative negative controls in our biochemical assays, we were surprised to find that suramin actually inhibits the *E. coli* GroEL/ES chaperonin system. This result could have profound implications on suramin's mechanism of action against *T. brucei* parasites.

We next evaluated chaperonin inhibitors for their ability to block the proliferation of *T. brucei brucei* parasites and found that most of the compounds are cytotoxic (Fig. 4C). The scatter in the correlation between IC_{50}/EC_{50} values could indicate that compounds hit another target in addition to the HSP60/10 chaperonin systems in parasites. It could also be that *E. coli* GroEL/ES is not a suitable surrogate to test with in lieu of the three *T. brucei* HSP60/10 systems. For instance, compounds may exhibit variable structure–activity relationships (SAR) against each of the three *T. brucei* HSP60 isoforms, which siRNA knock-down studies suggest would have differing effects on parasite viability.[17] In addition, localization differences between the three *T. brucei* HSP60 isoforms could significantly influence inhibitor effects against each and further complicate cytotoxicity profiles. We will investigate inhibitor mechanisms of action in parasites in future studies.

Through counter-screening against human mitochondrial HSP60/10, using procedures analogous to the GroEL/ES-based assays, we found that inhibitors are highly selective for bacterial GroEL/ES (Fig. 5A). However, the high selectivity we observe raises the question of why do these compounds not inhibit human HSP60 more potently than they do, considering *E. coli* GroEL and human HSP60 share ~95% amino acid identity in their ATP binding sites. We postulate this could be because these inhibitors bind to the *trans*-ring ATP pockets and allosterically lock up the double-ring GroEL, which functions through an obligate, two-stroke mechanism. This unique mode of inhibition would not be possible with human HSP60, which likely functions through a single-ring mechanism.[43–45] While we hoped that the lack of inhibition of human mitochondrial HSP60/10 in vitro would translate to low cytotoxicity to human cells, we found that many compounds are still moderately toxic to human liver (THLE-3) and kidney (HEK 293) cells (Fig. 5B). That many compounds are cytotoxic despite their being poor inhibitors of human HSP60/10 may suggest off-target effects

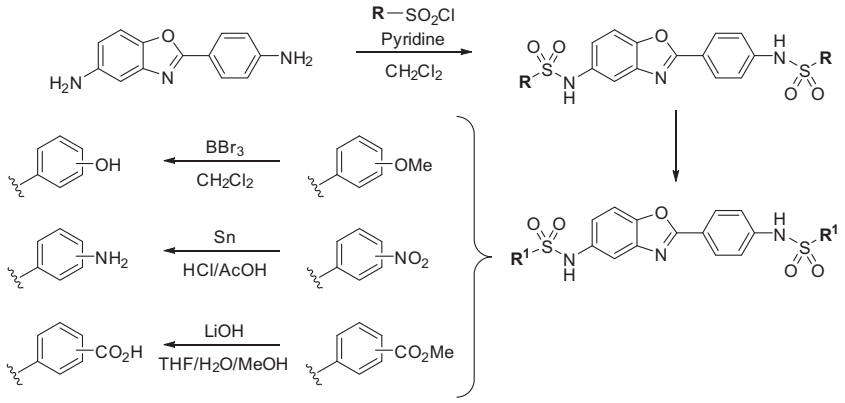
Table 1
Biochemical IC_{50} and cell viability EC_{50} results for chaperonin inhibitors based on the compound **2** scaffold where R = phenyl with variable *ortho*, *meta*, and *para*-substituents as presented. Results for the common HAT drugs are shown for comparison

Compound & substituent			Biochemical assay IC_{50} results (μM)					Cell viability EC_{50} results (μM)		
			Native MDH reporter	GroEL/ES-dMDH		HSP60/10-dMDH		*T. brucei*	THLE3 (Liver)	HEK 293 (Kidney)
				Refolding	ATPase	Refolding	ATPase			
$-CH_3$	**1**		>63	21	132	89	106	7.9	29	34
–H	**2a**		>63	3.9	4.6	>100	>250	6.4	19	15
–F	**2b**	*-o*	>63	22	>250	77	>250	19	55	31
		-m	>63	5.3	5.1	68	>250	10	20	24
		-p	>63	3.8	4.3	>100	>250	4.3	19	21
–Cl	**2c**	*-o*	>63	46	131	>100	>250	5.9	24	75
		-m	>63	18	>250	>100	>250	4.5	17	21
		-p	>63	23	35	96	>250	3.2	18	13
–Br	**2d**	*-o*	>63	63	144	>100	>250	28	37	28
		-m	>63	23	45	>100	>250	8.0	21	25
		-p	>63	24	19	>100	>250	2.4	16	22
$-CH_3$	**2e**	*-o*	>63	>100	194	>100	>250	1.8	17	25
		-m	>63	35	>250	>100	>250	4.9	18	65
		-p	>63	36	46	>100	>250	3.1	11	71
$-CF_3$	**2f**	*-o*	>63	25	21	>100	>250	28	50	66
		-m	>63	15	216	>100	>250	4.8	19	15
		-p	>63	35	>250	61	>250	4.1	21	>100
$-OCH_3$	**2g**	*-o*	>63	69	>250	>100	>250	2.7	>100	79
		-m	>63	48	>250	>100	>250	5.1	15	28
		-p	>63	36	84	>100	>250	3.3	>100	88
–OH	**2h**	*-o*	33	7.5	36	13	33	5.0	19	18
		-m	47	0.90	0.79	19	61	8.9	37	36
		-p	50	0.34	0.31	11	59	21	41	38
$-NO_2$	**2i**	*-o*	>63	3.6	3.1	75	194	>42	93	60
		-m	>63	11	>250	60	>250	11	37	46
		-p	>63	26	>250	65	>250	15	36	59
$-NH_2$	**2j**	*-o*	>63	10	>250	>100	>250	3.3	18	14
		-m	>63	2.8	9.6	87	127	15	30	58
		-p	>63	1.4	1.7	68	224	22	38	59
–CN	**2k**	*-o*	>63	32	>250	86	193	23	93	74
		-m	>63	7.6	4.1	91	>250	39	91	45
		-p	>63	37	>250	64	>250	16	79	45
$-CO_2CH_3$	**2l**	*-o*	>63	36	53	>100	>250	28	>100	>100
		-m	>63	11	>250	95	>250	>42	49	>100
		-p	>63	26	91	87	>250	23	>100	>100
$-CO_2H$	**2m**	*-o*	>63	>100	>250	>100	>250	>42	>100	>100
		-m	41	61	>250	81	>250	>42	>100	>100
		-p	>63	83	>250	>100	>250	>42	>100	>100
Pentamidine			>63	>100	>250	>100	>250	<0.019	19	>100
Suramin			>63	7.9	>250	11	>250	0.12	>100	>100
Nifurtimox			>63	>100	>250	>100	>250	2.8	>100	>100
Eflornithine			>63	>100	>250	>100	>250	>42	>100	>100

in human cells. We will identify potential off-target pathways that these inhibitors could be modulating in future studies.

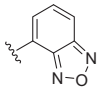
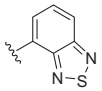
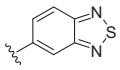
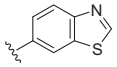
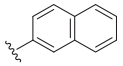
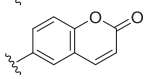
While a general trend is noted when comparing cytotoxicity of compounds to *T. brucei* parasites with human liver and kidney cells (Fig. 5C), we found that inhibitors are usually more selective for the parasites. A few compounds exhibit moderate to high selectivity for parasites over human cells: e.g., compounds **2c**-*o*, **2c**-*p*, **2d**-*p*, **2e**-*o*/*m*/*p*, **2g**-*o*, **2g**-*p*, **2l**-*o*, and **2l**-*p*, as well as the two naphthyl-containing analogs, **10** and **13**. Intriguingly, it appears that substituents extending outwards from the *ortho* and *para*-positions on the phenyl ring could provide an advantage for selectively targeting *T. brucei* parasites over human liver and kidney cells. These studies have importantly provided structural leads that we can pursue in future optimization studies. We will investigate how adding a variety of substituents to these and other aryls, as well as altering the sulfonamide linkers and the 2-phenylbenzoxazole core, will affect inhibitor potency and selectivity in future studies. We appreciate that lead inhibitors are pushing the higher limits of the Lipinski criteria (e.g., compound **10** has a MW of 606 g/mol and *c*log*P* of 7.3); therefore, to develop lead candidates that overcome the pharmacological deficiencies of current HAT therapeutics, we will also need to investigate inhibitor oral bioavailability, blood–brain barrier permeability, metabolic stability, and pharmacokinetic/pharmacodynamics profiles in vitro and in vivo.

In conclusion, we have developed a new series of chaperonin inhibitors that exhibit antibiotic effects against *Trypanosoma brucei* parasites in cell culture. While many of these initial analogs exhibit moderate cytotoxicity to human liver and kidney cells, the SAR generated from this study has provided valuable guidance on molecular substructures to pursue for increasing the therapeutic windows of these chaperonin-targeting antibiotic candidates. We are also exploring additional hits from our previous GroEL/ES high-throughput screening to identify alternative scaffolds that selectively kill *T. brucei* parasites. One of the most significant



Scheme 1. General methods to synthesize inhibitor analogs.[41,42] Coupling of sulfonyl chlorides with the 5-amino-2-(4-aminophenyl)benzoxazole core provided the primary bis-sulfonamide inhibitors. Three general secondary reactions were employed to further transform substituents: Series **2h**—methoxy deprotection to hydroxyls; Series **2j**—nitro reduction to amines; and Series **2m**—ester hydrolysis to carboxylic acids. Refer to the Supporting information for protocols and characterization data for specific compounds.

Table 2
Biochemical IC_{50} and cell viability EC_{50} results for chaperonin inhibitors where the sulfonamide end-capping R-groups are variable alkyl and aryl substructures as presented

Compound & substructures		Biochemical assay IC_{50} results (μM)					Cell viability EC_{50} results (μM)		
		Native MDH reporter	GroEL/ES-dMDH		HSP60/10-dMDH		*T. brucei*	THLE3 (Liver)	HEK 293 (Kidney)
			Refolding	ATPase	Refolding	ATPase			
	**2a**	>63	3.9	4.6	>100	>250	6.4	19	15
	**3**	>63	>100	>250	>100	>250	>42	>100	>100
	**4**	>63	40	>250	97	>250	>42	>100	>100
	**5**	>63	>100	>250	>100	>250	18	>100	>100
	**6**	>63	4.6	6.7	57	>250	17	44	26
	**7**	>63	55	208	>100	>250	13	29	19
	**8**	>63	32	>250	66	212	>42	58	45
	**9**	>63	22	127	98	152	>42	>100	52
	**10**	>63	23	54	>100	144	2.1	69	>100
	**11**	>63	3.7	1.7	62	127	>42	45	76
	**12**	>63	0.81	0.55	49	>250	25	30	95
	**13**	>63	40	67	>100	127	3.7	55	>100
	**14**	>63	18	83	70	201	>42	>100	>100

findings from this study is that the first-line therapeutic for African sleeping sickness, suramin, also inhibits both *E. coli* GroEL/ES and human HSP60/10. This suggests that suramin can inhibit one or all of the three *T. brucei* HSP60 isoforms in parasites; however, this may not be suramin's primary mechanism of action as it has been found to interact with several biological pathways.[46–52] Indeed, suramin's promiscuity against several different targets (i.e., polypharmacological effects) may be why this drug has been successful against *T. brucei* parasites for the past 100 years. It will be intriguing to investigate the contribution that inhibiting the three *T. brucei* HSP60 isoforms makes to the antibiotic efficacy of suramin. Importantly, these new findings further support accumulating evidence that chaperonin-targeting drugs can be developed even though they may inhibit human HSP60/10 biochemical functions in vitro. While we are using *T. brucei* as the model parasite to identify the viability of a chaperonin-targeting antibiotic strategy,

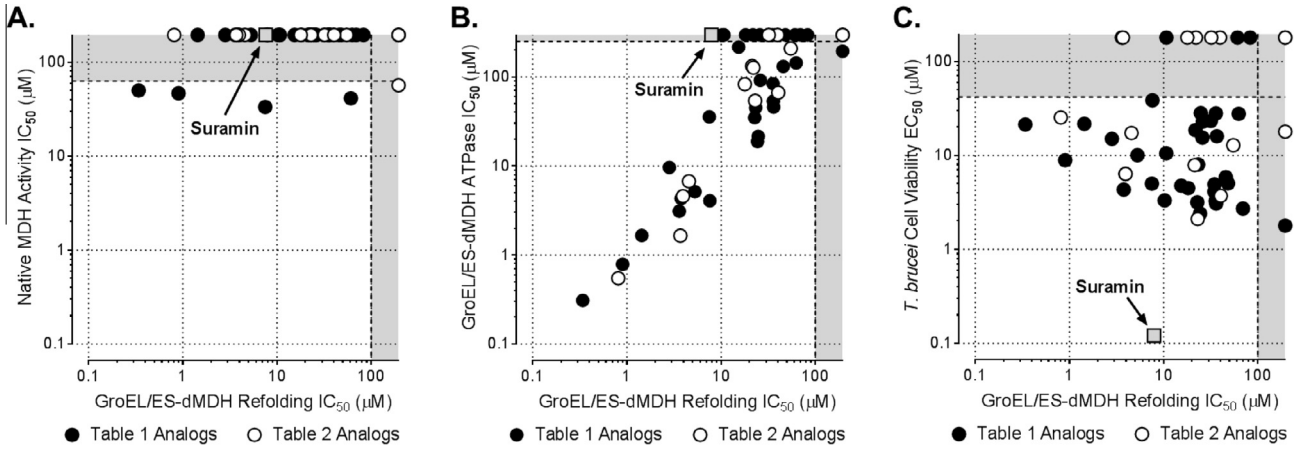


Figure 4. (A) Compounds selectively inhibit in the *E. coli* GroEL/ES-dMDH refolding assay without targeting the native MDH reporter reaction. (B) A strong correlation between IC_{50} values for the GroEL/ES-dMDH refolding and ATPase assays suggests the compounds interact directly with the chaperonin system, and are putatively binding to the ATP pockets. (C). Chaperonin inhibitors are cytotoxic to *T. brucei* parasites. Correlation plots include data from compounds in both Table 1 (black circles) and Table 2 (white circles). Data plotted in the gray zones represent results beyond the assay detection limits (i.e., >100 μM for the GroEL/ES-dMDH refolding assay, >63 μM for the native MDH activity assay, >250 μM for the chaperonin-mediated ATPase assay, and >42 μM for the *T. brucei* cell viability assay). Results for suramin (gray square), which is a HAT drug that was found to be a potent GroEL/ES inhibitor, are shown for comparison.

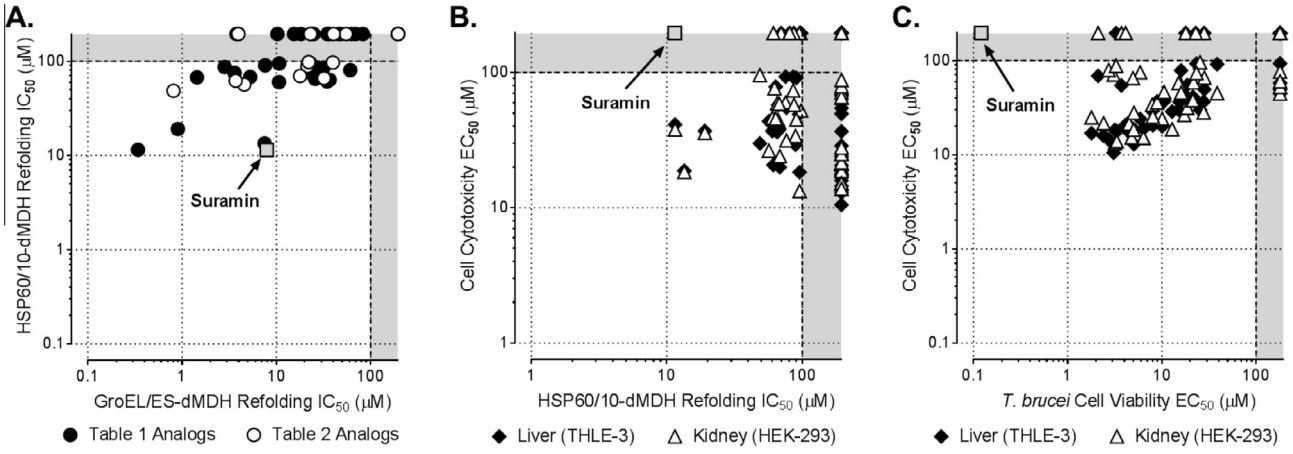


Figure 5. (A) Compounds selectively inhibit the refolding cycles of the *E. coli* GroEL/ES over the human HSP60/10 chaperonin system. (B). Many compounds exhibit moderate cytotoxicity to human liver and kidney cell lines, even though they do not inhibit the HSP60/10 refolding cycle in vitro. (C). Compounds are generally more cytotoxic to *T. brucei* parasites over human liver and kidney cells. Data plotted in the gray zones represent results beyond the assay detection limits (i.e., >100 μM for the chaperonin-mediated dMDH refolding assays, >100 μM for the human liver and kidney cell cytotoxicity assays, and >42 μM for the *T. brucei* cell viability assay). Correlation plots include data from both Tables 1 and 2 compounds.

our studies will open the possibility of targeting the chaperonin systems of a wide range of eukaryotic pathogens.

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Supplementary data

Supplementary data (tabulations of log(IC_{50}) and log(EC_{50}) results with standard deviations; experimental protocols for biochemical and cell-based assays; synthetic protocols and ^{1}H NMR, LC–MS, and HPLC characterization data for all compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.09.051.

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