

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

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Abstract

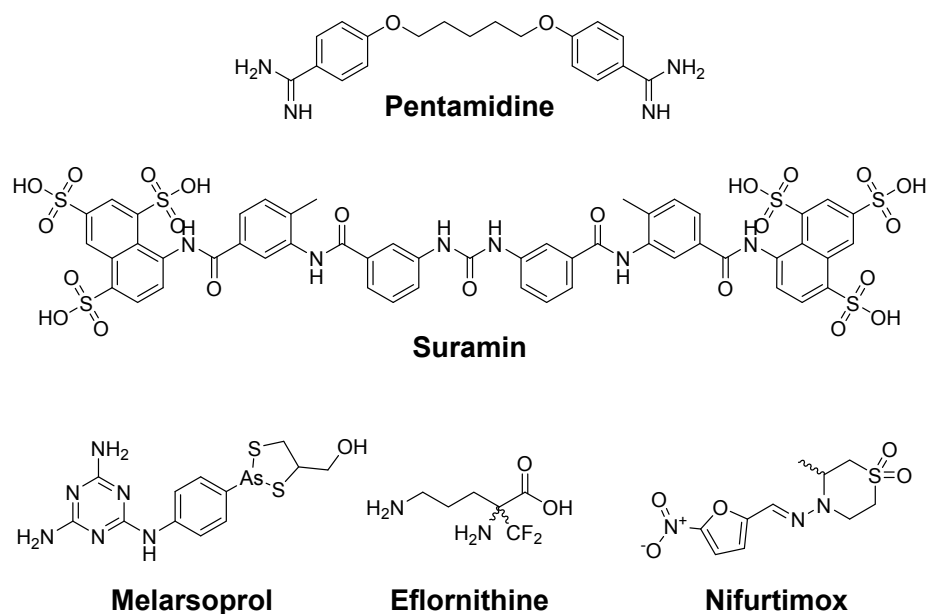
Trypanosoma brucei are protozoan parasites that cause African sleeping sickness in humans (also known as Human African Trypanosomiasis – HAT). Without treatment, *T. brucei* infections are fatal. There is an urgent need for new therapeutic strategies as current drugs are toxic, have complex treatment 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 *E. 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 $3.1 \mu M$, 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 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 (**Figure 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.

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



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 (**Figure 2A**).^{26, 27} 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, 28-30} 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 (**Figure 2B**).^{26, 27} 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.²⁶ 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, 28, 31, 32} 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.

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).²⁶

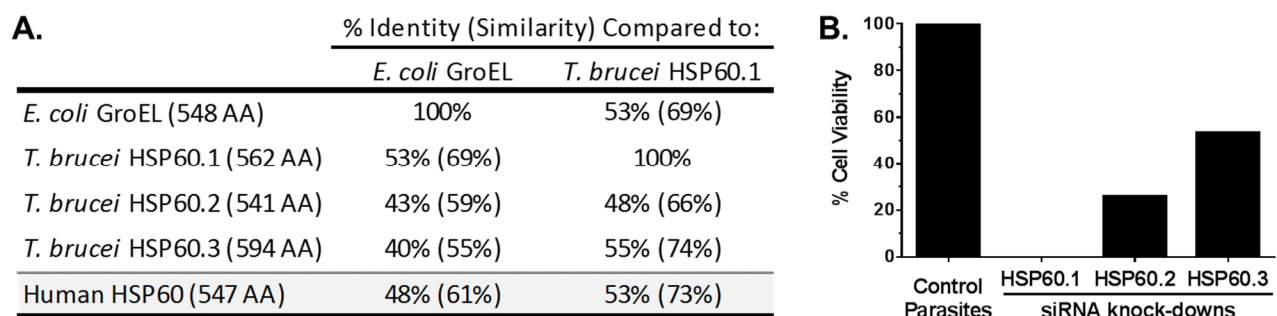
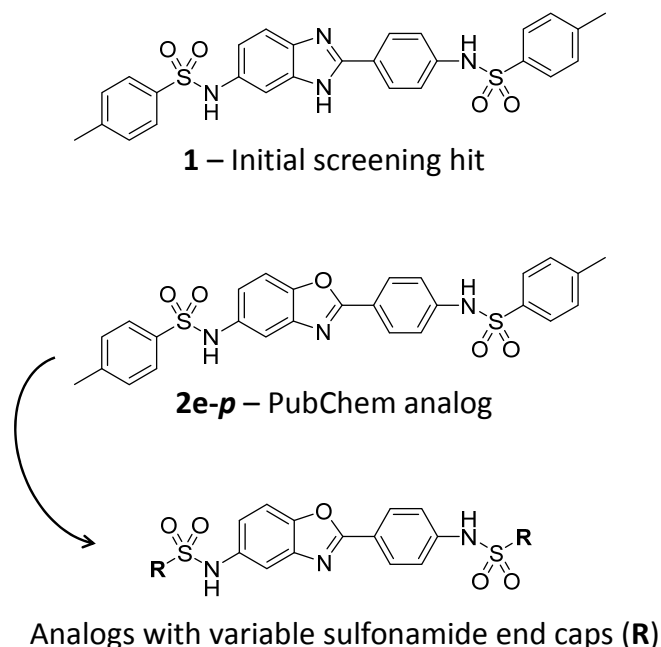


Figure 3. Compound **1** was an initial hit that emerged from our recent high-throughput screening for GroEL/ES inhibitors.³³ Compound **2e-p** is a related analog found in the PubChem database (CID #1098316) that has reported bioactivity in only 8 of the 285 assays it has been evaluated in.³⁴ One assay 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.



We previously performed high-throughput screening and discovered 235 small molecule inhibitors of the *E. coli* GroEL/ES chaperonin system.³³ We have since found that several of our chaperonin inhibitors exhibit antibiotic effects against Gram-positive and Gram-negative bacteria.³⁵ One of the most potent GroEL/ES inhibitors that we discovered was compound **1** (**Figure 3**), which inhibited both the substrate refolding and ATPase functions of the chaperonin system.^{33, 35} Unfortunately, compound **1** was inactive against the panel of bacteria we tested against, suggesting it may not be a good candidate for antibacterial development.³⁵ However, we found a related analog in the PubChem database where the benzimidazole core is replaced by a benzoxazole (**Figure 3**, compound **2e-p**, PubChem CID #1098316).³⁴ 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*.

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 effects (EC_{50} 7.9 μ M, **Table 1**).^{36, 37} As controls for cell viability testing, we included the four primary HAT therapeutics pentamidine (EC_{50} < 0.019 μ M), suramin (EC_{50} = 0.12 μ M), nifurtimox (EC_{50} = 2.8 μ M), and eflornithine (EC_{50} > 42 μ M). 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.³⁸⁻⁴⁰ 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).⁴¹ 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.³⁵ 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**).^{42, 43} 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**).

Scheme 1. General methods to synthesize inhibitor analogs.^{42, 43} 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.

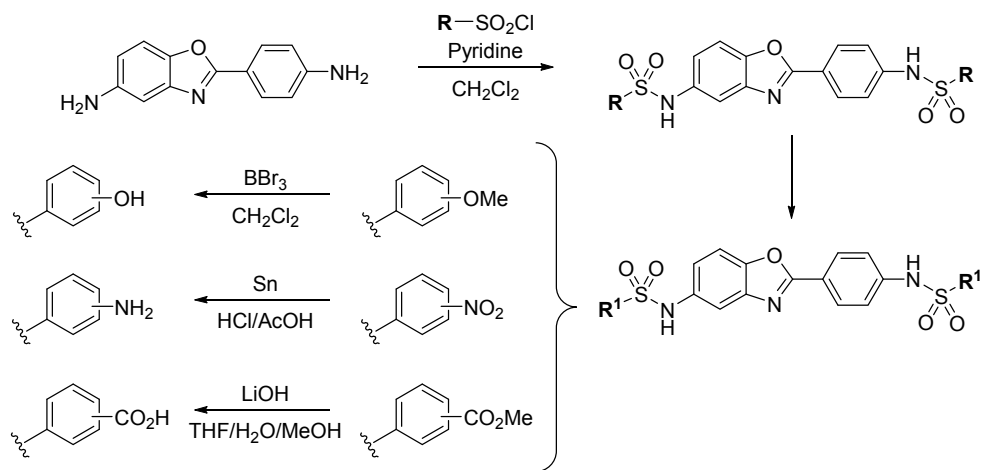















Table 1. Biochemical IC₅₀ and cell viability EC₅₀ results for chaperonin inhibitors based on the compound **2** scaffold where **R** = phenyl with variable *ortho*, *meta*, and *para*-substituents as presented. Results for the common HAT drugs are shown for comparison.

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 ₂ CH 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 ₂ H	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	

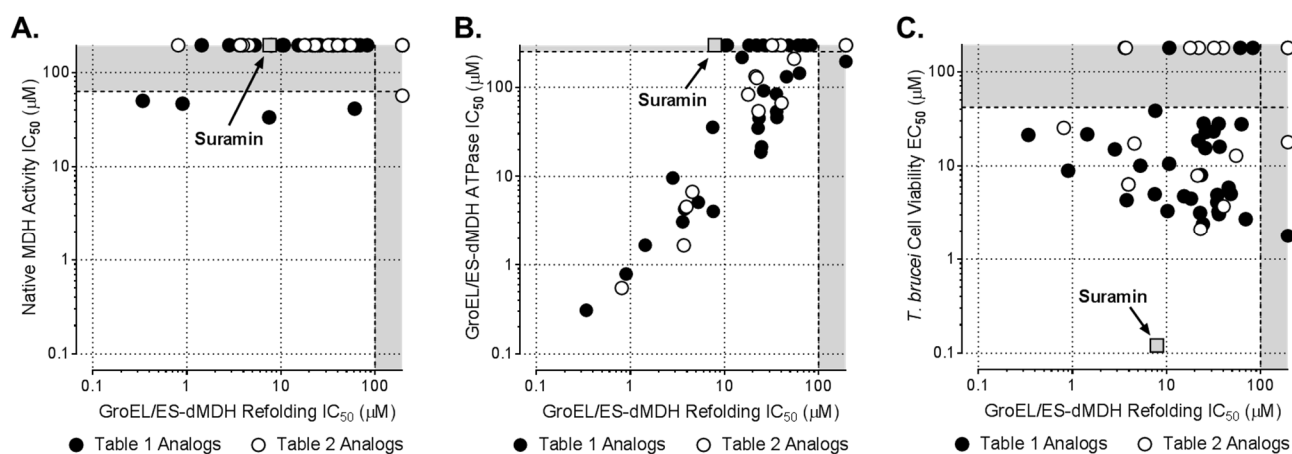
Table 2. Biochemical IC₅₀ and cell viability EC₅₀ results for chaperonin inhibitors where the sulfonamide end-capping **R**-groups are variable alkyl and aryl substructures as presented.

Compound & Substructures		Biochemical Assay IC ₅₀ Results (μM)					Cell Viability EC ₅₀ Results (μM)		
		Native MDH Reporter	GroEL/ES-dMDH		HSP60/10-dMDH		<i>T. brucei</i>	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

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.³⁵ 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 IC₅₀ 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 (**Figure 4A**). Furthermore, there is a strong correlation between inhibiting the refolding and ATPase functions of the chaperonin system (**Figure 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 -NH₂ 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.

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 IC₅₀ 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 grey zones represent results beyond the assay detection limits (i.e. >100 μ M for the GroEL/ES-dMDH refolding assay, >63 μ M for the native MDH activity assay, >250 μ M for the chaperonin-mediated ATPase assay, and >42 μ M for the *T. brucei* cell viability assay). Results for suramin (grey square), which is a HAT drug that was found to be a potent GroEL/ES inhibitor, are shown for comparison.

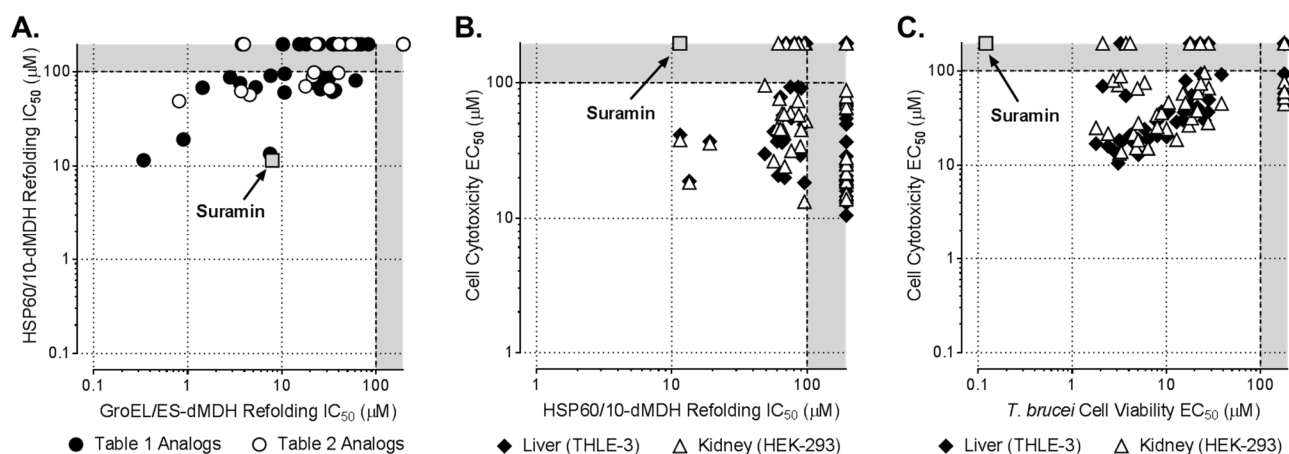


We next evaluated chaperonin inhibitors for their ability to block the proliferation of *T. brucei* parasites and found that most of the compounds are cytotoxic (**Figure 4C**). The scatter in the correlation between IC_{50}/EC_{50} values could indicate that compounds hit another target in addition 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.²⁶ 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 (**Figure 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.⁴⁴⁻⁴⁶ 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 (**Figure 5B**). That many compounds are cytotoxic despite their being poor inhibitors of human HSP60/10 may suggest off-target effects in human cells. We will identify potential off-target pathways that these inhibitors could be modulating in future studies.

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 grey zones represent results beyond the assay detection limits (i.e. >100 μ M for the chaperonin-mediated dMDH refolding assays, >100 μ M for the human liver and kidney cell cytotoxicity assays, and >42 μ M for the *T. brucei* cell viability assay). Correlation plots include data from both **Table 1** and **2** compounds.



While a general trend is noted when comparing cytotoxicity of compounds to *T. brucei* parasites with human liver and kidney cells (**Figure 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 clogP 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 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.⁴⁷⁻⁵³ 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, our studies will open the possibility of targeting the chaperonin systems of a wide range of eukaryotic pathogens.

Supporting Information: Supporting information associated with this article can be found in the online version, which includes tabulations of log(IC₅₀) and log(EC₅₀) results with standard deviations; experimental protocols for biochemical and cell-based assays; synthetic protocols and ¹H-NMR, LC-MS, and HPLC characterization data for all compounds.

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Supporting Information

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

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




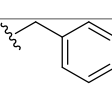
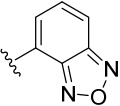
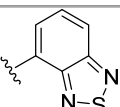
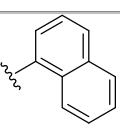
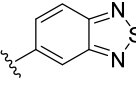
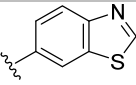
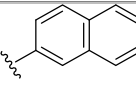
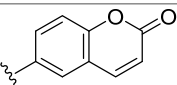
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Table S1. Log-transformed values for Table 1 IC₅₀ and EC₅₀ results. Results are presented as log(IC₅₀ or EC₅₀ / μ M) values \pm their standard deviations (SD).

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	1e	- p	>1.8	1.33 ± 0.68	2.12 ± 0.10	1.95 ± 0.08	2.02 ± 0.16	0.90 ± 0.11	1.47 ± 0.23	1.53 ± 0.24	
-H	2a		>1.8	0.60 ± 0.33	0.66 ± 0.06	>2	>2.4	0.80 ± 0.08	1.28 ± 0.02	1.18 ± 0.13	
-F	2b	- o	>1.8	1.34 ± 0.27	>2.4	1.88 ± 0.12	>2.4	1.27 ± 0.04	1.74 ± 0.05	1.50 ± 0.03	
		- m	>1.8	0.72 ± 0.17	0.71 ± 0.06	1.83 ± 0.13	>2.4	1.00 ± 0.09	1.30 ± 0.04	1.38 ± 0.07	
		- p	>1.8	0.58 ± 0.23	0.63 ± 0.09	>2	>2.4	0.64 ± 0.12	1.27 ± 0.07	1.33 ± 0.11	
-Cl	2c	- o	>1.8	1.66 ± 0.18	2.12 ± 0.07	>2	>2.4	0.77 ± 0.05	1.38 ± 0.10	1.87 ± 0.10	
		- m	>1.8	1.26 ± 0.43	>2.4	>2	>2.4	0.65 ± 0.13	1.24 ± 0.06	1.33 ± 0.08	
		- p	>1.8	1.36 ± 0.30	1.54 ± 0.17	1.98 ± 0.03	>2.4	0.50 ± 0.14	1.26 ± 0.12	1.12 ± 0.08	
-Br	2d	- o	>1.8	1.80 ± 0.11	2.16 ± 0.05	>2	>2.4	1.44 ± 0.14	1.56 ± 0.03	1.45 ± 0.08	
		- m	>1.8	1.37 ± 0.13	1.65 ± 0.07	>2	>2.4	0.90 ± 0.10	1.31 ± 0.09	1.40 ± 0.13	
		- p	>1.8	1.39 ± 0.27	1.28 ± 0.19	>2	>2.4	0.38 ± 0.15	1.20 ± 0.16	1.33 ± 0.18	
-CH ₃	2e	- o	>1.8	>2	2.29 ± 0.11	>2	>2.4	0.25 ± 0.42	1.23 ± 0.17	1.40 ± 0.14	
		- m	>1.8	1.54 ± 0.23	>2.4	>2	>2.4	0.69 ± 0.06	1.25 ± 0.11	1.81 ± 0.06	
		- p	>1.8	1.56 ± 0.23	1.66 ± 0.04	>2	>2.4	0.49 ± 0.16	1.02 ± 0.03	1.85 ± 0.17	
-CF ₃	2f	- o	>1.8	1.39 ± 0.23	1.33 ± 0.02	>2	>2.4	1.45 ± 0.16	1.69 ± 0.11	1.82 ± 0.12	
		- m	>1.8	1.19 ± 0.08	2.33 ± 0.16	>2	>2.4	0.68 ± 0.11	1.28 ± 0.03	1.18 ± 0.06	
		- p	>1.8	1.54 ± 0.36	>2.4	1.79 ± 0.35	>2.4	0.61 ± 0.10	1.32 ± 0.14	>2	
-OCH ₃	2g	- o	>1.8	1.86 ± 0.15	>2.4	>2	>2.4	0.44 ± 0.19	>2	1.90 ± 0.17	
		- m	>1.8	1.68 ± 0.07	>2.4	>2	>2.4	0.70 ± 0.09	1.16 ± 0.06	1.44 ± 0.15	
		- p	>1.8	1.55 ± 0.10	1.92 ± 0.01	>2	>2.4	0.51 ± 0.12	>2	1.94 ± 0.16	
-OH	2h	- o	1.52 ± 0.05	0.88 ± 0.17	1.55 ± 0.16	1.13 ± 0.19	1.52 ± 0.07	0.70 ± 0.08	1.27 ± 0.09	1.27 ± 0.12	
		- m	1.67 ± 0.04	-0.05 ± 0.24	-0.10 ± 0.13	1.28 ± 0.24	1.79 ± 0.07	0.95 ± 0.13	1.57 ± 0.05	1.55 ± 0.04	
		- p	1.70 ± 0.10	-0.47 ± 0.44	-0.50 ± 0.16	1.06 ± 0.31	1.77 ± 0.09	1.33 ± 0.10	1.62 ± 0.08	1.58 ± 0.06	
-NO ₂	2i	- o	>1.8	0.56 ± 0.23	0.49 ± 0.11	1.88 ± 0.11	2.29 ± 0.10	>1.6	1.97 ± 0.05	1.78 ± 0.05	
		- m	>1.8	1.03 ± 0.67	>2.4	1.78 ± 0.03	>2.4	1.02 ± 0.08	1.57 ± 0.06	1.66 ± 0.06	
		- p	>1.8	1.41 ± 0.15	>2.4	1.82 ± 0.24	>2.4	1.19 ± 0.04	1.56 ± 0.05	1.77 ± 0.07	
-NH ₂	2j	- o	>1.8	1.01 ± 0.26	>2.4	>2	>2.4	0.52 ± 0.17	1.25 ± 0.16	1.14 ± 0.14	
		- m	>1.8	0.45 ± 0.35	0.98 ± 0.58	1.94 ± 0.10	2.10 ± 0.10	1.18 ± 0.05	1.48 ± 0.02	1.76 ± 0.27	
		- p	>1.8	0.16 ± 0.29	0.22 ± 0.15	1.83 ± 0.15	2.35 ± 0.08	1.34 ± 0.07	1.58 ± 0.20	1.77 ± 0.18	
-CN	2k	- o	>1.8	1.50 ± 0.17	>2.4	1.93 ± 0.08	2.29 ± 0.04	1.37 ± 0.11	1.97 ± 0.04	1.87 ± 0.19	
		- m	>1.8	0.88 ± 0.20	0.61 ± 0.03	1.96 ± 0.03	>2.4	1.59 ± 0.12	1.96 ± 0.03	1.65 ± 0.04	
		- p	>1.8	1.56 ± 0.11	>2.4	1.80 ± 0.19	>2.4	1.20 ± 0.13	1.89 ± 0.08	1.66 ± 0.11	
-CO ₂ CH ₃	2l	- o	>1.8	1.55 ± 0.10	1.73 ± 0.10	>2 ±	>2.4	1.45 ± 0.25	>2	>2	
		- m	>1.8	1.03 ± 0.47	>2.4	1.98 ± 0.04	>2.4	>1.6	1.69 ± 0.22	>2	
		- p	>1.8	1.42 ± 0.11	1.96 ± 0.03	1.94 ± 0.08	>2.4	1.36 ± 0.18	>2	>2	
-CO ₂ H	2m	- o	>1.8	>2	>2.4	>2	>2.4	>1.6	>2	>2	
		- m	1.62 ± 0.05	1.78 ± 0.13	>2.4	1.91 ± 0.12	>2.4	>1.6	>2	>2	
		- p	>1.8	1.92 ± 0.14	>2.4	>2 ±	>2.4	>1.6	>2	>2	
Pentamidine		>1.8	>2	>2.4	>2	>2.4	<-1.7	1.27 ± 0.11	>2		
Suramin		>1.8	0.90 ± 0.19	>2.4	1.06 ± 0.09	>2.4	-0.92 ± 0.15	>2	>2		
Nifurtimox		>1.8	>2	>2.4	>2	>2.4	0.45 ± 0.09	>2	>2		
Eflornithine		>1.8	>2	>2.4	>2	>2.4	>1.6	>2	>2		

Table S2. Log-transformed values for Table 2 IC₅₀ and EC₅₀ results. Results are presented as log(IC₅₀ or EC₅₀ / μ M) values \pm their standard deviations (SD).

Compound & Substructures		Biochemical Assay IC_{50} Results (μM)					Cell Viability EC_{50} Results (μM)		
		Native MDH Reporter	GroEL/ES-dMDH		HSP60/10-dMDH		<i>T. brucei</i>	THLE3 (Liver)	HEK 293 (Kidney)
			Refolding	ATPase	Refolding	ATPase			
	2a	>1.8	0.60 \pm 0.33	0.66 \pm 0.06	>2	>2.4	0.80 \pm 0.08	1.28 \pm 0.02	1.18 \pm 0.13
	3	>1.8	>2	>2.4	>2	>2.4	>1.6	>2	>2
	4	>1.8	1.60 \pm 0.16	>2.4	1.99 \pm 0.04	>2.4	>1.6	>2	>2
	5	>1.8	>2	>2.4	>2	>2.4	1.25 \pm 0.06	>2	>2
	6	>1.8	0.66 \pm 0.24	0.83 \pm 0.21	1.75 \pm 0.17	>2.4	1.24 \pm 0.09	1.64 \pm 0.03	1.42 \pm 0.08
	7	>1.8	1.74 \pm 0.12	2.32 \pm 0.06	>2	>2.4	1.11 \pm 0.14	1.46 \pm 0.03	1.27 \pm 0.05
	8	>1.8	1.51 \pm 0.19	>2.4	1.82 \pm 0.15	2.33 \pm 0.09	>1.6	1.76 \pm 0.05	1.65 \pm 0.01
	9	>1.8	1.34 \pm 0.50	2.10 \pm 0.10	1.99 \pm 0.05	2.18 \pm 0.09	>1.6	>2	1.72 \pm 0.19
	10	>1.8	1.36 \pm 0.64	1.73 \pm 0.14	>2	2.16 \pm 0.11	0.32 \pm 0.06	1.84 \pm 0.28	>2
	11	>1.8	0.57 \pm 0.55	0.22 \pm 0.08	1.80 \pm 0.31	2.10 \pm 0.06	>1.6	1.65 \pm 0.18	1.88 \pm 0.12
	12	>1.8	-0.09 \pm 0.55	-0.26 \pm 0.09	1.69 \pm 0.31	>2.4	1.40 \pm 0.11	1.48 \pm 0.01	1.98 \pm 0.09
	13	>1.8	1.61 \pm 0.17	1.82 \pm 0.15	>2	2.10 \pm 0.11	0.57 \pm 0.17	1.74 \pm 0.29	>2
	14	>1.8	1.25 \pm 0.11	1.92 \pm 0.18	1.84 \pm 0.15	2.30 \pm 0.13	>1.6	>2	>2

General Materials and Methods.

DH5 α and BL21 (DE3) *E. coli* cells were purchased from New England Biolabs, and Rosetta™ 2 (DE3) *E. coli* cells from EMD Millipore. *Trypanosoma brucei brucei* Plimmer and Bradford parasites (Lister 427 VSG 221 [TetR T7RNAP] transgenic bloodstream form) were obtained from the ATCC (PRA-383). HEK 293 kidney and THLE-3 liver cells were obtained from the ATCC (CRL-1573 and CRL-11233, respectively). Antibiotics were used in following concentrations when appropriate; Kanamycin (34 $\mu\text{g/mL}$), ampicillin (50 $\mu\text{g/mL}$), chloramphenicol (30 $\mu\text{g/mL}$) and streptomycin (100 $\mu\text{g/mL}$). Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification. All test compounds were synthesized according to literature procedures for similar molecules.^{1,2} Reaction progress was monitored by thin-layer chromatography on silica gel 60 F254 coated glass plates (EM Sciences). Flash chromatography was performed using a Biotage Isolera One flash chromatography system and eluting through Biotage KP-Sil Zip or Snap silica gel columns for normal phase separations (hexanes:EtOAc gradients) or Snap KP-C18-HS columns for reverse phase separations (H₂O:MeOH gradients). Reverse phase high performance liquid chromatography (RP-HPLC) was performed using a Waters 1525 binary pump, 2489 tunable UV/Vis detector (254 and 280 nm detection), and 2707 autosampler. For preparatory HPLC purification, samples were chromatographically separated using a Waters XSelect CSH C18 OBD prep column (part number 186005422, 130 Å pore size, 5 μm particle size, 19x150 mm), eluting with a H₂O:CH₃CN gradient solvent system. Linear gradients were run from either 100:0, 80:20, or 60:40 A:B to 0:100 A:B (A = 95:5 H₂O:CH₃CN, 0.05% TFA; B = 5:95 H₂O:CH₃CN, 0.05% TFA). For primary purity analyses (HPLC-1), samples were chromatographically separated using a Waters XSelect CSH C18 column (part number 186005282, 130 Å pore size, 5 μm particle size, 3.0x150 mm), eluting with the above H₂O:CH₃CN gradient solvent systems. For secondary purity analyses (HPLC-2), samples were chromatographically separated using a Waters XBridge C18 column (part number 186003027, 130 Å pore size, 3.5 μm particle size, 3.0x100 mm), eluting with a H₂O:MeOH gradient solvent system. Linear gradients were run from either 100:0, 80:20, 60:40, or 20:80 A:B to 0:100 A:B (A = 95:5 H₂O:MeOH, 0.05% TFA; B = 5:95 H₂O:MeOH, 0.05% TFA). Test compounds were found to be >95% in purity from both RP-HPLC analyses. Mass spectrometry data were collected using an Agilent analytical LC-MS at the IU Chemical Genomics Core Facility (CGCF). ¹H-NMR spectra were recorded on either a Bruker 300 MHz or Bruker 500 MHz spectrometer. Chemical shifts are reported in parts per million and calibrated to the *d*₆-DMSO solvent peaks at 2.50 ppm.

Protein Expression and purification.

E. coli GroEL and GroES, and human mitochondrial HSP60 and HSP10 were expressed and purified as previously reported.³ Protein concentrations were determined using a Coomassie Protein Assay Kit (Thermo Scientific). Proteins were stored at 4°C in 50 mM Tris-HCl, pH 7.4, 300 mM NaCl, and 1 mM DTT. *E. coli* GroEL and GroES proteins were discarded after 30 days, and human HSP60 and HSP10 were discarded after 10 days.

Calculation of IC₅₀ and EC₅₀ values.

All IC₅₀ (or EC₅₀) values reported are averages of IC₅₀ (or EC₅₀) values determined from individual dose-response curves in replicate assays as follows: 1) Individual IC₅₀ values from replicate assays were first log-transformed and the average log(I/EC₅₀) values and standard deviations (SD) calculated; 2) Replicate log(I/EC₅₀) values were evaluated for outliers using the ROUT method in GraphPad Prism 6 (Q of 10%); and 3) Average IC₅₀ (or EC₅₀) values were then back-calculated from the average log(I/EC₅₀) values.

GroEL/ES and HSP60/10-mediated dMDH refolding assay protocols.

The GroEL/ES-dMDH and HSP60/10-dMDH refolding assays were conducted as previously reported,³ with one minor procedural difference: instead of quenching the refolding reactions with EDTA at the 60 minute time point, the refolding reactions were quenched when they reached ~90% completion (as determined from refolding time-course control experiments – generally ~20-40 min for GroEL/ES, and ~40-60 min for

HSP60/10). Compounds were tested in 8-point, 3-fold dilution series (100 μ M to 46 nM) in clear, flat-bottom 384-well microtiter plates. DMSO was used as negative control, and previously discovered chaperonin inhibitors were used as positive controls.^{3,4} IC₅₀ values for the test compounds were obtained by plotting the % inhibition results in GraphPad Prism 6 and analyzing by non-linear regression using the log (inhibitor) vs. response (variable slope) equation. Results presented represent the averages of IC₅₀ values obtained from at least triplicate experiments.

Native MDH enzymatic activity counter-screen assay protocol.

This assay was performed as described above for the GroEL/ES-dMDH refolding assay, but the compounds were pin-transferred after the EDTA quench step. Thus, only the enzymatic portion of the assay was in the presence of test compounds to identify their effects on the native MDH reporter substrate. Compounds were tested in 8-point, 3-fold dilution series (62.5 μ M to 29 nM) in clear, flat-bottom 384-well microtiter plates. DMSO was used as negative control, and previously discovered native MDH inhibitors were used as positive controls.^{3,4} IC₅₀ values for the test compounds were obtained by plotting the % inhibition results in GraphPad Prism 6 and analyzing by non-linear regression using the log (inhibitor) vs. response (variable slope) equation. Results presented represent the averages of IC₅₀ values obtained from at least triplicate experiments.

Chaperonin-dependent ATPase activity assay protocol.

The GroEL/ES-dMDH and HSP60/10-dMDH ATPase assays were conducted as previously reported,³ with the procedural differences as noted above that the refolding reactions were quenched when they reached ~90% completion (as determined from refolding time-course control experiments – generally ~20-40 min for GroEL/ES, and ~40-60 min for HSP60/10). Compounds were tested in 8-point, 3-fold dilution series (250 μ M to 114 nM) in clear, flat-bottom 384-well microtiter plates. DMSO was used as negative control, and previously discovered chaperonin inhibitors were used as positive controls.^{3,4} A second set of baseline control plates were prepared analogously, but without binary solution, to correct for possible interference from compound absorbance or turbidity. IC₅₀ values for the test compounds were obtained by plotting the OD₆₀₀ results in GraphPad Prism 6 and analyzing by non-linear regression using the log(inhibitor) vs. response (variable slope) equation. Results presented represent the averages of IC₅₀ values obtained from at least triplicate experiments.

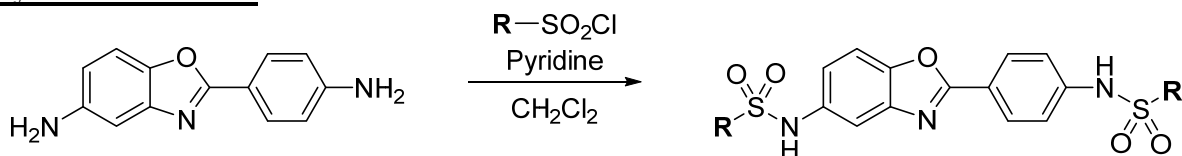
***T. brucei* cell viability assay protocol.**

Test compounds were evaluated using a robust *T. brucei* cell viability assay in 384-well plate format as previously reported.^{5,6} Briefly, 55 μ L of 2000 parasites/mL (110 parasites/well) of *Trypanosoma brucei brucei* (strain BF427) in HMI-9 medium were dispensed in to clear, 384-well polystyrene plates (BRAND cell culture grade plates, 781980). Plates were sealed with "Breathe Easy" oxygen permeable membranes (Diversified Biotech) and incubated at 37°C, 5% CO₂ for 24 h. Next, 1 μ L of the compound stocks (10 mM to 4.6 μ M, 3-fold dilutions in DMSO) were pre-diluted by pin-transfer into 20 μ L HMI-9 medium, then 5 μ L of these diluted compounds were added to the parasite assay plates to give an inhibitor concentration range of 42 μ M to 19 nM during the assay (the final DMSO concentration of 0.42% was maintained during the assay). Parasites were incubated for an additional 48 h at 37°C and 5% CO₂. Cell viability was then measured by adding 10 μ L of Alamar Blue reagent to give 10% v/v in the assay. Plates were incubated for 3 h at 37°C and 5% CO₂, then for another 22 h in dark at room temperature. Sample fluorescence (535 nm excitation, 590 nm emission) was read using a Molecular Devices FlexStation II 384-well plate reader, and cell viability was calculated as per vendor instructions. EC₅₀ values for the test compounds were obtained by plotting the % Alamar Blue reduction results in GraphPad Prism 6 and analyzing by non-linear regression using the log(inhibitor) vs. response (variable slope) equation. DMSO was used as negative control, and pentamidine, suramin, and nifurtimox (drugs used to treat HAT) were used as positive controls. Results presented represent the averages of EC₅₀ values obtained from at least triplicate experiments

HEK 293 and THLE-3 cytotoxicity assay protocol.

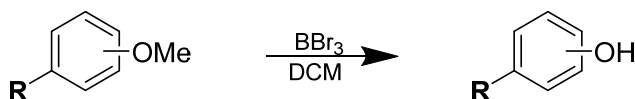
Cell cytotoxicity assays were performed using the Alamar Blue reporter reagents as previously described.^{3, 5, 6} Compounds were tested in 8-point, 3-fold dilution series (100 μ M to 46 nM) in 384-well plates (BRAND cell culture grade plates, 781980). DMSO was used as negative control, and previously discovered cytotoxic chaperonin inhibitors were used as positive controls.^{3, 4} Sample fluorescence (535 nm excitation, 590 nm emission) was read using a Molecular Devices FlexStation II 384-well plate reader, and cell viability was calculated as per vendor instructions. EC₅₀ values for the test compounds were obtained by plotting the % Alamar Blue reduction results in GraphPad Prism 6 and analyzing by non-linear regression using the log(inhibitor) vs. response (variable slope) equation. Results presented represent the averages of EC₅₀ values obtained from at least triplicate experiments.

General Synthetic Methods.



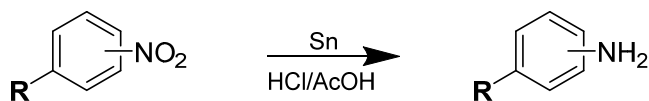
Synthetic Protocol A: General procedure for the synthesis of bis-sulfonamide analogues.

To a stirring mixture of 5-amino-2-(4-aminophenyl) benzoxazole (1 eq.) in anhydrous CH₂Cl₂ (5 mL) was added the respective sulfonyl chloride (2.1 eq.) followed by anhydrous pyridine (2.1 eq.). The reaction was allowed to stir at room temperature for 18 h and was then chromatographed over silica and concentrated. If necessary, the product was further purified by preparatory RP-HPLC (H₂O:CH₃CN gradient), concentrated, and lyophilized. Refer below for individual compound synthesis and characterization data.



Synthetic Protocol B: General procedure for methoxy-to-hydroxy deprotections.

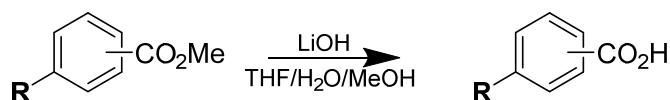
To a stirring mixture of the respective bis-sulfonamide (1 eq.) *or* mono-sulfonamide (1 eq.) in anhydrous CH₂Cl₂ (5 mL), was added BBr₃ (6 eq. *or* 3 eq., respectively, in CH₂Cl₂). The reaction was allowed to stir at room temperature for 18 h and then diluted drop-wise with MeOH (2 mL). The reaction was then washed with brine and extracted into EtOAc. The organics were dried over Na₂SO₄, filtered, and concentrated. The crude product was chromatographed over silica and concentrated. If necessary, the product was further purified by preparatory RP-HPLC (H₂O:CH₃CN gradient), concentrated, and lyophilized. Refer below for individual compound synthesis and characterization data.



Synthetic Protocol C: General procedure for nitro-to-amine reductions.

To the respective bis-sulfonamide (1 eq.) *or* mono-sulfonamide (1 eq.) was added tin powder (6 eq. *or* 3 eq., respectively), followed by a 1:10 mixture of HCl:AcOH (generally 0.2:2.0 mL). The reaction was allowed to stir at room temperature for 18 h, then diluted with EtOAc and H₂O, neutralized with NaHCO₃, and filtered. The filtrate was extracted with EtOAc and the organics dried over Na₂SO₄, filtered, and concentrated. The crude product was then chromatographed over silica and concentrated. If necessary, the product was further purified

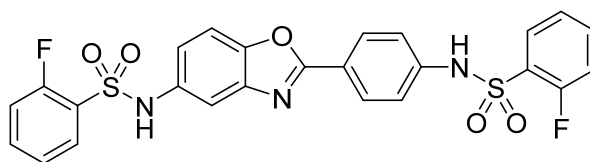
by preparatory RP-HPLC (H₂O:CH₃CN gradient), concentrated, and lyophilized. Refer below for individual compound synthesis and characterization data.



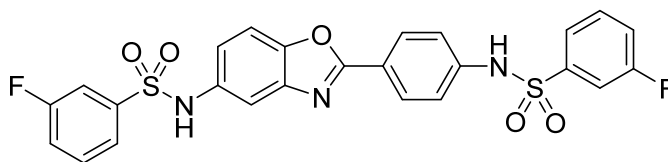
Synthetic Protocol D: General procedure for ester-to-acid hydrolyses.

To a stirring mixture of the respective methyl ester compound (1 eq.) in THF (1.5 mL), MeOH (0.5 mL), and H₂O (0.5 mL), was added LiOH•H₂O (~6-10 eq.). The reaction was allowed to stir at room temperature for 18 h and then was diluted with H₂O (10 mL) and acidified with 1M HCl. The precipitate was filtered, washed with H₂O, and dried. If necessary, the product was further purified by preparatory RP-HPLC (H₂O:CH₃CN gradient), concentrated, and lyophilized. Refer below for individual compound synthesis and characterization data.

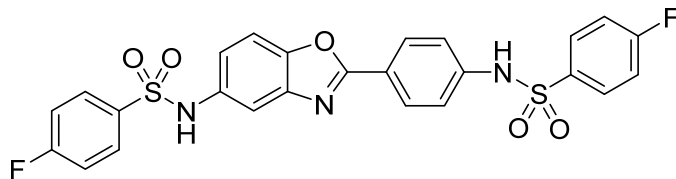
Synthesis of specific test molecules.



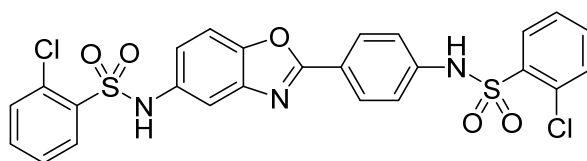
2b-o: 2-fluoro-N-(4-(5-((2-fluorophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (47.0 mg, 0.209 mmol), 2-fluorobenzenesulfonyl chloride (69.0 μ L, 0.52 mmol), and anhydrous pyridine (42.5 μ L, 0.52 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2b-o** as a white solid (50.5 mg, 45% yield). ¹H-NMR (500 MHz, *d*₆-DMSO) δ 11.21 (br s, 1H), 10.66 (br s, 1H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.92 (t, *J* = 6.9 Hz, 1H), 7.76-7.84 (m, 1H), 7.63-7.70 (m, 2H), 7.60 (d, *J* = 8.8 Hz, 1H), 7.35-7.44 (m, 4H), 7.32 (td, *J* = 7.6, 1.1 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 2H), 7.10 (dd, *J* = 8.7, 2.0 Hz, 1H); MS (ESI) 539.8 *m/z* [M-H⁺]⁻, C₂₅H₁₆F₂N₃O₅S₂ requires 540.0; HPLC-1 = >99%; HPLC-2 = 97%.



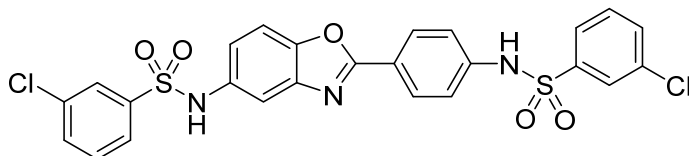
2b-m: 3-fluoro-N-(4-(5-((2-fluorophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (59.8 mg, 0.265 mmol), 3-fluorobenzenesulfonyl chloride (89.0 μ L, 0.663 mmol), and anhydrous pyridine (54.0 μ L, 0.662 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2b-m** as a white solid (131 mg, 91% yield). ¹H-NMR (300 MHz, *d*₆-DMSO) δ 10.99 (br s, 1H), 10.47 (br s, 1H), 8.03 (d, *J* = 8.8 Hz, 2H), 7.40-7.77 (m, 10H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.08 (dd, *J* = 8.7, 2.1 Hz, 1H); MS (ESI) 542.0 *m/z* [MH⁺], C₂₅H₁₈F₂N₃O₅S₂ requires 542.1; HPLC-1 = 100%; HPLC-2 = 98%.



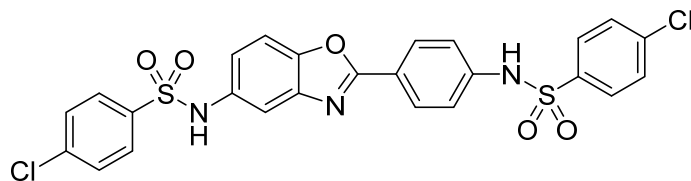
2b-p: 4-fluoro-N-(4-(5-((4-fluorophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (55.3 mg, 0.246 mmol), 4-fluorobenzenesulfonyl chloride (124 mg, 0.637 mmol), and anhydrous pyridine (50.0 μ L, 0.613 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2b-p** as a white solid (108 mg, 81% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.93 (br s, 1H), 10.35 (br s, 1H), 8.00 (d, J = 8.8 Hz, 2H), 7.83-7.92 (m, 2H), 7.75-7.80 (m, 2H), 7.61 (d, J = 8.8 Hz, 1H), 7.33-7.43 (m, 5H), 7.27 (d, J = 8.8 Hz, 2H), 7.06 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 542.0 m/z [MH^+], $\text{C}_{25}\text{H}_{18}\text{F}_2\text{N}_3\text{O}_5\text{S}_2$ requires 542.1; HPLC-1 = 99%; HPLC-2 = 98%.



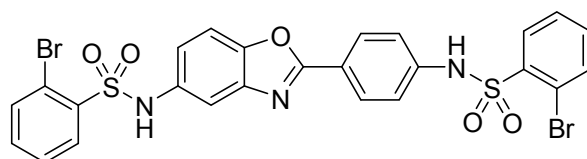
2c-o: 2-chloro-N-(4-(5-((2-chlorophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (59.7 mg, 0.265 mmol), 2-chlorobenzenesulfonyl chloride (90.0 μ L, 0.660 mmol), and anhydrous pyridine (54.0 μ L, 0.662 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2c-o** as a white solid (74.3 mg, 49% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.21 (s, 1H), 10.67 (s, 1H), 8.11-8.15 (m, 1H), 8.02 (dd, J = 7.7, 1.4 Hz, 1H), 7.97 (d, J = 8.8 Hz, 2H), 7.53-7.65 (m, 6H), 7.46-7.50 (m, 1H), 7.39 (d, J = 2.2 Hz, 1H), 7.26 (d, J = 8.5 Hz, 2H), 7.11 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 571.8 m/z [M-H^+], $\text{C}_{25}\text{H}_{16}\text{Cl}_2\text{N}_3\text{O}_5\text{S}_2$ requires 572.0; HPLC-1 = 99%; HPLC-2 = 98%.



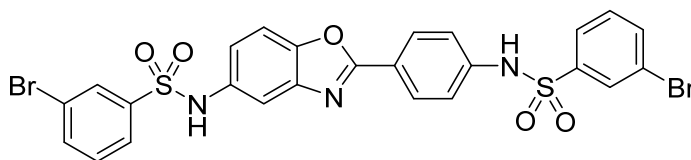
2c-m: 3-chloro-N-(4-(5-((3-chlorophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (55.6 mg, 0.247 mmol), 3-chlorobenzenesulfonyl chloride (87.0 μ L, 0.618 mmol), and anhydrous pyridine (50.0 μ L, 0.613 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2c-m** as a white solid (94.0 mg, 66% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.98 (br s, 1H), 10.45 (br s, 1H), 8.03 (d, J = 8.8 Hz, 2H), 7.83 (t, J = 1.9 Hz, 1H), 7.53-7.80 (m, 8H), 7.41 (d, J = 2.2 Hz, 1H), 7.31 (d, J = 8.8 Hz, 2H), 7.07 (dd, J = 8.5, 2.2 Hz, 1H); MS (ESI) 571.7 m/z [M-H^+], $\text{C}_{25}\text{H}_{16}\text{Cl}_2\text{N}_3\text{O}_5\text{S}_2$ requires 572.0; HPLC-1 = 99%; HPLC-2 = 99%.



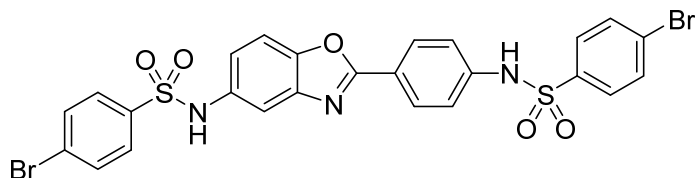
2c-p: 4-chloro-N-(4-(5-((4-chlorophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (62.7 mg, 0.278 mmol), 4-chlorobenzenesulfonyl chloride (146 mg, 0.693 mmol), and anhydrous pyridine (57.0 μ L, 0.699 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2c-p** as a white solid (133 mg, 83% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.95 (br s, 1H), 10.42 (br s, 1H), 8.00-8.04 (m, 2H), 7.81-7.85 (m, 2H), 7.70-7.74 (m, 2H), 7.59-7.67 (m, 5H), 7.40 (d, J = 1.9 Hz, 1H), 7.27-7.32 (m, 2H), 7.07 (dd, J = 8.7, 2.0 Hz, 1H); MS (ESI) 571.8 m/z $[\text{M-H}^+]$, $\text{C}_{25}\text{H}_{16}\text{Cl}_2\text{N}_3\text{O}_5\text{S}_2$ requires 572.0; HPLC-1 = 98%; HPLC-2 = 97%.



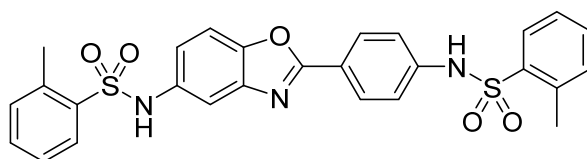
2d-o: 2-bromo-N-(4-(5-((2-bromophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (49.0 mg, 0.218 mmol), 2-bromobenzenesulfonyl chloride (78.5 μ L, 0.544 mmol), and anhydrous pyridine (44.0 μ L, 0.543 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2d-o** as a pink-white solid (84.1 mg, 46% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.22 (s, 1H), 10.60 (s, 1H), 8.16 (dd, J = 7.9, 1.6 Hz, 1H), 8.07 (dd, J = 7.6, 1.9 Hz, 1H), 7.98 (d, J = 8.5 Hz, 2H), 7.82 (td, J = 8.0, 1.1 Hz, 2H), 7.57-7.63 (m, 2H), 7.54 (td, J = 7.7, 1.3 Hz, 2H), 7.47-7.51 (m, 1H), 7.28 (d, J = 2.2 Hz, 1H), 7.26 (d, J = 8.5 Hz, 2H), 7.12 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 659.7 m/z $[\text{M-H}^+]$, $\text{C}_{25}\text{H}_{16}\text{Br}_2\text{N}_3\text{O}_5\text{S}_2$ requires 659.9; HPLC-1 = >99%; HPLC-2 = >99%.



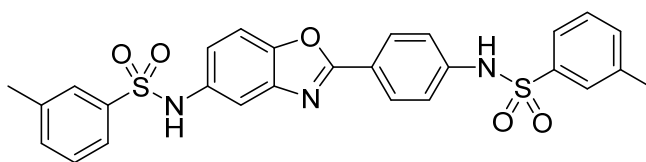
2d-m: 3-bromo-N-(4-(5-((3-bromophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (49.0 mg, 0.218 mmol), 3-bromobenzenesulfonyl chloride (78.5 μ L, 0.544 mmol), and anhydrous pyridine (44.5 μ L, 0.543 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2d-m** as a white solid (41.7 mg, 29% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.97 (br s, 1H), 10.43 (br s, 1H), 8.03 (d, J = 8.8 Hz, 2H), 7.98 (s, 1H), 7.88 (t, J = 1.7 Hz, 1H), 7.80-7.86 (m, 3H), 7.69 (dq, J = 7.9, 0.8 Hz, 1H), 7.64 (d, J = 8.5 Hz, 1H), 7.53 (t, J = 8.0 Hz, 1H), 7.48 (t, J = 8.0 Hz, 1H), 7.40 (d, J = 1.9 Hz, 1H), 7.30 (d, J = 7.9 Hz, 2H), 7.07 (dd, J = 8.7, 2.0 Hz, 1H); MS (ESI) 659.7 m/z $[\text{M-H}^+]$, $\text{C}_{25}\text{H}_{16}\text{Br}_2\text{N}_3\text{O}_5\text{S}_2$ requires 659.9; HPLC-1 = 98%; HPLC-2 = 98%.



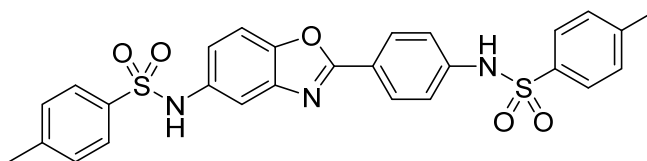
2d-p: 4-bromo-N-(4-(5-((4-bromophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (53.6 mg, 0.238 mmol), 4-bromobenzenesulfonyl chloride (155 mg, 0.606 mmol), and anhydrous pyridine (48.5 μ L, 0.595 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2d-p** as a white solid (38.6 mg, 24% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.95 (br s, 1H), 10.42 (br s, 1H), 8.02 (d, J = 8.8 Hz, 2H), 7.77-7.82 (m, 2H), 7.73-7.77 (m, 4H), 7.61-7.65 (m, 3H), 7.40 (d, J = 1.9 Hz, 1H), 7.29 (d, J = 8.8 Hz, 2H), 7.06 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 661.8 m/z [MH^+], $\text{C}_{25}\text{H}_{18}\text{Br}_2\text{N}_3\text{O}_5\text{S}_2$ requires 661.9; HPLC-1 = 98%; HPLC-2 = 98%.



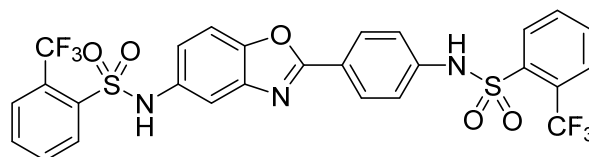
2e-o: 2-methyl-N-(4-(5-((2-methylphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (51.9 mg, 0.230 mmol), *o*-toluenesulfonyl chloride (83.0 μ L, 0.575 mmol), and anhydrous pyridine (47.0 μ L, 0.576 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2e-o** as a white solid (64.5 mg, 53% yield). $^1\text{H-NMR}$ (300 MHz, d_6 -DMSO) δ 11.03 (s, 1H), 10.47 (s, 1H), 7.94-8.00 (m, 3H), 7.86 (d, J = 7.6 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 7.30-7.55 (m, 7H), 7.24 (d, J = 8.8 Hz, 2H), 7.06 (dd, J = 8.8, 2.1 Hz, 1H), 2.60 (s, 3H), 2.59 (s, 3H); MS (ESI) 534.0 m/z [MH^+], $\text{C}_{27}\text{H}_{24}\text{N}_3\text{O}_5\text{S}_2$ requires 534.1; HPLC-1 = 99%; HPLC-2 = 99%.



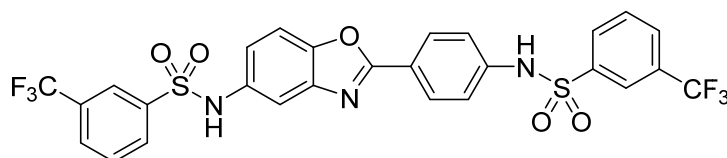
2e-m: 3-methyl-N-(4-(5-((2-methylphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (52.8 mg, 0.234 mmol), *m*-toluenesulfonyl chloride (85.0 μ L, 0.586 mmol), and anhydrous pyridine (48.0 μ L, 0.589 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2e-m** as a white solid (101 mg, 81% yield). $^1\text{H-NMR}$ (300 MHz, d_6 -DMSO) δ 10.86 (br s, 1H), 10.32 (br s, 1H), 8.00 (d, J = 8.8 Hz, 2H), 7.38-7.69 (m, 10H), 7.30 (d, J = 8.8 Hz, 2H), 7.07 (dd, J = 8.7, 2.1 Hz, 1H), 2.34 (s, 3H), 2.31 (s, 3H); MS (ESI) 534.0 m/z [MH^+], $\text{C}_{27}\text{H}_{24}\text{N}_3\text{O}_5\text{S}_2$ requires 534.1; HPLC-1 = 98%; HPLC-2 = 98%.



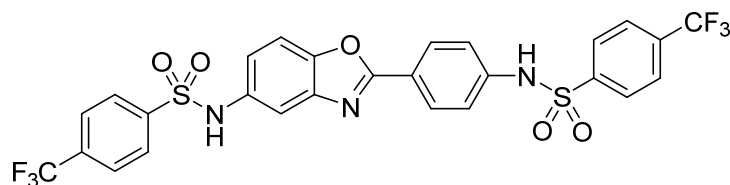
2e-p: 4-methyl-N-(4-(5-((4-methylphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (45.9 mg, 0.204 mmol), *p*-toluenesulfonyl chloride (85.1 mg, 0.466 mmol), and anhydrous pyridine (35.0 μ L, 0.429 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2e-p** as a yellow solid (29.5 mg, 27% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.82 (br s, 1H), 10.26 (br s, 1H), 7.76-8.02 (m, 2H), 7.70-7.75 (m, 2H), 7.57-7.63 (m, 3H), 7.34-7.39 (m, 3H), 7.26-7.34 (m, 4H), 7.07 (dd, J = 8.5, 2.2 Hz, 1H), 2.32 (s, 3H), 2.30 (s, 3H); MS (ESI) 531.9 m/z $[\text{M-H}]^+$, $\text{C}_{27}\text{H}_{22}\text{N}_3\text{O}_5\text{S}_2$ requires 532.1; HPLC-1 = >99%; HPLC-2 = 98%.



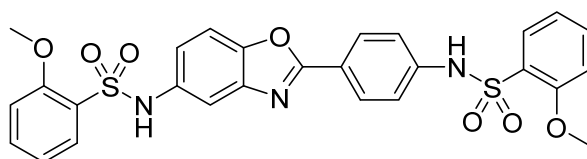
2f-o: 2-(trifluoromethyl)-N-(4-(5-((2-(trifluoromethyl)phenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (45.4 mg, 0.202 mmol), 2-trifluoromethyl-benzenesulfonyl chloride (117 μ L, 0.785 mmol), and anhydrous pyridine (54.0 μ L, 0.662 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2f-o** as a white solid (124 mg, 96% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.24 (br s, 1H), 10.73 (br s, 1H), 8.13-8.17 (m, 1H), 8.10 (d, J = 7.6 Hz, 1H), 8.00-8.05 (m, 3H), 7.98 (dd, J = 7.4, 1.4 Hz, 1H), 7.78-7.90 (m, 4H), 7.64 (d, J = 8.8 Hz, 1H), 7.41 (d, J = 1.9 Hz, 1H), 7.29 (d, J = 8.8 Hz, 2H), 7.12 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 641.9 m/z $[\text{MH}]^+$, $\text{C}_{27}\text{H}_{18}\text{F}_6\text{N}_3\text{O}_5\text{S}_2$ requires 642.1; HPLC-1 = >99%; HPLC-2 = 99%.



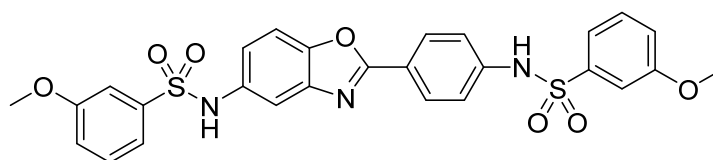
2f-m: 3-(trifluoromethyl)-N-(4-(5-((3-(trifluoromethyl)phenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (48.5 mg, 0.215 mmol), 3-trifluoromethylbenzenesulfonyl chloride (86.0 μ L, 0.536 mmol), and anhydrous pyridine (39.0 μ L, 0.478 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2f-m** as a white solid (74.0 mg, 54% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.01 (br s, 1H), 10.48 (br s, 1H), 8.05-8.13 (m, 2H), 7.95-8.05 (m, 6H), 7.83 (t, J = 7.9 Hz, 1H), 7.77 (t, J = 7.9 Hz, 1H), 7.64 (d, J = 8.8 Hz, 1H), 7.40 (d, J = 1.9 Hz, 1H), 7.31 (d, J = 8.8 Hz, 2H), 7.06 (dd, J = 8.7, 2.0 Hz, 1H); MS (ESI) 639.9 m/z $[\text{M-H}]^+$, $\text{C}_{27}\text{H}_{16}\text{F}_6\text{N}_3\text{O}_5\text{S}_2$ requires 640.0; HPLC-1 = >99%; HPLC-2 = 98%.



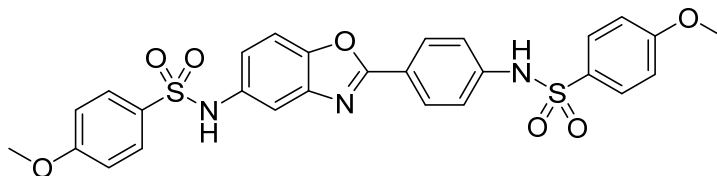
2f-p: 4-(trifluoromethyl)-N-(4-(5-((4-(trifluoromethyl)phenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (52.7 mg, 0.234 mmol), 4-trifluoromethylbenzenesulfonyl chloride (144 mg, 0.589 mmol), and anhydrous pyridine (47.5 μ L, 0.582 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2f-p** as a yellow solid (56.6 mg, 38% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.09 (br s, 1H), 10.60 (br s, 1H), 8.03 (dd, J = 8.5, 6.0 Hz, 4H), 7.97 (d, J = 8.5 Hz, 2H), 7.93 (s, 4H), 7.63 (d, J = 8.8 Hz, 1H), 7.43 (d, J = 2.2 Hz, 1H), 7.31 (d, J = 8.8 Hz, 2H), 7.07 (dd, J = 8.7, 2.0 Hz, 1H); MS (ESI) 641.9 m/z [MH^+], $\text{C}_{27}\text{H}_{18}\text{F}_6\text{N}_3\text{O}_5\text{S}_2$ requires 642.1; HPLC-1 = 98%; HPLC-2 = 98%.



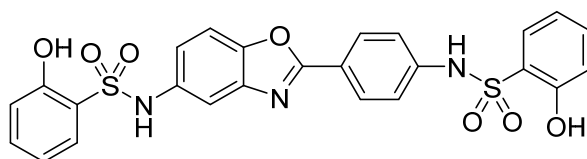
2g-o: 2-methoxy-N-(4-(5-((2-methoxyphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (50.5 mg, 0.224 mmol), 2-methoxybenzenesulfonyl chloride (113 mg, 0.548 mmol), and anhydrous pyridine (45.5 μ L, 0.558 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2g-o** as a white solid (66.5 mg, 52% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.61 (br s, 1H), 10.04 (s, 1H), 7.92-7.96 (m, 2H), 7.85 (dd, J = 7.7, 1.7 Hz, 1H), 7.73 (dd, J = 7.7, 1.7 Hz, 1H), 7.50-7.58 (m, 3H), 7.36 (d, J = 1.9 Hz, 1H), 7.23-7.27 (m, 2H), 7.15 (dd, J = 7.9, 4.4 Hz, 2H), 7.10 (dd, J = 8.8, 2.2 Hz, 1H), 7.04-7.07 (m, 1H), 6.96-7.00 (m, 1H), 3.89 (s, 3H), 3.84 (s, 3H); MS (ESI) 566.1 m/z [MH^+], $\text{C}_{27}\text{H}_{24}\text{N}_3\text{O}_7\text{S}_2$ requires 566.1; HPLC-1 = 97%; HPLC-2 = 96%.



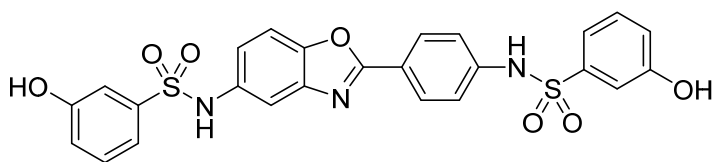
2g-m: 3-methoxy-N-(4-(5-((3-methoxyphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (56.3 mg, 0.250 mmol), 3-methoxybenzenesulfonyl chloride (89.0 μ L, 0.629 mmol), and anhydrous pyridine (51.0 μ L, 0.625 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2g-m** as a white solid (126 mg, 89% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.84 (br s, 1H), 10.32 (s, 1H), 7.99-8.04 (m, 2H), 7.61 (d, J = 8.8 Hz, 1H), 7.45-7.50 (m, 1H), 7.37-7.44 (m, 3H), 7.26-7.33 (m, 4H), 7.23-7.25 (m, 1H), 7.19 (ddd, J = 8.3, 2.6, 0.8 Hz, 1H), 7.13-7.16 (m, 1H), 7.09 (dd, J = 8.8, 2.2 Hz, 1H), 3.77 (s, 3H), 3.74 (s, 3H); MS (ESI) 566.0 m/z [MH^+], $\text{C}_{27}\text{H}_{24}\text{N}_3\text{O}_7\text{S}_2$ requires 566.1; HPLC-1 = >99%; HPLC-2 = >99%.



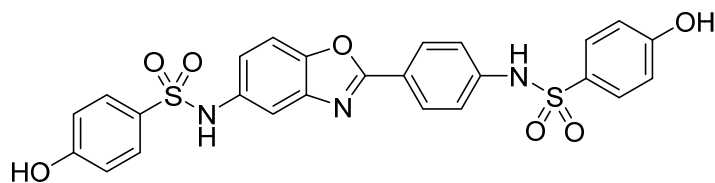
2g-p: 4-methoxy-N-(4-(5-((4-methoxyphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (57.5 mg, 0.255 mmol), 4-methoxybenzenesulfonyl chloride (130 mg, 0.630 mmol), and anhydrous pyridine (48.0 μ L, 0.589 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2g-p** as a white solid (112 mg, 78% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.73 (br s, 1H), 10.20 (s, 1H), 7.97-8.02 (m, 2H), 7.75-7.80 (m, 2H), 7.64-7.68 (m, 2H), 7.60 (d, J = 8.8 Hz, 1H), 7.38 (d, J = 2.2 Hz, 1H), 7.26-7.31 (m, 2H), 7.05-7.10 (m, 3H), 7.00-7.05 (m, 2H), 3.78 (s, 3H), 3.76 (s, 3H); MS (ESI) 566.1 m/z [MH^+], $\text{C}_{27}\text{H}_{24}\text{N}_3\text{O}_7\text{S}_2$ requires 566.1; HPLC-1 = 98%; HPLC-2 = 97%.



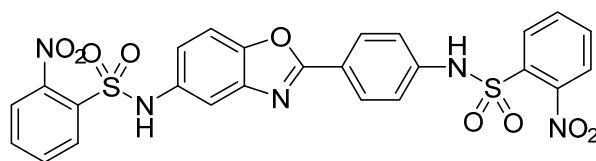
2h-o: 2-hydroxy-N-(4-(5-((2-hydroxyphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from **2g-o** (189 mg, 0.335 mmol) and boron tribromide (2.00 mL, 2.00 mmol) according to synthetic protocol B. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2h-o** as a tan solid (96.0 mg, 53% yield). $^1\text{H-NMR}$ (300 MHz, d_6 -DMSO) δ 10.91 (br s, 2H), 10.70 (br s, 1H), 9.99 (br s, 1H), 7.93 (d, J = 8.8 Hz, 2H), 7.78 (dd, J = 8.1, 1.6 Hz, 1H), 7.65 (dd, J = 7.9, 1.5 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 7.31-7.44 (m, 3H), 7.26 (d, J = 8.8 Hz, 2H), 7.13 (dd, J = 8.8, 2.0 Hz, 1H), 6.87-6.96 (m, 3H), 6.79-6.86 (m, 1H); MS (ESI) 538.0 m/z [MH^+], $\text{C}_{25}\text{H}_{20}\text{N}_3\text{O}_7\text{S}_2$ requires 538.1; HPLC-1 = 95%; HPLC-2 = 96%.



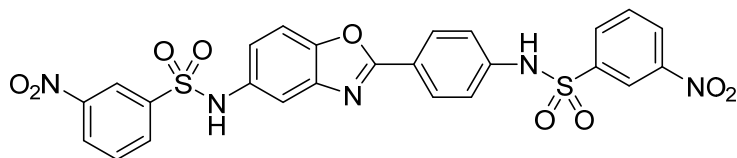
2h-m: 3-hydroxy-N-(4-(5-((3-hydroxyphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from **2g-m** (78.1 mg, 0.138 mmol) and boron tribromide (0.83 mL, 0.83 mmol) according to synthetic protocol B. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2h-m** as a yellow solid (31.8 mg, 43% yield). $^1\text{H-NMR}$ (300 MHz, d_6 -DMSO) δ 10.84 (s, 1H), 10.30 (s, 1H), 10.19 (br s, 1H), 10.09 (br s, 1H), 8.02 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.8 Hz, 1H), 7.23-7.42 (m, 6H), 7.04-7.21 (m, 4H), 6.90-7.01 (m, 2H); MS (ESI) 538.0 m/z [MH^+], $\text{C}_{25}\text{H}_{20}\text{N}_3\text{O}_7\text{S}_2$ requires 538.1; HPLC-1 = 97%; HPLC-2 = 97%.



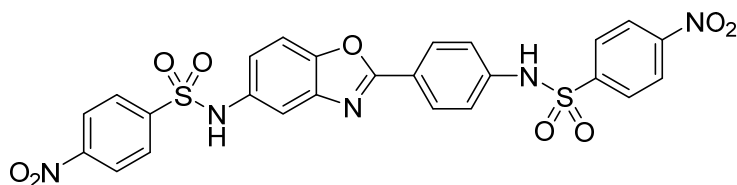
2h-p: 4-hydroxy-N-(4-(5-((4-hydroxyphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from **2g-p** (74.5 mg, 0.132 mmol) and boron tribromide (0.80 mL, 0.80 mmol) according to synthetic protocol B. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2h-p** as a white solid (45.2 mg, 64% yield). ¹H-NMR (500 MHz, *d*₆-DMSO) δ 10.41 (br s, 2H), 10.07 (br s, 1H), 7.96 (d, *J* = 8.2 Hz, 2H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.53-7.60 (m, 3H), 7.35 (d, *J* = 1.9 Hz, 1H), 7.22 (d, *J* = 8.2 Hz, 2H), 7.04 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.79-6.86 (m, 4H); MS (ESI) 535.8 *m/z* [M-H⁺]⁻, C₂₅H₁₈N₃O₇S₂ requires 536.1; HPLC-1 = 95%; HPLC-2 = 95%.



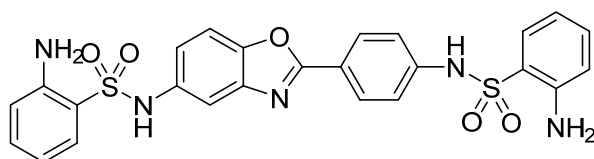
2i-o: 2-nitro-N-(4-(5-((2-nitrophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (53.6 mg, 0.238 mmol), 2-nitrobenzenesulfonyl chloride (185 μL, 0.839 mmol), and anhydrous pyridine (61.0 μL, 0.748 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient), followed by preparatory RP-HPLC purification, afforded **2i-o** as a yellow solid (143 mg, 101% yield). ¹H-NMR (500 MHz, *d*₆-DMSO) δ 11.28 (br s, 1H), 10.78 (br s, 1H), 8.03-8.07 (m, 3H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.96 (td, *J* = 7.5, 1.4 Hz, 2H), 7.77-7.87 (m, 4H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.43 (d, *J* = 2.2 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 2H), 7.13 (dd, *J* = 8.8, 2.2 Hz, 1H); MS (ESI) 593.8 *m/z* [M-H⁺]⁻, C₂₅H₁₆N₅O₉S₂ requires 594.0; HPLC-1 = 98%; HPLC-2 = 99%.



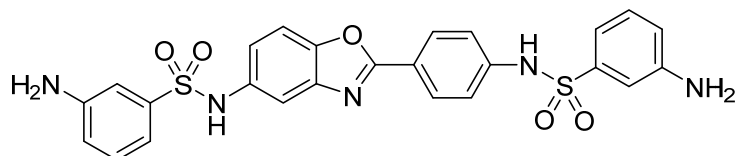
2i-m: 3-nitro-N-(4-(5-((3-nitrophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (53.9 mg, 0.239 mmol), 3-nitrobenzenesulfonyl chloride (132 μL, 0.593 mmol), and anhydrous pyridine (45.0 μL, 0.552 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient), followed by preparatory RP-HPLC purification, afforded **2i-m** as a yellow-orange solid (42.9 mg, 30% yield). ¹H-NMR (500 MHz, *d*₆-DMSO) δ 11.11 (br s, 1H), 10.63 (br s, 1H), 8.54 (t, *J* = 1.9 Hz, 1H), 8.49 (t, *J* = 1.9 Hz, 1H), 8.40-8.45 (m, 2H), 8.21 (dq, *J* = 7.9, 0.8 Hz, 1H), 8.07 (dq, *J* = 7.9, 0.8 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.77-7.87 (m, 2H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.2 Hz, 1H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.08 (dd, *J* = 8.8, 2.2 Hz, 1H); MS (ESI) 596.1 *m/z* [MH⁺], C₂₅H₁₈N₅O₉S₂ requires 596.1; HPLC-1 = 95%; HPLC-2 = 95%.



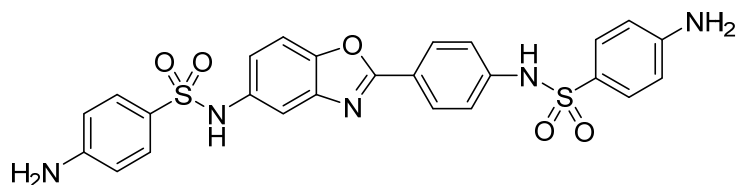
2i-p: 4-nitro-N-(4-(5-((4-nitrophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (112 mg, 0.497 mmol), 4-nitrobenzenesulfonyl chloride (566 mg, 0.486 mmol), and anhydrous pyridine (100 μ L, 0.486 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2i-p** as a yellow solid (91.0 mg, 23% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.16 (br s, 1H), 10.67 (br s, 1H), 8.36-8.40 (m, 2H), 8.32-8.36 (m, 2H), 8.06-8.10 (m, 2H), 8.01-8.05 (m, 2H), 7.94-7.98 (m, 2H), 7.64 (d, J = 8.8 Hz, 1H), 7.43 (d, J = 1.9 Hz, 1H), 7.30-7.34 (m, 2H), 7.08 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 593.9 m/z [M-H^+], $\text{C}_{25}\text{H}_{16}\text{N}_5\text{O}_9\text{S}_2$ requires 594.0; HPLC-1 = 99%; HPLC-2 = >99%.



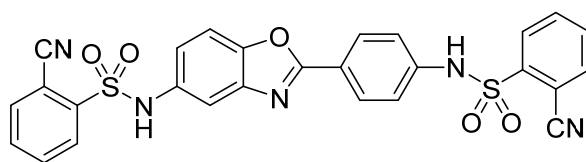
2j-o: 2-amino-N-(4-(5-((2-aminophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from **2i-o** (51.9 mg, 0.0871 mmol) and tin powder (62.1 mg, 0.523 mmol) according to synthetic protocol C. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2j-o** as a white solid (35.7 mg, 77% yield). $^1\text{H-NMR}$ (300 MHz, d_6 -DMSO) δ 10.80 (br s, 1H), 10.29 (br s, 1H), 7.98 (d, J = 8.8 Hz, 2H), 7.55-7.64 (m, 2H), 7.43-7.48 (m, 1H), 7.32-7.36 (m, 1H), 7.15-7.25 (m, 4H), 7.04 (dd, J = 8.7, 2.1 Hz, 1H), 6.70-6.78 (m, 2H), 6.48-6.60 (m, 2H), 6.00 (br s, 4H); MS (ESI) 536.0 m/z [MH^+], $\text{C}_{25}\text{H}_{22}\text{N}_5\text{O}_5\text{S}_2$ requires 536.1; HPLC-1 = >99%; HPLC-2 = 98%.



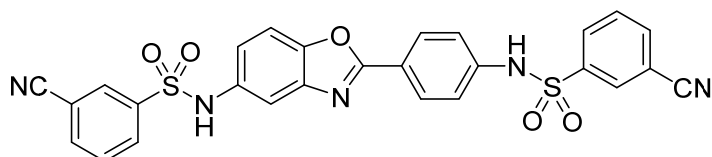
2j-m: 3-amino-N-(4-(5-((3-aminophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized using a different reduction procedure, with addition of NaBH_4 (62.3 mg, 1.65 mmol), to a stirring mixture of **2i-m** (135 mg, 0.227 mmol) and NiSO_4 hexahydrate (11.9 mg, 0.045 mmol). After 15 minutes, the reaction was diluted with 1 M HCl and the precipitate was filtered, rinsed with water, and collected. Preparatory RP-HPLC purification afforded **2j-m** as a white solid (29.0 mg, 24% yield). $^1\text{H-NMR}$ (300 MHz, d_6 -DMSO) δ 10.76 (s, 1H), 10.23 (s, 1H), 7.97-8.04 (m, 2H), 7.57-7.64 (m, 1H), 7.39 (d, J = 2.0 Hz, 1H), 7.25-7.32 (m, 2H), 7.12-7.22 (m, 2H), 7.04-7.10 (m, 2H), 6.88-7.00 (m, 3H), 6.72-7.79 (m, 2H); MS (ESI) 536.1 m/z [MH^+], $\text{C}_{25}\text{H}_{22}\text{N}_5\text{O}_5\text{S}_2$ requires 536.0; HPLC-1 = >99%; HPLC-2 = 98%.



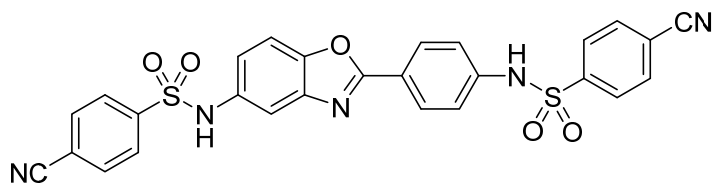
2j-p: 4-amino-N-(4-(5-((4-aminophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from **2i-p** (72.4 mg, 0.122 mmol) and tin powder (126 mg, 1.06 mmol) according to synthetic protocol C. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2j-p** as a pink solid (32.3 mg, 49% yield). ¹H-NMR (500 MHz, *d*₆-DMSO) δ 10.50 (br s, 1H), 9.88 (s, 1H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.8 Hz, 1H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.34-7.37 (m, 3H), 7.23 (d, *J* = 8.5 Hz, 2H), 7.04 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.54 (d, *J* = 8.8 Hz, 2H), 6.50 (d, *J* = 8.8 Hz, 2H), 6.00 (br s, 2H), 5.95 (br s, 2H); MS (ESI) 536.0 *m/z* [MH⁺], C₂₅H₂₂N₅O₅S₂ requires 536.1; HPLC-1 = 98%; HPLC-2 = 97%.



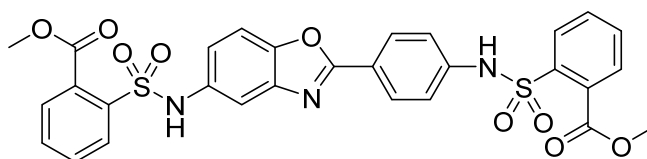
2k-o: 2-cyano-N-(4-(5-((2-cyanophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (55.7 mg, 0.247 mmol), 2-cyanobenzenesulfonyl chloride (127 mg, 0.627 mmol), and anhydrous pyridine (50.5 μL, 0.619 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2k-o** as a white solid (59.9 mg, 44% yield). ¹H-NMR (500 MHz, *d*₆-DMSO) δ 11.40 (s, 1H), 10.81 (s, 1H), 8.07-8.14 (m, 2H), 8.01-8.06 (m, 4H), 7.77-7.94 (m, 4H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.40 (d, *J* = 2.2 Hz, 1H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.08 (dd, *J* = 8.7, 2.0 Hz, 1H); MS (ESI) 556.1 *m/z* [MH⁺], C₂₇H₁₈N₅O₅S₂ requires 556.1; HPLC-1 = 95%; HPLC-2 = 95%.



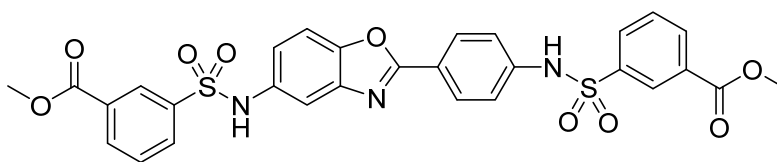
2k-m: 3-cyano-N-(4-(5-((3-cyanophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (49.4 mg, 0.219 mmol), 3-cyanobenzenesulfonyl chloride (109 mg, 0.540 mmol), and anhydrous pyridine (39.0 μL, 0.478 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2k-m** as an off-white solid (89.2 mg, 73% yield). ¹H-NMR (500 MHz, *d*₆-DMSO) δ 11.05 (br s, 1H), 10.52 (br s, 1H), 8.28 (s, 1H), 8.16 (s, 1H), 8.07-8.14 (m, 3H), 8.03 (d, *J* = 8.8 Hz, 2H), 7.98 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.79 (t, *J* = 8.0 Hz, 1H), 7.74 (t, *J* = 7.9 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.42 (d, *J* = 2.2 Hz, 1H), 7.32 (d, *J* = 8.8 Hz, 2H), 7.07 (dd, *J* = 8.8, 2.2 Hz, 1H); MS (ESI) 556.0 *m/z* [MH⁺], C₂₇H₁₈N₅O₅S₂ requires 556.1; HPLC-1 = >99%; HPLC-2 = >99%.



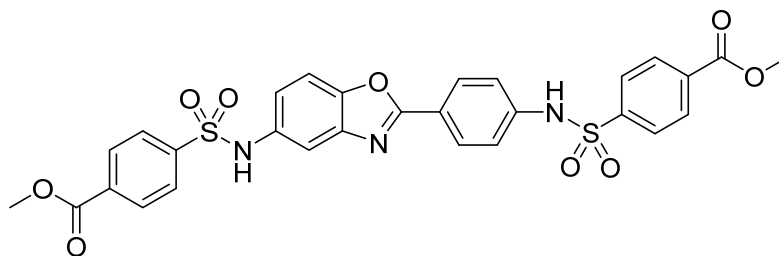
2k-p: 4-cyano-N-(4-(5-((4-cyanophenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (44.3 mg, 0.197 mmol), 4-cyanobenzenesulfonyl chloride (96.0 mg, 0.476 mmol), and anhydrous pyridine (32.0 μ L, 0.392 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2k-p** as a yellow-orange solid (80.3 mg, 73% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.11 (br s, 1H), 10.60 (br s, 1H), 8.05-8.09 (m, 2H), 8.00-8.05 (m, 4H), 7.97-8.00 (m, 2H), 7.87 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 1.9 Hz, 1H), 7.31 (d, J = 8.8 Hz, 2H), 7.07 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 553.9 m/z $[\text{M-H}^+]$, $\text{C}_{27}\text{H}_{16}\text{N}_5\text{O}_5\text{S}_2$ requires 554.1; HPLC-1 = >99%; HPLC-2 = 98%.



2l-o: methyl 2-(N-(4-(5-((2-methoxycarbonyl)phenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)sulfamoyl)benzoate was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (55.4 mg, 0.246 mmol), methyl-2-chlorosulfonylbenzoate (147 mg, 0.627 mmol), and anhydrous pyridine (50.0 μ L, 0.613 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2l-o** as a white solid (69.3 mg, 45% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.84 (br s, 1H), 10.29 (br s, 1H), 8.00 (d, J = 8.8 Hz, 2H), 7.91-7.94 (m, 1H), 7.84-7.87 (m, 1H), 7.58-7.72 (m, 7H), 7.38 (d, J = 2.2 Hz, 1H), 7.26 (d, J = 8.5 Hz, 2H), 7.08 (dd, J = 8.7, 2.0 Hz, 1H), 3.87 (s, 3H), 3.83 (s, 3H); MS (ESI) 622.0 m/z $[\text{MH}^+]$, $\text{C}_{29}\text{H}_{24}\text{N}_3\text{O}_9\text{S}_2$ requires 622.1; HPLC-1 = 99%; HPLC-2 = 99%.

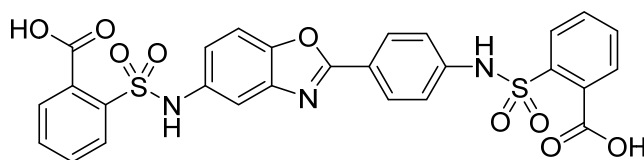


2l-m: methyl 3-(N-(4-(5-((3-methoxycarbonyl)phenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)sulfamoyl)benzoate was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (59.5 mg, 0.264 mmol), 3-chlorosulfonylbenzoic acid methyl ester (156 mg, 0.664 mmol), and anhydrous pyridine (54.0 μ L, 0.662 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2l-m** as a white solid (121 mg, 74% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.00 (br s, 1H), 10.48 (br s, 1H), 8.37 (d, J = 1.6 Hz, 1H), 8.30 (d, J = 1.3 Hz, 1H), 8.10-8.18 (m, 2H), 8.07 (dd, J = 7.9, 0.9 Hz, 1H), 8.00 (d, J = 8.5 Hz, 2H), 7.91-7.95 (m, 1H), 7.73 (t, J = 7.9 Hz, 1H), 7.67 (t, J = 7.9 Hz, 1H), 7.61 (d, J = 8.8 Hz, 1H), 7.38 (s, 1H), 7.29 (d, J = 8.5 Hz, 2H), 7.05 (dd, J = 8.7, 2.0 Hz, 1H), 3.87 (s, 3H), 3.85 (s, 3H); MS (ESI) 622.0 m/z $[\text{MH}^+]$, $\text{C}_{29}\text{H}_{24}\text{N}_3\text{O}_9\text{S}_2$ requires 622.1; HPLC-1 = 98%; HPLC-2 = 99%.

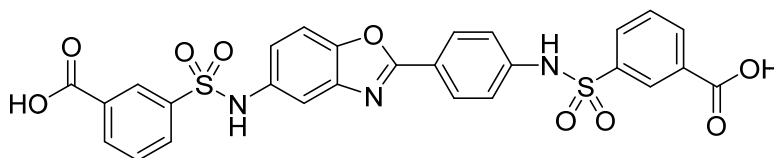


2l-p: methyl 4-(N-(4-(5-((4-(methoxycarbonyl)phenyl)sulfonamido)benzo[d]oxazol-2-

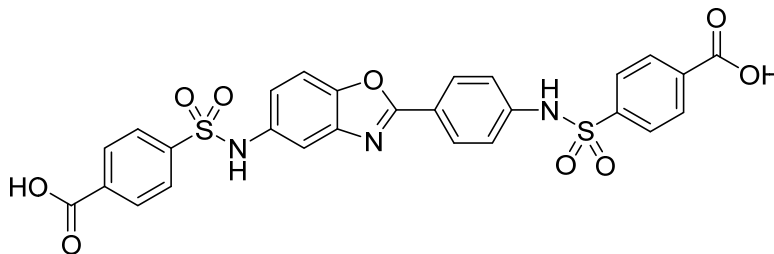
9yl)phenyl)sulfamoyl)benzoate was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (60.8 mg, 0.270 mmol), methyl-4-chlorosulfonylbenzoate (163 mg, 0.694 mmol), and anhydrous pyridine (55.0 μ L, 0.674 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2l-p** as a yellow solid (19.7 mg, 20% yield). $^1\text{H-NMR}$ (300 MHz, d_6 -DMSO) δ 11.05 (s, 1H), 10.51 (s, 1H), 7.93-8.13 (m, 8H), 7.82-7.87 (m, 2H), 7.62 (d, J = 8.8 Hz, 1H), 7.39 (d, J = 2.0 Hz, 2H), 7.30 (d, J = 8.8 Hz, 1H), 7.06 (dd, J = 8.8, 2.1 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H); MS (ESI) 622.2 m/z [MH^+], $\text{C}_{29}\text{H}_{24}\text{N}_3\text{O}_9\text{S}_2$ requires 622.1; HPLC-1 = 95%; HPLC-2 = 95%.



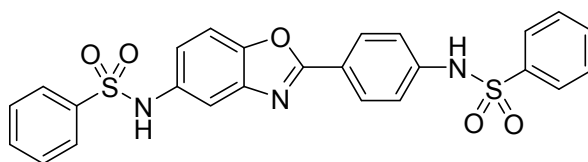
2m-o: 2-(N-(4-(5-((2-carboxyphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)sulfamoyl)benzoic acid was synthesized from **2l-o** (147 mg, 0.236 mmol) and lithium hydroxide monohydrate (100 mg, 2.39 mmol) according to synthetic protocol D. Filtration of the precipitate, followed by preparatory RP-HPLC purification, afforded **2m-o** as a white solid (48.7 mg, 35% yield). $^1\text{H-NMR}$ (300 MHz, d_6 -DMSO) δ 13.65 (br s, 1H), 10.66 (br s, 1H), 10.05 (s, 1H), 8.00 (m, J = 8.8 Hz, 2H), 7.89 (d, J = 7.5 Hz, 1H), 7.78 (d, J = 7.5 Hz, 1H), 7.53-7.70 (m, 7H), 7.41 (d, J = 2.0 Hz, 1H), 7.30 (m, J = 8.8 Hz, 2H), 7.10 (dd, J = 8.8, 2.1 Hz, 1H); MS (ESI) 591.8 m/z [M-H^+], $\text{C}_{27}\text{H}_{18}\text{N}_3\text{O}_9\text{S}_2$ requires 592.1; HPLC-1 = >99%; HPLC-2 = 95%.



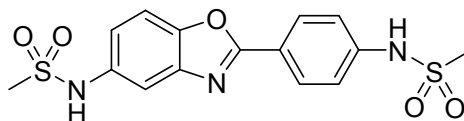
2m-m: 3-(N-(4-(5-((3-carboxyphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)sulfamoyl)benzoic acid was synthesized from **2l-m** (76.8 mg, 0.124 mmol) and lithium hydroxide monohydrate (29.6 mg, 0.705 mmol) according to synthetic protocol D. Filtration of the precipitate afforded **2m-m** as a white solid (64.8 mg, 88% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 13.52 (br s, 2H), 10.99 (br s, 1H), 10.45 (s, 1H), 8.36 (t, J = 1.6 Hz, 1H), 8.29 (t, J = 0.9 Hz, 1H), 8.15 (d, J = 7.9 Hz, 1H), 8.11 (d, J = 7.9 Hz, 1H), 8.00-8.07 (m, 3H), 7.91 (d, J = 8.5 Hz, 1H), 7.71 (t, J = 7.9 Hz, 1H), 7.60-7.67 (m, 2H), 7.39 (d, J = 2.2 Hz, 1H), 7.30 (d, J = 8.8 Hz, 2H), 7.06 (dd, J = 8.8, 1.9 Hz, 1H); MS (ESI) 594.0 m/z [MH^+], $\text{C}_{27}\text{H}_{20}\text{N}_3\text{O}_9\text{S}_2$ requires 594.1; HPLC-1 = 99%; HPLC-2 = 98%.



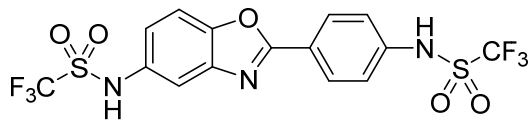
2m-p: 4-(N-(4-(5-((4-carboxyphenyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)sulfamoyl)benzoic acid was synthesized from **2l-p** (316 mg, 0.508 mmol) and lithium hydroxide monohydrate (245 mg, 5.84 mmol) according to synthetic protocol D. Filtration of the precipitate afforded **2m-p** as a pale-yellow solid (281 mg, 85% yield). ¹H-NMR (300 MHz, *d*₆-DMSO) δ 13.44 (br s, 2H), 11.04 (s, 1H), 10.50 (s, 1H), 8.00-8.12 (m, 6H), 7.94 (d, *J* = 8.6 Hz, 2H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.40 (d, *J* = 2.0 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 2H), 7.07 (dd, *J* = 8.8, 2.0 Hz, 1H); MS (ESI) 591.8 *m/z* [M-H]⁺, C₂₇H₂₀N₃O₉S₂ requires 592.1; HPLC-1 = 96%; HPLC-2 = 96%.



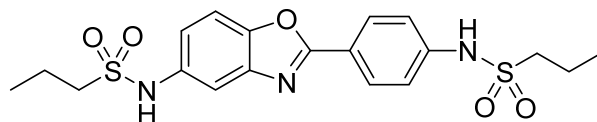
2a: N-(4-(5-(phenylsulfonamido)benzo[d]oxazol-2-yl)phenyl)benzenesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (43.1 mg, 0.191 mmol), benzenesulfonyl chloride (55.0 μL, 0.431 mmol), and anhydrous pyridine (30.0 μL, 0.368 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **6** as a white solid (79.5 mg, 82% yield). ¹H-NMR (500 MHz, *d*₆-DMSO) δ 10.90 (br s, 1H), 10.34 (br s, 1H), 8.00 (d, *J* = 8.8 Hz, 2H), 7.82-7.86 (m, 2H), 7.72-7.76 (m, 2H), 7.50-7.65 (m, 7H), 7.39 (d, *J* = 2.2 Hz, 1H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.07 (dd, *J* = 8.8, 2.2 Hz, 1H); MS (ESI) 506.0 *m/z* [MH]⁺, C₂₅H₂₀N₃O₅S₂ requires 506.1; HPLC-1 = 100%; HPLC-2 = 99%.



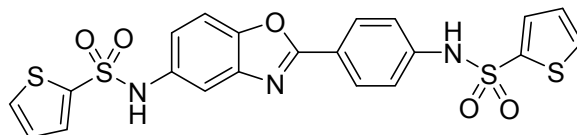
3: N-(4-(5-(methylsulfonamido)benzo[d]oxazol-2-yl)phenyl)methanesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (104.3 mg, 0.463 mmol), methanesulfonyl chloride (108 μL, 1.39 mmol), and anhydrous pyridine (113 μL, 1.39 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **2** as a pinkish-white solid (79.1 mg, 45% yield). ¹H-NMR (500 MHz, *d*₆-DMSO) δ 10.38 (s, 1H), 9.79 (s, 1H), 8.15 (d, *J* = 8.9 Hz, 1H), 7.85 (d, *J* = 8.7, 1H), 7.75 (d, *J* = 9.1 Hz, 1H), 7.60 (d, *J* = 2.2 Hz, 1H), 7.40 (d, *J* = 8.9 Hz, 1H), 7.26 (dd, *J* = 8.6, 2.1 Hz, 2H), 3.33 (s, 6H); MS (ESI) 382.0 *m/z* [MH]⁺, C₁₅H₁₆N₃O₅S₂ requires 382.1; HPLC-1 = 100%; HPLC-2 = 99%.



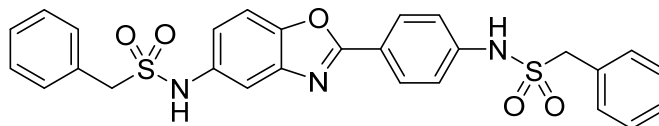
4: 1,1,1-trifluoro-N-(4-(5-((trifluoromethyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)methanesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (52.6 mg, 0.234 mmol), trifluoromethanesulfonic anhydride (90.0 μ L, 0.535 mmol), and anhydrous pyridine (38.0 μ L, 0.466 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **3** as an orange solid (102 mg, 89% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 8.19 (d, J = 8.5 Hz, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.67 (d, J = 2.1, 1H), 7.46 (d, J = 8.7 Hz, 2H), 7.31 (dd, J = 8.7, 2.1 Hz, 2H); MS (ESI) 490.1 m/z [MH^+], $\text{C}_{15}\text{H}_{10}\text{F}_6\text{N}_3\text{O}_5\text{S}_2$ requires 490.0; HPLC-1 = 97%; HPLC-2 = 97%.



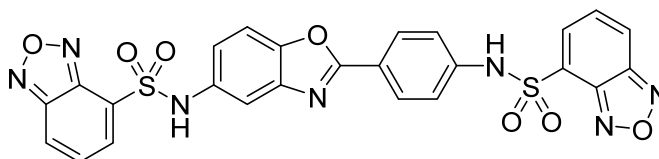
5: N-(4-(5-(propylsulfonamido)benzo[d]oxazol-2-yl)phenyl)propane-1-sulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (51.7 mg, 0.230 mmol), n -propylsulfonyl chloride (112 μ L, 0.999 mmol), and anhydrous pyridine (76.0 μ L, 0.932 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **4** as a white solid (59.4 mg, 59% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.41 (br s, 1H), 9.85 (br s, 1H), 8.12 (d, J = 8.2 Hz, 2H), 7.73 (d, J = 8.8 Hz, 1H), 7.58 (d, J = 2.2 Hz, 1H), 7.39 (d, J = 8.8 Hz, 2H), 7.25 (dd, J = 8.8, 2.2 Hz, 1H), 3.16-3.24 (m, 2H), 3.04-3.09 (m, 2H), 1.66-1.75 (m, 4H), 0.90-0.98 (m, 6H); MS (ESI) 438.2 m/z [MH^+], $\text{C}_{19}\text{H}_{24}\text{N}_3\text{O}_5\text{S}_2$ requires 438.1; HPLC-1 = 98%; HPLC-2 = 99%.



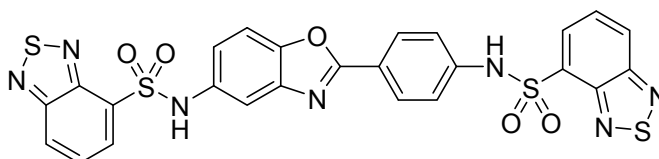
6: N-(4-(5-(thiophene-2-sulfonamido)benzo[d]oxazol-2-yl)phenyl)thiophene-2-sulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (45.0 mg, 0.200 mmol), 2-thiophenesulfonyl chloride (212 mg, 1.16 mmol), and anhydrous pyridine (66.0 μ L, 0.810 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **5** as a white solid (65.6 mg, 63% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.01 (s, 1H), 10.46 (s, 1H), 8.06 (d, J = 8.2 Hz, 2H), 7.92 (d, J = 4.7 Hz, 1H), 7.88 (dd, J = 4.9, 1.4 Hz, 1H), 7.65-7.68 (m, 2H), 7.51 (dd, J = 3.8, 1.6 Hz, 1H), 7.46 (d, J = 1.9 Hz, 1H), 7.35 (d, J = 8.5 Hz, 2H), 7.07-7.15 (m, 3H); MS (ESI) 517.9 m/z [MH^+], $\text{C}_{21}\text{H}_{16}\text{N}_3\text{O}_5\text{S}_4$ requires 518.0; HPLC-1 = 98%; HPLC-2 = 99%.



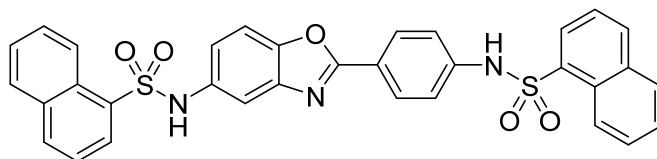
7: 1-phenyl-N-(4-(5-((phenylmethyl)sulfonamido)benzo[d]oxazol-2-yl)phenyl)methanesulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (41.1 mg, 0.182 mmol), α -toluenesulfonyl chloride (145 mg, 0.759 mmol), and anhydrous pyridine (51.0 μ L, 0.625 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **7** as a white solid (29.2 mg, 30% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 10.42 (br s, 1H), 9.92 (br s, 1H), 8.10-8.14 (m, 2H), 7.73 (d, J = 8.8 Hz, 1H), 7.59 (d, J = 1.9 Hz, 1H), 7.33-7.38 (m, 8H), 7.27-7.31 (m, 4H), 7.23 (dd, J = 8.8, 2.2 Hz, 1H), 4.60 (s, 2H), 4.49 (s, 2H); MS (ESI) 534.0 m/z [MH^+], $\text{C}_{27}\text{H}_{24}\text{N}_3\text{O}_5\text{S}_2$ requires 534.1; HPLC-1 = 99%; HPLC-2 = 97%.



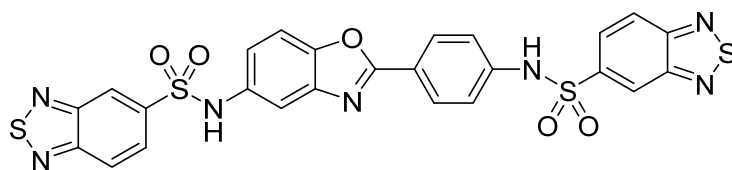
8: N-(4-(5-(benzo[c][1,2,5]oxadiazole-4-sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzo[c][1,2,5]oxadiazole-4-sulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (56.3 mg, 0.250 mmol), 2,1,3-benzoxadiazolesulfonyl chloride (119 mg, 0.546 mmol), and anhydrous pyridine (45.0 μ L, 0.552 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **8** as a yellow solid (115 mg, 78% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.56 (br s, 1H), 11.00 (br s, 1H), 8.31-8.36 (m, 2H), 8.23 (d, J = 5.0 Hz, 1H), 8.09-8.14 (m, 1H), 7.94 (d, J = 8.5 Hz, 2H), 7.70-7.75 (m, 1H), 7.65-7.69 (m, 1H), 7.56 (d, J = 8.8 Hz, 1H), 7.40 (d, J = 1.9 Hz, 1H), 7.27 (d, J = 7.9 Hz, 2H), 7.04 (dd, J = 8.7, 2.0 Hz, 1H); MS (ESI) 590.1 m/z [MH^+], $\text{C}_{15}\text{H}_{16}\text{N}_7\text{O}_7\text{S}_2$ requires 590.1; HPLC-1 = 95; HPLC-2 = 95%.



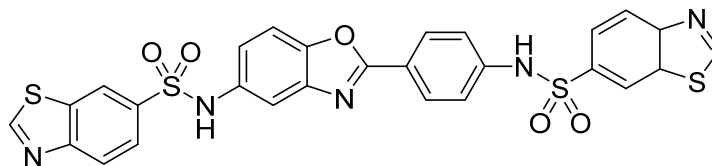
9: N-(4-(5-(benzo[c][1,2,5]thiadiazole-4-sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzo[c][1,2,5]thiadiazole-4-sulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (59.7 mg, 0.265 mmol), 2,1,3-benzothiadiazole-4-sulfonyl chloride (156.5 mg, 0.667 mmol), and anhydrous pyridine (54.0 μ L, 0.662 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **9** as a white solid (157 mg, 95% yield). $^1\text{H-NMR}$ (500 MHz, d_6 -DMSO) δ 11.34 (br s, 1H), 10.75 (br s, 1H), 8.33-8.38 (m, 3H), 8.24 (dd, J = 7.1, 1.1 Hz, 1H), 7.83-7.89 (m, 3H), 7.79 (dd, J = 8.8, 6.9 Hz, 1H), 7.47 (dd, J = 8.8 Hz, 1H), 7.33 (d, J = 1.9 Hz, 1H), 7.22-7.27 (m, 2H), 7.01 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 621.9 m/z [MH^+], $\text{C}_{23}\text{H}_{16}\text{N}_7\text{O}_5\text{S}_4$ requires 622.0; HPLC-1 = 98; HPLC-2 = 97%.



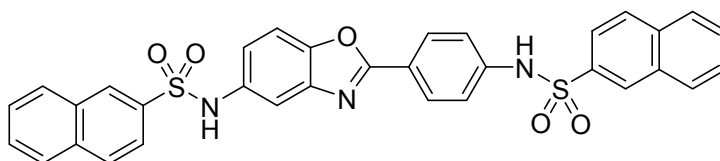
10: N-(4-(5-(naphthalene-1-sulfonamido)benzo[d]oxazol-2-yl)phenyl)naphthalene-1-sulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (54.2 mg, 0.241 mmol), 1-naphthalenesulfonyl chloride (135 mg, 0.594 mmol), and anhydrous pyridine (45.0 μ L, 0.552 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **10** as a white solid (139 mg, 95% yield). ^1H NMR (500 MHz, DMSO) δ 11.26 (br s, 1H), 10.70 (br s, 1H), 8.73 (d, J = 8.5 Hz, 2H), 8.29 (d, J = 7.3 Hz, 1H), 8.15-8.23 (m, 3H), 8.03-8.07 (m, 2H), 7.86 (d, J = 8.5 Hz, 2H), 7.70-7.76 (m, 2H), 7.61-7.67 (m, 3H), 7.57 (t, J = 7.7 Hz, 1H), 7.47 (d, J = 8.8 Hz, 1H), 7.26 (d, J = 1.9 Hz, 1H), 7.18 (d, J = 8.5 Hz, 2H), 6.95 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 606.0 m/z [MH^+], $\text{C}_{33}\text{H}_{24}\text{N}_3\text{O}_5\text{S}_2$ requires 606.1 HPLC-1 = 99; HPLC-2 = 99%.



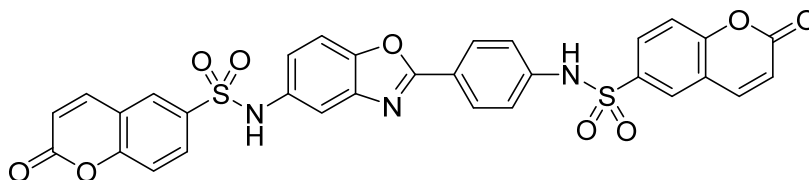
11: N-(4-(5-(benzo[c][1,2,5]thiadiazole-5-sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzo[c][1,2,5]thiadiazole-5-sulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (59.1 mg, 0.262 mmol), 2,1,3-benzothiadiazole-5-sulfonyl chloride (153 mg, 0.651 mmol), and anhydrous pyridine (53.5 μ L, 0.656 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **11** as a yellow solid (56.6 mg, 35% yield). ^1H -NMR (500 MHz, d_6 -DMSO) δ 11.18 (br s, 1H), 10.68 (br s, 1H), 8.59 (s, 1H), 8.45 (dd, J = 1.9, 0.6 Hz, 1H), 8.27-8.30 (m, 2H), 7.93-7.99 (m, 4H), 7.58 (d, J = 8.8 Hz, 1H), 7.44 (d, J = 1.9 Hz, 1H), 7.32 (d, J = 8.8 Hz, 2H), 7.10 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 621.9 m/z [MH^+], $\text{C}_{25}\text{H}_{16}\text{N}_7\text{O}_5\text{S}_4$ requires 622.1; HPLC-1 = 98; HPLC-2 = 98%.



12: N-(4-(5-(benzo[d]thiazole-6-sulfonamido)benzo[d]oxazol-2-yl)phenyl)benzo[d]thiazole-6-sulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (57.5 mg, 0.255 mmol), 1,3-benzothiazole-6-sulfonyl chloride (148 mg, 0.634 mmol), and anhydrous pyridine (52.0 μ L, 0.638 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **12** as a white solid (65.1 mg, 41% yield). ^1H -NMR (500 MHz, d_6 -DMSO) δ 11.01 (br s, 1H), 10.45 (br s, 1H), 9.60 (s, 1H), 9.57 (s, 1H), 8.80 (d, J = 1.9 Hz, 1H), 8.67 (d, J = 1.6 Hz, 1H), 8.23 (d, J = 8.5 Hz, 1H), 8.21 (d, J = 9.1 Hz, 1H), 7.95-7.98 (m, 2H), 7.92-7.95 (m, 1H), 7.84-7.88 (m, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.41 (d, J = 1.9 Hz, 1H), 7.30 (d, J = 8.8 Hz, 2H), 7.06 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 619.8 m/z [MH^+], $\text{C}_{27}\text{H}_{18}\text{N}_5\text{O}_5\text{S}_4$ requires 620.0; HPLC-1 = 99; HPLC-2 = 98%.



13: N-(4-(5-(naphthalene-2-sulfonamido)benzo[d]oxazol-2-yl)phenyl)naphthalene-2-sulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (56.1 mg, 0.249 mmol), 2-naphthalenesulfonyl chloride (142 mg, 0.624 mmol), and anhydrous pyridine (51.0 μ L, 0.625 mmol) according to synthetic protocol A. Flash chromatographic purification (hexanes:EtOAc gradient) afforded **13** as a white solid (127 mg, 84% yield). ^1H NMR (500 MHz, DMSO) δ 11.01 (br s, 1H), 10.43 (br s, 1H), 8.53 (s, 1H), 8.39 (s, 1H), 8.12-8.16 (m, 1H), 8.04-8.10 (m, 3H), 7.97 (t, J = 7.9 Hz, 2H), 7.92 (d, J = 8.8 Hz, 2H), 7.80 (dd, J = 8.8, 1.9 Hz, 1H), 7.75 (dd, J = 8.5, 1.9 Hz, 1H), 7.58-7.69 (m, 4H), 7.53 (d, J = 8.8 Hz, 1H), 7.40 (d, J = 2.2 Hz, 1H), 7.29 (d, J = 8.8 Hz, 2H), 7.06 (dd, J = 8.8, 2.2 Hz, 1H); MS (ESI) 606.2 m/z [MH^+], $\text{C}_{33}\text{H}_{24}\text{N}_3\text{O}_5\text{S}_2$ requires 606.1; HPLC-1 = 99%; HPLC-2 = 99%.



14: 2-oxo-N-(4-(5-((2-oxo-2H-chromene)-6-sulfonamido)benzo[d]oxazol-2-yl)phenyl)-2H-chromene-6-sulfonamide was synthesized from 5-amino-2-(4-aminophenyl)benzoxazole (52.7 mg, 0.234 mmol), coumarin-6-sulfonyl chloride (144 mg, 0.588 mmol), and anhydrous pyridine (47.5 μ L, 0.582 mmol) according to synthetic protocol A. Flash chromatographic purification (EtOAc:methanol gradient) afforded **14** as a tan solid (102 mg, 68% yield). ^1H -NMR (500 MHz, d_6 -DMSO) δ 11.04 (br s, 1H), 10.47 (br s, 1H), 8.28 (d, J = 2.2 Hz, 1H), 8.12-8.18 (m, 3H), 7.95-7.98 (m, 3H), 7.88 (dd, J = 8.8, 2.2 Hz, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.54 (dd, J = 8.5, 6.6 Hz, 2H), 7.42 (d, J = 1.9 Hz, 1H), 7.28 (d, J = 8.8 Hz, 2H), 7.06 (dd, J = 8.7, 2.0 Hz, 1H), 6.55-6.61 (m, 2H); MS (ESI) 641.9 m/z [MH^+], $\text{C}_{31}\text{H}_{20}\text{N}_3\text{O}_9\text{S}_2$ requires 642.1; HPLC-1 = 97%; HPLC-2 = 97%.

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