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

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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 to 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 threefold 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 is a concern. Melarsoprol is the most toxic, causing a reactive encephalopathy in 5−10% of treated patients, with a 1−5% mortality rate.[9]

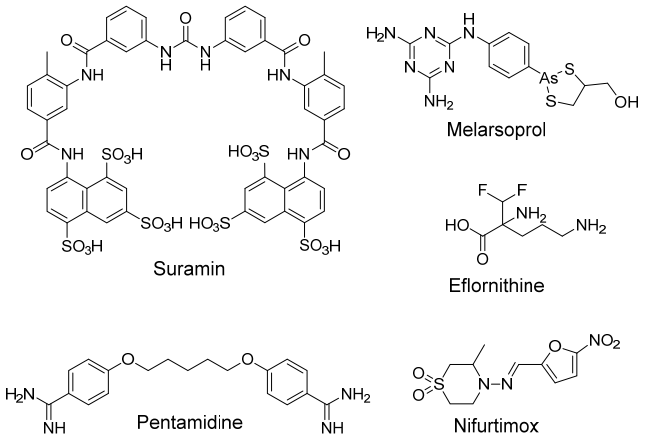


Figure 1. Older generation African sleeping sickness drugs.

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 mono-therapy but was easier and cheaper to administer.[11,12] 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 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]

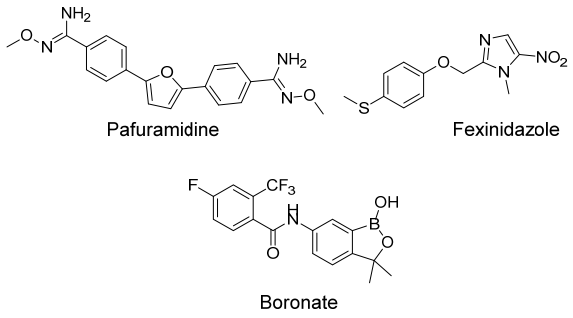


Figure 2. New generation of potential African sleeping sickness treatments.

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 non-human 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 non-human 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 twelve compounds as promising medicinal chemistry starting points for drug development.

This article discusses the synthesis and anti-trypanocidal activity of new analogues for the bis-sulfonamide hit, WEHI-1203255 (Figure 3), which showed an IC_{50} value of 1.3 μM with a SI of >32. This compound has excellent physiochemical 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. 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.

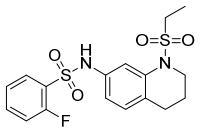
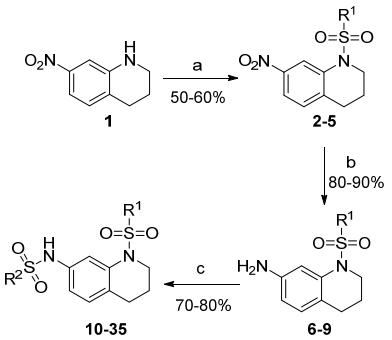


Figure 3. Lead compound (WEHI 1203255), IC_{50}= 1.3 μM.

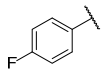
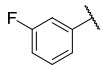
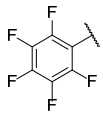
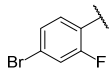
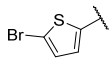
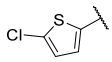
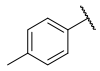
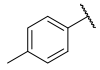
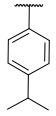
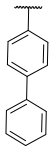
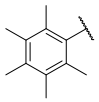
The strategy for the synthesis of sulfonamide analogues is summarised in Scheme 1, and started from 7-nitrotetrahydroquinoline which was sulfonated using the appropriate sulfonyl chloride in pyridine at room temperature. The nitro group was reduced, initially using acetic acid in ethanol in the presence of tin with sonication,[21] however, under these conditions, yields were between 50-60%. The use of Raney nickel in methanol in presence of hydrazine hydrate 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 in 70-80% yield, the final analogues. 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 timeframe, reliable yields as well and at reasonable cost.



Scheme 1. Reagents and conditions: (a) Dry pyridine, R^1SO_2Cl, rt, 24 h; (b) Methanol, $NH_2NH_2.H_2O$, Raney Nickel, reflux, 6-8 h; (c) Dry pyridine, R^2SO_2Cl, 0 °C to rt, 4-6 h.

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 μM 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 resynthesised and tested with IC_{50}/SI= 1.3 μM/>32, confirming the activity results from the initial HTS. Changing the *ortho*-fluoro substituent to the *para* (**11**) and *meta* (**12**) 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 mono-fluoro atom only in the *ortho* position, with no advantages with the presence of the extra fluorine atoms.

Table 1 R^1, R^2, molecular weight, ClogP, IC_{50} and Selectivity Index for compounds 10-35.

	R^1	R^2	Mwt	ClogP*	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

*Calculated using ACDLabs v.12.0 (ACD/Labs, Toronto, Canada), S.I. = selectivity index.

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 don't 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.

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 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 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 structure-activity relationships (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 2nd sulfonamide unit (red) must be aromatic, indicating that the π-electrons are likely to be significant. Aliphatic substituents abolish activity.

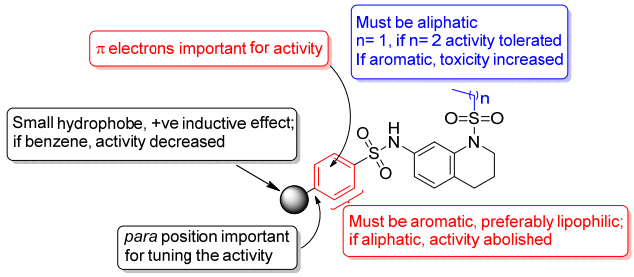


Figure 4. Structure-activity relationships for the bis-sulfonamides

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 mwt of synthesized derivatives low and ClogP were used as a guide to pharmacokinetic properties to maintain. 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*.

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SUPPORTING INFORMATION AVAILABLE Synthetic and biological experimental procedures, selected dose response curves (active compounds), full characterization of the synthesized compounds, and biological assay protocol data are available.

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