ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Facile Synthesis and Preliminary Structure–Activity Analysis of New Sulfonamides Against *Trypanosoma brucei*

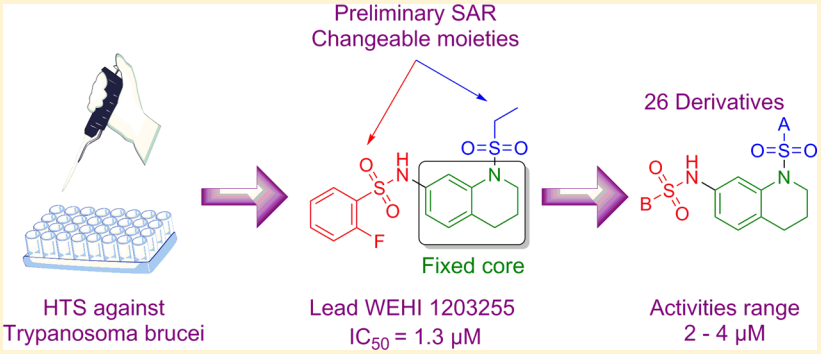
Adel A. Rashad,[†] Amy J. Jones,[‡] Vicky M. Avery,[‡] Jonathan Baell,*[,§] and Paul A. Keller*[,†]

[†]School of Chemistry, University of Wollongong, Wollongong 2522, Australia

[‡]Eskitis Institute for Drug Discovery, Griffith University, Brisbane Innovation Park, Don Young Road, Nathan 4111, Australia

[§]Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville 3052, Australia

Supporting Information



ABSTRACT: The high throughput screening of a library of over 87,000 drug-like compounds against the African sleeping sickness parasite resulted in the discovery of hits with a wide range of molecular diversity. We report here the medicinal chemistry development of one such hit, a tetrahydroisoquinoline disulfonamide, with the synthesis and testing of 26 derivatives against the trypanosome subspecies. Activities in the 2−4 μM range were revealed with a selectivity index suitable for further development.

KEYWORDS: *T. brucei, SAR, tetrahydroquinoline, sulfonamides*

Trypanosoma brucei gambiense and *T. brucei rhodesiense* are the causative agents of human African trypanosomiasis (HAT), also known as sleeping sickness. *T. b. rhodesiense* is found in Eastern and Southern Africa, whereas *T. b. gambiense* occurs in Western and Central Africa and is responsible for over 90% of all reported cases of infection.[1] This disease threatens about 70 million people living in sub-Saharan Africa and causes an estimated 25,000 deaths per year.[2,3] It has a major impact on the affected nations causing suffering and poverty, and if left untreated, the disease is usually fatal.[4] A lack of full-scale screening programs and poor diagnostic tools leads to an under-reporting of cases, which is likely to be at least 3-fold higher than the measured value.[5]

Both subspecies are transmitted by the bite of the infected tsetse fly. *T. b. gambiense* HAT is primarily a chronic disease, and it can be many months to years before patients succumb to the disease. In contrast, *T. b. rhodesiense* HAT is acute, with death occurring within months of infection. After the bite, the parasites start to multiply in the blood; that is, phase I. During this phase, the parasite lives within the bloodstream and subsequently migrates to other areas of the human body, such as the lymph nodes and spleen, causing febrile illness with symptoms similar to those caused by malaria (rash, fever, shaking chills, body aches, and general fatigue). If phase I is left untreated, the parasites penetrate the blood–brain barrier and invade the central nervous system (CNS) (phase II) causing neurological symptoms including progressive mental deterioration, sleep disturbances, long lasting coma, and finally death if not treated.[6]

Unfortunately, vaccines are not available and therefore, the main line of defense against the parasite is chemotherapeutics. The treatment options are limited with only four registered drugs available. Suramin and pentamidine are effective against early stage infections while melarsoprol (contains arsenic) and eflornithine are used to treat late-stage disease (Figure 1).[7,8] These drugs were developed approximately 30 years ago, and suffer high toxicity, lack of efficacy, and emerging resistance are concerns. Melarsoprol is the most toxic, causing a reactive encephalopathy in 5−10% of treated patients, with a 1−5% mortality rate.[9]

Recently, there has been some progress in the treatment of HAT[10] with a nifurtimox–eflornithine (Figure 1) combination (NECT) therapy developed, which is as effective as eflornithine monotherapy but was easier and cheaper to administer.[11,12]

Received: November 26, 2013
Accepted: March 10, 2014
Published: March 10, 2014

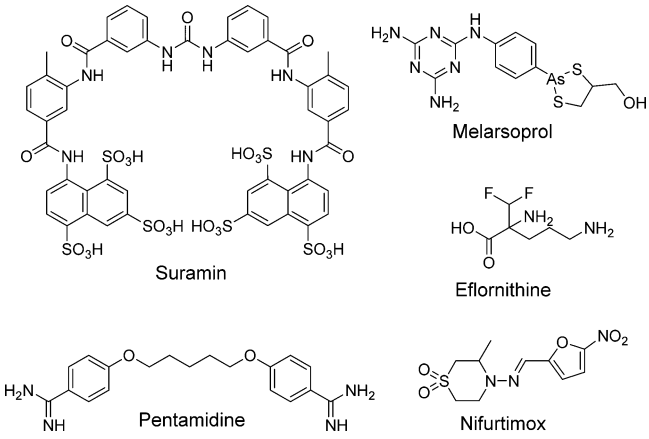


Figure 1. Older generation African sleeping sickness drugs.

However, NECT is not ideal due to the parental mode of administration, the need to hospitalize patients during treatment, and the possible development of resistance.[13,14]

Increased research and investment into HAT chemotherapy has resulted in the identification of numerous trypanocidal compounds, a number of which have entered or are in clinical development. The diminazene, pafuramidine[15] (Figure 2), was

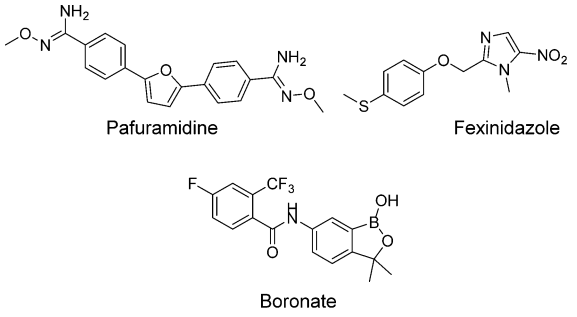


Figure 2. New generation of potential African sleeping sickness treatments.

the first oral drug to enter clinical development for early stage HAT in 2005. However, the observation of severe hepatic toxicity and renal insufficiency during a retrospective phase I trial in 2008 lead to the compound being abandoned. In 2009, a second orally available drug, fexinidazole[16] (Figure 2), entered phase I clinical trials for HAT. The drug is effective against both stages of the disease and subsequently progressed to phase II/III clinical development in 2012.

More recently, the novel boron-containing molecule (SCYx-7158) (Figure 2) emerged as an orally active drug candidate, with promising activity against both acute and CNS stage infections. The compound successfully completed preclinical studies in 2011 and entered phase I clinical trials in 2012.[17] The mechanism by which the boronate acts as a trypanocidal agent is still unknown. Overall, present treatment options for HAT are limited, and the high attrition rates in drug discovery means that new therapeutics with acceptable efficacies and safety profiles are urgently needed.[18]

High throughput screening (HTS) is one approach that can be used to identify new lead compounds for such neglected diseases. Therefore, the HTS library of 87,926 compounds (WEHI 2003)[19] was tested against the nonhuman infective trypanosome subspecies, *Trypanosoma brucei brucei* and against a mammalian cell line HEK293, to determine a selectivity index (SI) for each compound. Although *T. b. brucei* is nonhuman infective, it is frequently used in HAT drug discovery campaigns and lead optimization programs as a model for the human infective subspecies (*T. b. gambiense* and *T. b. rhodesiense*), which are more difficult to maintain and culture in vitro. Cluster analysis, considering chemical alerts such as toxicophores, the likelihood of CNS penetration, and drug-like structural features, yielded a subset of 12 compounds as promising medicinal chemistry starting points for drug development.

This communication discusses the synthesis and antitrypanocidal activity of new analogues for the bis-sulfonamide hit, WEHI-1203255 (Figure 3), which showed an IC_{50} value of 1.3

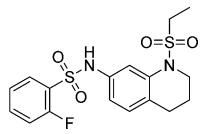
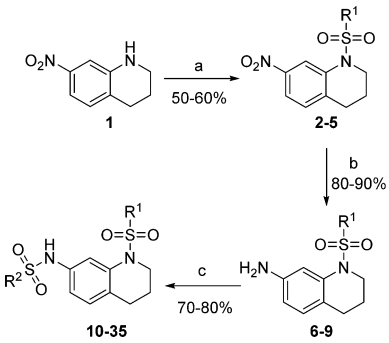


Figure 3. Lead compound (WEHI 1203255), IC_{50} = 1.3 μM.

μM with a SI > 32. This compound has excellent physicochemical properties, good calculated aqueous solubility of 100 μM, an acceptable polar surface area of 84 Å^2, and an acceptable CLogP value[20] of 2.5. The analogues were synthesized and tested for their ability to inhibit the growth of *T. b. brucei* limiting the changes to the two sulfonamide moieties to study the preliminary structure–activity relationships (SARs). The cytotoxicity profiles of the compounds were evaluated using HEK293 cell line, and SI was estimated for each analogue. The SI of the compounds was determined where possible by directly comparing the IC_{50} values from the *T. b. brucei* and HEK 293 assay. If this was not possible, an estimated SI value was calculated by comparing the IC_{50} in the *T. b. brucei* assay and the highest dose at which there was no activity (<50%) in the HEK 293 assay.

The strategy for the synthesis of sulfonamide analogues is summarized in Scheme 1 and started from 7-nitrotetrahydroquinoline, which was sulfonated using the appropriate sulfonyl chloride in pyridine at room temperature. The nitro group was reduced, initially using acetic acid in ethanol in the presence of tin with sonication;[21] however, under these conditions, yields were between 50 and 60%. The use of Raney nickel in methanol in the presence of hydrazine hydrate

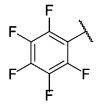
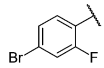
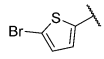
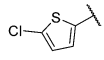
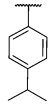
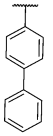
Scheme 1[a]



[a]Reagents and conditions: (a) dry pyridine, R^1SO_2Cl, rt, 24 h; (b) methanol, $NH_2NH_2{\cdot}H_2O$, Raney nickel, reflux, 6–8 h; (c) dry pyridine, R^2SO_2Cl, 0 °C to rt, 4–6 h.

dx.doi.org/10.1021/ml400487t | ACS Med. Chem. Lett. 2014, 5, 496–500

Table 1. R^1, R^2, Molecular Weight, ClogP, IC_{50} and Selectivity Index for Compounds 10−35

	R^1	R^2	Mwt	ClogP[a]	IC_{50} (μM)	S.I.
10	Et		398.5	2.5±0.8	1.3±0.6	32±3.1
11	Et		398.5	3.0±0.8	7.8±2.7	11.8±4.1
12	Et		398.5	3.1±0.8	7.8±2.2	11.2±2.5
13	Et		416.5	2.3±0.9	82% @ 10 μM	
14	Et		470.4	3.4±1.1	94% @ 10 μM	
15	Et		477.4	3.5±0.9	9.9±3.3	9.0±2.3
16	Et		386.5	2.3±0.8	4.0±0.6	20.8±2.9
17	Et		465.4	3.5±0.9	11.2±2.6	7.7±1.6
18	Et		421.0	3.3±0.9	13.1±4.3	6.9±2.2
19	Et		394.5	3.1±0.8	3.4±0.9	25.8±2.9
20	Et	Et	332.4	1.6±0.8	14% @ 10 μM	
21	Et		386.4	2.8±0.9	18% @ 10 μM	
22	Pr		408.5	3.7±0.8	1.84 ± 0.09	29 ± 25.4
23	Pr		436.6	4.5±0.8	1.71 ± 0.53	8.9 ± 1.5
24	Pr		470.6	4.9±0.8	5.7 ± 2.8	16.6 ± 8.2
25	Pr		412.5	3.0±0.8	3.5 ±0.8	24.3 ± 5.3
26	Pr		400.5	2.8±0.8	< 10% @ 10 μM	
27	Pr		408.5	3.7±0.8	11% @ 10 μM	
28	Pr		408.5	3.7±0.8	<10% @ 10 μM	
29	Pr		422.6	4.1±0.8	9 ± 4.3	10.4 ± 5
30	Pr		422.6	4.1±0.8	<10% @ 10 μM	
31	Pr		464.6	5.5±0.8	7.9 ±3.2	11.4±4.7
32			440.6	2.3±0.8	35% 10 μM	
33			448.6	3.1±0.8	3.1±0.9	2.5±0.5
34			448.6	3.1±0.8	7.6±2.6	1.7±0.5
35			456.6	3.9±0.8	3.9±0.9	1.8±0.4

[a]Calculated using ACDLabs v.12.0 (ACD/Laboratories, Toronto, Canada); S.I. = selectivity index.

as a source of hydrogen reliably gave the aniline derivatives in gram quantities in 80−90% yields. The amino group was then reacted with the different sulfonyl chlorides in pyridine at room temperature (Scheme 1), giving the final analogues in 70−80% yield. HPLC analysis of these bis-sulfonamides showed a purity range >95% for all the synthesized derivatives. This facile three step synthetic strategy enabled us to access 26 separate derivatives in a short time frame, reliable yields as well and at reasonable cost.

The results for the testing against *T. b. brucei*, the calculated ClogP, and the SI are listed in Table 1. The initial activity was determined by screening at 1 and 10 μM, and derivatives showing >80% activity at 10 μM and >50% activity at 1 μM were then tested to obtain the IC_{50} values. The first series of derivatives examined the changes in the aromatic sulfonyl moiety where the lead compound **10** has a fluorine atom in the *ortho* position. This lead compound was also resynthesized, confirming the activity results from the initial HTS. Changing the *ortho*-fluoro substituent to the *para* (**11**) and *meta* (**12**)

dx.doi.org/10.1021/ml400487t | ACS Med. Chem. Lett. 2014, 5, 496−500

positions did not improve activity where the IC_{50}/SI profile was 7.8 μM/>10 for both derivatives, indicating a slightly decreased trypanocidal activity and increased toxicity compared to **10**. Increasing the number of the fluorine atoms had a negative effect on the activity, where the addition of a second fluoro substituent into the adjacent *ortho* position (**13**) resulted in a decreased activity (82% activity at 10 μM) as did the presence of five fluoro substituents (**14**, 94% activity at 10 μM). This implied the importance of the monofluoro atom only in the *ortho* position, with no advantages with the presence of the extra fluorine atoms.

The fluorine atom is the smallest halogen size; therefore, it was interesting to test the presence of other halogen atoms. The addition of a bromo substituent at the *para* position (compound **15**) resulted in increased toxicity and did not improve the activity (IC_{50}/SI = 9.9 μM/>8).

Replacing the benzene moiety with its bioisostere thiophene[22,23] (**16**) resulted in a similar activity (IC_{50}/SI = 4.0 μM/>20) compared to the lead **10**. The same activity profile of **10** and the simpler **16** might be attributed to the lipophilic nature of the thiophene ring in **16** that might have the same effect as the fluorine atom in **10**. The addition of another halogen to this thiophene (Br, **17**) showed a decreased trypanocidal activity (IC_{50}/SI = 11.2 μM/>7) and a concurrent reduction in the SI value compared to the unsubstituted thiophene analogue **16**. Replacement of the bulky bromo substituent in **17** by a chloro substituent (**18**) was also not tolerated, with a similar decrease in activity (IC_{50}/SI = 13.1 μM/>6). The activity of **18** was 3-fold less than that of **16** but similar to that of **17**.

Replacing the aromatic moiety with aliphatic chains as in compounds **20** and **21** completely abolished the activity, which indicates the importance of the aromatic ring in that position for the trypanocidal activity. The π system of the aromatic ring may be involved in the interaction site, whereas with aliphatic side chains such interactions do not exist. Interestingly, the introduction of a small hydrophobe such as a methyl group on the *para* position on the phenyl ring of the aromatic sulfonyl moiety (compound **19**) made little difference to the activity (IC_{50}/SI = 3.4 μM/>24) compared to **10**. As the mode of action and the target of the compounds are not yet known, the role of this methyl group cannot be assured; however, it might be involved in a hydrophobic interaction within the target site.

The extension of the ethyl side chain of the other sulfonyl group (second changeable moiety) to a propyl group, as in compound **22**, was also tolerated (IC_{50}/SI = 1.84 μM/>29) with no significant difference in the activity compared to **19**. However, in the case of compound **26**, the extra length of this substituent was not tolerated. When the *p*-tolyl group of **22** was replaced by a thiophene ring (compound **26**), the activity was completely abolished due to the propyl group compared to **16** (with an ethyl side chain).

The *para* position of the methyl hydrophobe in **22** was important for activity as can be indicated by the inactivity of compounds **27** (*ortho* position) and **28** (*meta* position). The relatively good activity of **29** and **31** is moderated by a poor SI when compared to **10** or **22**. The inactivity of **30** (no *p*-methyl group) also gives indications about the importance of the *para* position. Figure 4 shows the SAR for this series of bis-sulfonamides.

The addition of the hydrophobic methyl groups on either the ethyl side chain or on the aromatic sulfonyl moieties seemed to act as a tuner for the activity. Compound **26** was completely

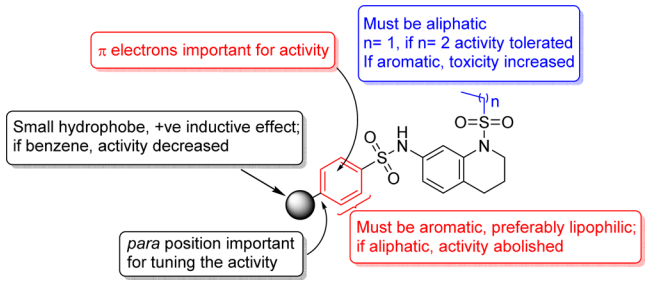


Figure 4. Structure–activity relationships for the bis-sulfonamides.

inactive, whereas compound **25** showed similar activity and selectivity (IC_{50}/SI = 3.5 μM/>24) compared to the lead **10**. Increasing the bulkiness of compound **22** to **23** (replacing the small methyl hydrophobe with the more bulky isopropyl group) resulted in a similar activity profile but increased toxicity (IC_{50}/SI = 1.71 μM/>8). Further increases in the bulkiness by replacing the isopropyl in **23** by a phenyl ring (**24**) decreased the activity (IC_{50}/SI = 5.7 μM/>16) by 3-fold compared to **23**. This difference could be attributed to a size effect. This also confirms that the π system of the aromatic ring (directly attached to the sulfonamide group) might be involved in the activity.

When the aliphatic sulfonyl side chain (sulfonyl group attached to the tetrahydroquinoline N) was replaced with aromatic ring, the activity was either completely abolished as in the case of compound **32** or toxicity was increased as in compounds **33**–**35**. Interestingly, compound **33** carrying the *para*-methyl group was the best in this series (IC_{50}/SI = 3.1 μM/>2), confirming the importance of the *para* position on this aromatic moiety. The aliphatic sulfonyl groups directly attached to the tetrahydroquinoline ring is important for activity rather than an aromatic replacement.

An initial SAR model can be generated from this information (Figure 4). Treating the tetrahydroisoquinoline unit as a scaffold, toxicity is minimized if the sulfonamide (blue) is aliphatic with the hydrophobicity tolerated up to 3 methylene units. Larger moieties reduce the activity. In contrast, the second sulfonamide unit (red) must be aromatic, indicating that the π-electrons are likely to be significant. Aliphatic substituents abolish activity.

The activity is maximized when this aromatic unit is substituted in the *para* position. The substituent is best as a small hydrophobic unit with a positive inductive effect.

This study revealed a new structural class of *T. brucei* inhibitors with a good selectivity index, suitable for further investigations. Close adherence to drug-like properties throughout the study kept the molecular weight of synthesized derivatives low, and ClogP values were used as a guide to pharmacokinetic properties that needed to be maintained. Initial SAR studies confirm the initial hit compound and enabled basic design principles to be observed. Further optimization of this sulfonamide series is possible, in particular the *para* position of the terminal aryl sulfonamide group, and further studies in this direction will be forthcoming. Therefore, the discovery of the bis-sulfonamides as a novel class of antiparasitic agents offers a new medicinal chemistry opportunity for targeting *T. brucei spp*.

dx.doi.org/10.1021/ml400487t | *ACS Med. Chem. Lett.* 2014, 5, 496–500

■ ASSOCIATED CONTENT

Supporting Information

Synthetic and biological experimental procedures, selected dose−response curves (active compounds), full characterization of the synthesized compounds, and biological assay protocol data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(J.B.) E-mail: Jonathan.Baell@monash.edu.

*(P.A.K.) E-mail: keller@uow.edu.au.

Funding

This research was supported by the National Health and Medical Research Fund (AUSTRALIA) Project Grant 1025581 and a University Postgraduate Award to AR from the University of Wollongong.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Cecchi, G.; Paone, M.; Franco, J. R.; Fevre, E. M.; Diarra, A.; Ruiz, J. A.; Mattioli, R. C.; Simarro, P. P. Towards the Atlas of human African trypanosomiasis. *Int. J. Health. Geogr.* **2009**, *8*, 15.

(2) Brun, R.; Blum, J.; Chappuis, F.; Burri, C. Human African trypanosomiasis. *Lancet* **2010**, *375*, 148−159.

(3) Simarro, P. P.; Cecchi, G.; Franco, J. R.; Paone, M.; Diarra, A.; Ruiz-Postigo, J. A.; Fevre, E. M.; Mattioli, R. C.; Jannin, J. G. Estimating and mapping the population at risk of sleeping sickness. *PLoS Negl. Trop. Dis.* **2012**, *6*, e1859.

(4) Popejoy, M. W. Working to Overcome the Global Impact of Neglected Tropical Diseases. *Perspect. Public Heal.* **2012**, *132*, 192−192.

(5) Wastling, S. L.; Welburn, S. C. Diagnosis of human sleeping sickness: sense and sensitivity. *Trends Parasitol.* **2011**, *27*, 394−402.

(6) Phillips, M. A. Stoking the drug target pipeline for human African trypanosomiasis. *Mol. Microbiol.* **2012**, *86*, 10−14.

(7) Croft, S. L.; Barrett, M. P.; Urbina, J. A. Chemotherapy of trypanosomiases and leishmaniasis. *Trends Parasitol.* **2005**, *21*, 508−512.

(8) Renslo, A. R.; McKerrow, J. H. Drug discovery and development for neglected parasitic diseases. *Nat. Chem. Biol.* **2006**, *2*, 701−710.

(9) Pepin, J.; Milord, F. The treatment of human African trypanosomiasis. *Adv. Parasitol.* **1994**, *33*, 1−47.

(10) Barrett, M. P. Potential new drugs for human African trypanosomiasis: some progress at last. *Curr. Opin. Infect. Dis.* **2010**, *23*, 603−608.

(11) Priotto, G.; Kasparian, S.; Mutombo, W.; Ngouama, D.; Ghorashian, S.; Arnold, U.; Ghabri, S.; Baudin, E.; Buard, V.; Kazadi-Kyanza, S.; Ilunga, M.; Mutangala, W.; Pohlig, G.; Schmid, C.; Karunakara, U.; Torreele, E.; Kande, V. Nifurtimox-eflornithine combination therapy for second-stage African *Trypanosoma brucei* gambiense trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. *Lancet* **2009**, *374*, 56−64.

(12) Yun, O.; Priotto, G.; Tong, J.; Flevaud, L.; Chappuis, F. NECT is next: Implementing the new drug combination therapy for *Trypanosoma brucei* gambiense sleeping sickness. *PLoS Neglect. Trop. Dis.* **2010**, *4*, e720.

(13) Delespaux, V.; de Koning, H. P. Drugs and drug resistance in African trypanosomiasis. *Drug Resist. Update* **2007**, *10*, 30−50.

(14) Rodgers, J. Human African trypanosomiasis, chemotherapy and CNS disease. *J. Neuroimmunol.* **2009**, *211*, 16−22.

(15) Wenzler, T.; Boykin, D. W.; Ismail, M. A.; Hall, J. E.; Tidwell, R. R.; Brun, R. New treatment option for second-stage African sleeping sickness: In vitro and in vivo efficacy of aza analogs of DB289. *Antimicrob. Agents Chemother.* **2009**, *53*, 4185−4192.

(16) Torreele, E.; Trunz, B. B.; Tweats, D.; Kaiser, M.; Brun, R.; Mazue, G.; Bray, M. A.; Pecoul, B. Fexinidazole: A new oral nitroimidazole drug candidate entering clinical development for the treatment of sleeping sickness. *PLoS Neglect. Trop. Dis.* **2010**, *4*, e923.

(17) Jacobs, R. T.; Nare, B.; Wring, S. A.; Orr, M. D.; Chen, D.; Sligar, J. M.; Jenks, M. X.; Noe, R. A.; Bowling, T. S.; Mercer, L. T.; Rewerts, C.; Gaukel, E.; Owens, J.; Parham, R.; Randolph, R.; Beaudet, B.; Bacchi, C. J.; Yarlett, N.; Plattner, J. J.; Freund, Y.; Ding, C.; Akama, T.; Zhang, Y. K.; Brun, R.; Kaiser, M.; Scandale, I.; Don, R. SCYX-7158, an orally-active benzoxaborole for the treatment of stage 2 human African trypanosomiasis. *PLoS Neglect. Trop. Dis.* **2011**, *5*, e1151.

(18) Ettari, R.; Tamborini, L.; Angelo, I. C.; Micale, N.; Pinto, A.; De Micheli, C.; Conti, P. Inhibition of Rhodesain as a Novel Therapeutic Modality for Human African Trypanosomiasis. *J. Med. Chem.* **2013**, *56*, 5637−5658.

(19) Sykes, M. L.; Baell, J. B.; Kaiser, M.; Chatelain, E.; Moawad, S. R.; Ganame, D.; Ioset, J. R.; Avery, V. M. Identification of compounds with anti-proliferative activity against *Trypanosoma brucei* brucei Strain 427 by a whole cell viability based HTS campaign. *PLoS Neglect. Trop. Dis.* **2012**, *6*, e1896.

(20) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2001**, *46*, 3−26.

(21) Gamble, A. B.; Garner, J.; Gordon, C. P.; O'Conner, S. M. J.; Keller, P. A. Aryl nitro reduction with iron powder or stannous chloride under ultrasonic irradiation. *Synth. Commun.* **2007**, *37*, 2777−2786.

(22) Buchstaller, H. P.; Siebert, C. D.; Steinmetz, R.; Frank, I.; Berger, M. L.; Gottschlich, R.; Leibrock, J.; Krug, M.; Steinhilber, D.; Noe, C. R. Synthesis of thieno[2,3-b]pyridinones acting as cytoprotectants and as inhibitors of [H-3]Glycine binding to the *N*-methyl-D-aspartate (NMDA) receptor. *J. Med. Chem.* **2006**, *49*, 864−871.

(23) Hwang, K. J.; Lee, T. S.; Kim, K. W.; Kim, B. T.; Lee, C. M.; Park, E. Y.; Woo, R. S. 4-Hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b] pyrimidine derivatives: Synthesis and their biological evaluation for the glycine site acting on the *N*-methyl-D-aspartate (NMDA) receptor. *Arch. Pharm. Res.* **2001**, *24*, 270−275.