RESEARCH ARTICLE

Cite this: *RSC Med. Chem.*, 2020, **11**, 1413

Received 4th August 2020,
Accepted 10th September 2020

DOI: 10.1039/d0md00277a

rsc.li/medchem

Investigation of thiazolyl-benzothiophenamides as potential agents for African sleeping sickness†

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African sleeping sickness is a potentially fatal neglected disease affecting sub-Saharan Africa. High-throughput screening identified the thiazolyl-benzothiophenamide **1** to be active against the causative parasite, *Trypanosoma brucei*. This work establishes structure–activity relationships of **1**, guiding the design of second generation derivatives. After screening against the clinically relevant species *T. b. rhodesiense*, the derivative **16** was identified as a suitable candidate for further investigation.

African sleeping sickness, or Human African trypanosomiasis (HAT), is a neglected parasitic disease affecting sub-Saharan Africa.^{1,2} The causative trypanosome is *Trypanosoma brucei*, a protozoan species which can be further sub-divided into the subspecies *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense* and is transmitted to humans through the bite of the tsetse fly. The trypanosomes cross the blood–brain barrier (BBB) to initiate the second stage of the disease, which causes the characteristic sleep disorder and is ultimately fatal if left untreated. Current chemotherapeutics (Fig. 1) include pentamidine and suramin (stage I), and melarsoprol or a combination of eflornithine and nifurtimox (stage II).^{3,4} In addition to the potential of parasitic resistance to drugs such as pentamidine and melarsoprol,⁵ most of the current treatments are stage and species-specific, require intravenous administration and cause serious side effects, resulting in an urgent need for alternatives.^{2a} Promising methods of identifying new treatments for HAT include drug repurposing (including the incorporation of organometallic moieties),⁶ high-throughput screening (HTS) and the mining of existing pharmaceutical libraries: for example, fexinidazole (Fig. 1) was originally discovered by Hoechst AG as an antiprotozoal agent⁷ and abandoned in the 1980s, but was approved for use in November 2018 as the first new treatment for stage II HAT

in thirty years.⁸ Screening of benzoxaboroles by Anacor Pharmaceuticals similarly afforded acoziborole⁹ (Fig. 1), which is in phase III clinical trials as of 2020.¹⁰

This work establishes the structure–activity relationships (SAR) of the thiazolyl-benzothiophenamide scaffold, WEHI-0086109 (**1**, Fig. 2), identified during a HTS conducted in 2012.^{11a,12} It should be noted that other benzothiophene-2-carboxamides have been included in medicine for Malaria Venture's Malaria Box¹³ and developed as antimalarial agents

Fig. 1 Current drug treatments for African sleeping sickness showing stage and species specificity.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/d0md00277a

Fig. 2 Lead compound WEHI-0086109 (1) showing bioactivity against *T. b. brucei* and SAR components to be investigated.

in subsequent SAR studies.¹⁴ For this SAR study, investigation of the scaffold **1** was split into three components: the thiazole fragment, the benzothiophene fragment, and the amide linker (Fig. 2). A series of derivatives were synthesised and tested *in vitro* against *Trypanosoma brucei brucei* (a non-human infective surrogate for *T. b. gambiense* and *T. b. rhodesiense*), and selected derivatives were tested against *T. b. rhodesiense*, and the selectivity over mammalian cells evaluated. These results were used to determine the SAR of the thiazolyl-benzothiophenamide scaffold. The lead compound **1** exhibits bioactivity against *T. b. brucei* in the low micromolar range ($IC_{50} = 2.50 \mu M$) with selectivity over human (HEK 293) cells ($SI > 48$) and is adequately druglike under Lipinski guidelines¹⁵ ($MW = 362.85 \text{ g mol}^{-1}$, $ClogP = 1.70$, one H-bond donor, four H-bond acceptors) (Table 1).

The overall strategy for synthesising derivatives of **1** began with preparation of the thiazole fragment **2** (Scheme 1). This

Scheme 1 Synthesis of 3-substituted derivatives of WEHI-0086109 (1). a. Br_2 in CH_2Cl_2 , $0^\circ C - r.t.$ b. Thiourea, pyridine in MeOH, reflux. c. $R'-B(OH)_2$, $Ni(dppe)Cl_2$, K_3PO_4 in toluene, reflux. d. NaOH in dioxane/ H_2O , r.t. e. $SOCl_2$ in dioxane, reflux. ii. **2**, pyridine in toluene, reflux.

required bromination of the appropriate diketone to facilitate the following Hantzsch thiazole synthesis, with both steps being achieved in mostly good yields on a gram scale. The ready availability of dimedone as the starting diketone allowed the incorporation of a 5-*gem*-dimethyl substituent, which is commonly used in drug design.¹⁶ The thiazole fragment **2** was then introduced *via* a Schotten-Baumann amidation with a range of acyl chlorides derived from the readily accessible building block **3**.¹⁷ After purity analysis *via*

Table 1 Bioactivity against *T. b. brucei* 427 (IC_{50}) and selectivity index (SI) over HEK 293 cells of 3-substituted WEHI-0086109 (1) derivatives (see Scheme 1). Assays were performed in triplicate. Controls (IC_{50}/SI): pentamidine ($2.13 \pm 0.59 \text{ nM}/307$); diminazene ($64.10 \pm 14.48 \text{ nM}/672$); puromycin ($51.97 \pm 14.85 \text{ nM}/8$)

Compound	R	R'	Yield (%) (amidation)	MW (g mol^{-1})	$ClogP^b$	IC_{50}^c (μM) <i>T. b. brucei</i>	IC_{50}^c (μM) HEK293	SI
1	H		>99	362.85	1.70	2.50 ± 0.18^a	<50% activity at $83.3 \mu M$	>48
4	Me		86	390.90	2.73	0.73 ± 0.12	10.4 ± 0.96	14
5	H		13	404.50	3.24	77% activity at $83.3 \mu M$	< 50% activity at $83.3 \mu M$	—
6	Me		58	432.56	4.28	93% at $41.7 \mu M$	< 50% activity at $41.7 \mu M$	—
7	H		47	472.50	4.12	97% activity at $41.7 \mu M$	59% at $83.3 \mu M$	—
8	Me		82	500.55	5.16	<50% activity at $83.3 \mu M$	<50% activity at $83.3 \mu M$	—
9	H		49	434.53	3.16	7.23 ± 1.06	88% at $83.3 \mu M$	>12
10	Me		35	462.58	4.20	2.91 ± 0.04	<50% activity at $83.3 \mu M$	>29
11	H		52	434.53	3.16	9.39 ± 1.54	88% at $83.3 \mu M$	>9
12	Me		44	462.58	4.20	2.21 ± 0.33	<50% activity at $83.3 \mu M$	>38
13	H		56	454.56	4.41	6.38 ± 1.10	4.63 ± 1.69	1
14	Me		47	482.62	5.45	100% activity at $83.3 \mu M$	52% activity at $83.3 \mu M$	—
15^d	H	H	42	328.40	1.81	97% activity at $79.4 \mu M$	<50% activity at $79.4 \mu M$	—
16^d	Me	H	55	356.46	2.85	0.75 ± 0.08	<50% activity at $79.4 \mu M$	>106

^a Average of results from two separate assays ($1.75 \pm 0.48 \mu M$, $3.22 \pm 0.31 \mu M$). ^b Calculated using ChemDraw v. 16.0. ^c Percentage activity at highest concentration has been given where IC_{50} could not be accurately determined. ^d Prepared from benzothiophene-2-carboxylic acid, using EDC·HCl, DMAP and **2** in CH_2Cl_2 at room temperature.

HPLC, the bioactivity of the derivatives with respect to *T. b. brucei* was determined *via* an *in vitro* resazurin viability assay, based on an Alamar Blue assay previously developed by Sykes and Avery.^{11b}

The first component of the scaffold to be investigated was the benzothiophene fragment, which contains a convenient activated heteroaryl chloride moiety that can be subjected to cross-coupling reactions with boronic acids, enabling facile variation of R' for SAR purposes. A number of aryl groups were therefore introduced to **3a** in good yields *via* the nickel-catalysed cross-coupling developed by the Miura group for this specific system.¹⁸ Upon amidation with **2a** or **2b**, the derivatives **4–16** were prepared in varying yields, in the presence or absence of a 5-*gem*-dimethyl substituent. Additionally, amidation of **2** and commercially available benzothiophene-2-carboxylic acid (*via* a coupling reagent) gave the derivatives **15** and **16**, in which the 3-substituent was absent entirely. Screening against *T. b. brucei* revealed that the 5-*gem*-dimethyl substituent improved biological activity (IC₅₀ = 0.73 μM (**4**) *vs.* 2.50 μM (**1**) and similarly **10** *vs.* **9** and **12** *vs.* **11**). Furthermore, despite extensive derivatisation of the 3-chloro handle with aryl groups, bioactivity was maximised when this was absent entirely (IC₅₀ = 0.75 μM (**16**)). It is important to note that variation of the 3-chloro position of **1** appears to control selectivity over human cells, in that a 5-*gem*-dimethyl substituent alone depleted selectivity (SI = 14 (**4**)), while simultaneous 3-substitution replenished this (SI > 29 (**10**), >38 (**12**)) while maintaining bioactivity. This was reflected most prominently in derivative **16**, which contained both a 5-*gem*-dimethyl and 3-hydro substituent and was four times as potent as the lead compound (IC₅₀ = 0.75 μM) and twice as selective (SI > 106). Critically, this derivative exhibited almost identical druglikeness to the lead compound **1**, with slightly higher lipophilicity (ClogP = 2.85) and a similar molecular weight (356.46 g mol⁻¹).

Further derivatisation about the benzothiophene fragment was carried out by treating substituted cinnamic acids with thionyl chloride (Scheme 2), however the type and position of substituents around the benzene ring was limited. For example, the use of *meta*-substituted cinnamic acids gave a regioisomeric mixture of 5- and 7-substituted benzothiophenes (**3e** and **3f**) and, though the major regioisomer could be selectively recrystallised, the yield suffered. The 6-substituted derivatives of **1** (**17–20**) were therefore more straightforward to synthesise (from **3b–d**), however these were mostly detrimental to bioactivity upon screening against *T. b. brucei* (**17–20**, Table 2). These results reinforced the benefit of the 5-*gem*-dimethyl substituent (IC₅₀/SI = 2.27 μM/>37 (**18**) *vs.* 7.77 μM/>11 (**17**) and similarly **20** *vs.* **19**) and subsequent derivatives therefore incorporated this unit. While a 5-methoxy substituent was detrimental to bioactivity (**21**), the introduction of a 7-bromo substituent (**22**) led to an eight-fold increase in bioactivity (IC₅₀ = 0.32 μM) and a three-fold increase in selectivity (SI > 130) with respect to **1**, warranting further investigation of this position. This derivative is more lipophilic than the lead

Scheme 2 Synthetic strategy for variation of the benzothiophene fragment of WEHI-0086109 (**1**). a. SOCl₂, pyridine in chlorobenzene, reflux. b. SOCl₂, DMAP in heptane, reflux. A mixture of the 5-X and 7-X isomers was obtained, from which the major regioisomer was isolated *via* selective recrystallisation in each case. c. **2**, pyridine in toluene, reflux.

compound (ClogP = 3.60) while still maintaining druglikeness under Lipinski guidelines. Removal of the benzene ring from the benzothiophene fragment of **1** depleted bioactivity (**24**), revealing its necessity. Replacing the entire fragment with the structurally similar benzothiazole, however, led to a two-fold increase in bioactivity (IC₅₀ = 1.41 μM (**23**)) while maintaining selectivity (SI > 56). In terms of physiochemical properties, this derivative possesses an additional H-bond acceptor with respect to the lead compound **1**, while maintaining a similar partition coefficient (ClogP = 1.91), rendering it an arguably more suitable drug molecule.

The importance of the thiazole fragment of the thiazolyl-thiophenamide scaffold was similarly investigated, as was that of the amide linker (Table 3). Though *N*-methylation of **1** was successfully carried out, the resulting product was insoluble in most solvents. The *N*-allyl derivative **25** was therefore synthesised *via* the corresponding *N*-allylaminothiazole fragment in good yield and found to be inactive against *T. b. brucei*, indicating that a secondary amide was essential for bioactivity. The ketone substituent of the thiazole fragment was also found to be essential as its removal (**26**) or replacement with other related heterocyclic fragments (**27–28**) depleted bioactivity.

Investigation of the three components of the thiazolyl-thiophenamide scaffold of **1** allowed a preliminary SAR with respect to *T. b. brucei* to be established (Fig. 3). The existing thiazole fragment and secondary amide of **1** were both found to be essential, with the ketone proving to be particularly important. The introduction of a 5-*gem*-dimethyl substituent improved bioactivity, though simultaneous derivatisation of the benzothiophene fragment was required to maintain selectivity. The benzene component of the thiophene fragment proved to be essential, though bioactivity and

Table 2 Bioactivity against *T. b. brucei* 427 (IC₅₀) and selectivity index (SI) over HEK 293 cells of WEHI-0086109 (1) derivatives in which the benzothienophene fragment is varied (see Scheme 2). Assays were performed in triplicate. Controls (IC₅₀/SI): pentamidine (2.13 ± 0.59 nM/307); diminazene (64.10 ± 14.48 nM/672); puromycin (51.97 ± 14.85 nM/8)

Compound	R	Het	Yield (%) (amidation)	MW (g mol ⁻¹)	ClogP ^b	IC ₅₀ ^c (μM) <i>T. b. brucei</i>	IC ₅₀ ^c (μM) HEK293	SI
1	H		>99	362.85	1.70	2.50 ± 0.18 ^a	<50% activity at 83.3 μM	>48
17	H		94	376.87	2.19	7.77 ± 2.01	<50% activity at 83.3 μM	>11
18	Me		94	404.93	3.23	2.27 ± 0.54	<50% activity at 83.3 μM	>37
19	H		58	441.74	2.56	78% activity at 79.4 μM	<50% activity at 79.4 μM	—
20	Me		66	469.80	3.60	6.53 ± 0.38	<50% activity at 79.4 μM	>12
21	Me		41	420.93	2.65	5.86 ± 1.74	<50% activity at 83.3 μM	>14
22	Me		50	469.80	3.60	0.32 ± 0.23	<50% activity at 41.7 μM	>130
23 ^d	Me		62	357.45	1.91	1.41 ± 0.17	<50% activity at 79.4 μM	>56
24 ^e	Me		75	306.40	1.50	90% activity at 79.4 μM	<50% activity at 79.4 μM	—

^a Average of results from two separate assays (1.75 ± 0.48 μM, 3.22 ± 0.31 μM). ^b Calculated using ChemDraw v. 16.0. ^c Percentage activity at highest concentration has been given where IC₅₀ could not be accurately determined. ^d Prepared from benzothiazole-2-carboxylic acid, using EDC-HCl, DMAP and the appropriate amine fragment in CH₂Cl₂ at room temperature. ^e Prepared from 2-thiophenecarbonyl chloride, **2b** and pyridine in toluene at 100 °C.

Table 3 Bioactivity against *T. b. brucei* 427 (IC₅₀) and selectivity index (SI) over HEK 293 cells of WEHI-0086109 (1) derivatives in which the thiazole fragment is varied. Assays were performed in triplicate. Controls (IC₅₀/SI): pentamidine (2.13 ± 0.59 nM/307); diminazene (64.10 ± 14.48 nM/672); puromycin (51.97 ± 14.85 nM/8)

Compound	Structure	Yield (%) (amidation)	MW (g mol ⁻¹)	ClogP ^b	IC ₅₀ ^c (μM) <i>T. b. brucei</i>	IC ₅₀ ^c (μM) HEK293	SI
1		>99	362.85	1.70	2.50 ± 0.18 ^a	<50% activity at 83.3 μM	>48
25 ^d		60	402.91	3.03	<50% activity at 83.3 μM	<50% activity at 83.3 μM	—
26 ^e		64	348.86	3.28	89% activity at 83.3 μM	<50% activity at 83.3 μM	—
27 ^f		30	344.83	3.31	<50% activity at 83.3 μM	<50% activity at 83.3 μM	—
28 ^g		49	294.77	1.71	96% activity at 83.3 μM	<50% activity at 83.3 μM	—

^a Average of results from two separate assays (1.75 ± 0.48 μM, 3.22 ± 0.31 μM). ^b Calculated using ChemDraw v. 16.0. ^c Percentage activity at highest concentration has been given where IC₅₀ could not be accurately determined. ^d Prepared from 2-bromocyclohexane-1,3-dione, *N*-allylthiourea and pyridine in MeOH at reflux, followed by acylation with **3b** and pyridine in acetone at reflux. ^e Prepared from 2-chlorocyclohexanone and thiourea in EtOH at reflux, followed by acylation with **3b** and pyridine in toluene at reflux. ^f Prepared from 2-aminobenzothiazole and **3b** in acetone at reflux. ^g Prepared from 2-aminothiazole, **3b** and pyridine in acetone at reflux.

Fig. 3 Structure–activity relationships of the thiazolyl-benzothiophenamide scaffold of WEHI-0086109 (**1**) with respect to *T. b. brucei* and selectivity over human cells.

selectivity could be improved *via* replacement of the entire fragment with a benzothiazole unit, and maximised in the presence of a 7-bromo substituent and absence of the 3-chloro handle.

Analysis of the SAR allowed for the design of a second generation of potential antitrypanosomal agents, with the base structure possessing a 5-*gem*-dimethyl, 3-hydro and 7-bromo substituent (as in **29**, Scheme 3). Preparation of the proposed derivative **29** required the synthesis of the appropriate 7-bromo thiophene fragment **3g** in which the 3-chloro handle was absent, and this was achieved by the base-catalysed addition of methyl thioglycolate to the appropriate benzaldehyde in excellent yield. The derivative **29** was accessed *via* a coupling reagent in fair yield, however the highly insoluble nature of this compound in most solvents (impeding full characterisation) implied that this derivative was not suitable as a drug molecule. This was addressed by utilising the bromo handle of **3g** in a Suzuki–Miyaura cross-coupling to introduce a 7-phenyl group to give **3i**, which was found to be tolerated in terms of bioactivity and selectivity upon screening against *T. b. brucei* ($IC_{50}/SI = 2.79 \mu M / >28$ (**31**), Table 4). Despite this, the druglikeness of this derivative was less than ideal due to its high lipophilicity ($ClogP = 4.74$). A 7-methoxy derivative (**30**, $ClogP = 2.80$) was therefore

synthesised, with an improvement in yield from the 7-bromo analogue, however this derivative exhibited similar solubility issues and therefore could not be profiled in the *in vitro* *T. b. brucei* and HEK293 assays.

It should be reiterated that the SAR had been established with respect to the non-human infective species, *T. b. brucei*. In order to evaluate how the bioactivity translated to a more clinically relevant, human infective species, a select panel of the most promising derivatives were screened *in vitro* against *T. b. rhodesiense*^{19a} (Table 5). The lead compound **1** was almost four times less active against this strain ($IC_{50} = 8.96 \mu M$) and sixteen times less selective over mammalian cells ($SI = 3$). Though all derivatives exhibited bioactivity in the low micromolar range, this was decreased by up to twenty-two-fold (**22**) with respect to the non-human infective species. The selectivity over skeletal muscle cells (L6) was low to moderate for these compounds ($SI < 10$ for compounds **1**, **22**, **23** and **30**). The most promising derivative was **16**, which was almost twice as bioactive as the lead compound against *T. b. rhodesiense* ($IC_{50} = 5.04 \mu M$) and four times as selective ($SI = 13$), leaving this a suitable candidate for further SAR studies. For the compounds tested in Table 5, the blood brain barrier (BBB) score²⁰ was also calculated and in each case the value was in the 4–6 range needed for an optimal CNS drug. For comparison, the BBB scores for fexinidazole and acoziborole were also calculated and found to be 4.91 and 3.58, respectively.

Conclusions

This work established structure–activity relationships (SAR) of WEHI-0086109 (**1**) with respect to the non-human infective species *T. b. brucei*. The resulting novel thiazolyl-benzothiophenamides can progress in the drug discovery pipeline as a novel scaffold with bioactivity against parasite species causing African sleeping sickness. A number of these compounds exhibited greater bioactivity and selectivity than the lead compound **1**, particularly the 3-hydro, 5-*gem*-dimethyl derivative **16**, which was more bioactive and selective against *T. b. brucei* ($IC_{50}/SI = 0.75 \mu M / >106$) and the human infective species *T. b. rhodesiense* ($IC_{50}/SI = 5.04 \mu M / 13$). While the SI and bioactivity was lower for benzothiazole **23**, further substituted variants of this system warrant further investigation, alongside related heterocyclic systems, including benzoxazoles and benzimidazoles. The structure–activity relationships can now be used to design second generation derivatives, which was initiated within this work, and this can be continued with a focus on substitution at the 7-position of the thiazolyl-benzothiophenamide scaffold. Critically, this work maintained an attention to Lipinski guidelines ($ClogP$, MW, H-bond donors and acceptors) and blood–brain barrier penetration, and continuing this will ensure that future derivatives exhibit a suitable physiochemical profile to maximise their druglikeness. Future work should also take into account current target product profiles formulated by the Drugs for Neglected Diseases Initiative, where the ideal candidate would be

Scheme 3 Synthesis of second generation WEHI-0086109 (**1**) derivatives. a. Methyl thioglycolate and K_2CO_3 in DMF, 70 °C. b. i. NaOH in dioxane/ H_2O , r.t. ii. **2b**, EDC·HCl and DMAP in CH_2Cl_2 , r.t. c. Ph-B(OH)₂, Pd(dppf)Cl₂· CH_2Cl_2 and K_2CO_3 in dioxane/ H_2O , reflux. d. i. NaOH in dioxane/ H_2O , r.t. ii. $SOCl_2$ in dioxane, reflux. iii. **2b** and pyridine in toluene, reflux.

Table 4 Bioactivity against *T. b. brucei* 427 (IC₅₀) and selectivity index (SI) over HEK 293 cells of second generation WEHI-0086109 (1) derivatives. Assays were performed in triplicate. Controls as per Tables 1–3

Compound	MW (g mol ⁻¹)	Clog P ^a	IC ₅₀ ^b (μM) <i>T. b. brucei</i>	IC ₅₀ ^b (μM) HEK293	SI
1	362.85	1.70	2.50 ± 0.18 ^c	< 50% activity at 83.3 μM	>48
30^d	386.48	2.77	—	—	—
31	432.56	4.74	2.79 ± 1.04	<50% activity at 79.4 μM	>28

^a Calculated using ChemDraw v. 16.0. ^b Percentage activity at highest concentration has been given where IC₅₀ could not be accurately determined.^c Average of results from two separate assays (1.75 ± 0.48 μM, 3.22 ± 0.31 μM). ^d Bioactivity not determined due to solubility issues.**Table 5** Bioactivity against *T. b. rhodesiense* STIB 900 (IC₅₀) and selectivity index (SI) over L6 cells of selected WEHI-0086109 (1) derivatives. Assays were performed in triplicate. Controls: melarsoprol, IC₅₀ (*T. b. rhodesiense*) = 14 ± 3 nM; podophyllotoxin, IC₅₀ (L6 cells) = 11 ± 1 nM

Compound	Structure	IC ₅₀ (μM) <i>T. b. brucei</i>	SIHEK 293	IC ₅₀ (μM) <i>T. b. rhodesiense</i>	IC ₅₀ (μM)L6	SIL6	BBB score
1		2.50 ± 0.18 ^a	>48	8.96 ± 3.10	24.95 ± 2.47	3	5.08
16		0.75 ± 0.08	>106	5.04 ± 1.21	65.4 ± 19.10	13	5.06
22		0.32 ± 0.23	>130	7.13 ± 2.91	25.15 ± 2.62	4	4.93
23		1.41 ± 0.17	>56	10.58 ± 3.18	30.40 ± 6.79	3	4.89
30^b		—	—	8.68 ± 1.27	54.45 ± 17.47	6	4.95

^a Average of results from two separate assays (1.75 ± 0.48 μM, 3.22 ± 0.31 μM). ^b Bioactivity against *T. b. brucei* and HEK293 not determined due to solubility issues. BBB score = blood brain barrier score and is calculated using an algorithm reported by Weaver and co-workers.²⁰

effective against both stages of the disease and both target species (*T. b. gambiense* and *T. b. rhodesiense*).²¹ Other critical aspects to consider in this context are the cost of the drug – which should be reasonable considering the simple and cheap starting materials used herein – and long term stability in tropical climates.

Experimental

Selected general and representative procedures are disclosed herein: a comprehensive experimental section and NMR spectra for novel compounds are provided in the ESI†. All reactions were carried out under a nitrogen atmosphere in dry glassware. Reactions were monitored *via* thin-layer chromatography (TLC) analysis using aluminium-backed silica gel sheets coated with fluorescent indicator F254 and visualized *via* UV light (254 nm) and potassium permanganate or anisaldehyde stain. Column chromatography was performed using flash grade silica gel (40–60 nm). NMR spectroscopy was conducted using a 500, 400 or 300 MHz spectrometer. Coupling constants (*J*) are

reported in hertz (Hz) and multiplicities abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets) or m (multiplet) and some denoted app. (apparent) or br. (broad). High resolution mass spectrometry (HRMS) was performed on a triple quadrupole mass spectrometer and infrared data collected on an FTIR instrument. Purity analysis was conducted *via* HPLC under reversed-phase conditions and C18 column, with detection at 254 nm or 280 nm. The precatalyst Ni(dppe)Cl₂ (ref. 19) was prepared as per the literature, as were the thiazole and benzothiophene building blocks (see ESI†). Biological assays were performed within the groups of Prof Vicky Avery (*T. brucei*/HEK293 cells)^{11b} and Dr Marcel Kaiser (*T. b. rhodesiense*/L6 cells)^{19a} *via* their previously reported methods.

General procedure for the Ni(0)-catalysed synthesis of cross-coupled 3-arylbenzothiophenes¹⁶

Typically carried out on a 100 mg scale. Methyl 3-chlorobenzo[*b*]thiophene-2-carboxylate (1.0 equiv.), the appropriate arylboronic acid (1.5 equiv.), Ni(dppe)Cl₂ (5

mol%) and K_3PO_4 (2.0 equiv.) were heated at 120 °C in toluene in a capped reaction vial for 18 hours. If methyl 3-chlorobenzo[*b*]thiophene-2-carboxylate could still be detected *via* TLC analysis (R_f = 0.28, EtOAc/hexane, 5:95) after this time, a further portion of arylboronic acid and $Ni(dppe)Cl_2$ was added and the reaction continued for a further 18 hours to consume starting material and enable purification. The reaction mixture was diluted with CH_2Cl_2 and filtered, before the solvents were removed *in vacuo*. The crude material was subjected to column chromatography (typically EtOAc/hexane, 5:95) to give the pure product. A representative product was methyl 3-(3-methoxyphenyl)benzo[*b*]thiophene-2-carboxylate: white solid, 84%. Mp 126–127 °C. **HRMS-ASAP** (m/z): $[M + H]^+$ calcd for $C_{17}H_{15}O_3S$, 299.0736, found, 299.0737. **FTIR** $\bar{\nu}_{max}$ (cm^{-1}): 2944 (br., vw), 1723 (s, CO), 1226 (vs, OMe), 756 (s), 737 (s). **1H NMR** (500 MHz, $CDCl_3$): δ 7.89 (d, J = 8.1 Hz, 1H, HetCH), 7.58 (d, J = 8.2 Hz, 1H, HetCH), 7.48 (app. t, J = 7.9 Hz, 1H, ArCH or HetCH), 7.41 (app. t, J = 7.9 Hz, 1H, ArCH or HetCH), 7.36 (app. t, J = 7.9 Hz, 1H, ArCH or HetCH), 7.05–6.98 (m, 2H, ArCH), 6.96 (br. s, 1H, ArCH), 3.85 (s, 3H, OMe), 3.80 (s, 3H, OMe). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 162.8 (QC, CO), 159.2 (QC, C-OMe), 143.9 (QC, HetC), 140.3 (QC, HetC), 140.0 (QC, HetC), 135.8 (QC, HetC), 129.0 (CH, ArCH or HetCH), 127.9 (QC, ArC), 127.2 (CH, ArCH or HetCH), 125.3 (CH, ArCH or HetCH), 124.8 (CH, ArCH or HetCH), 122.4 (CH, ArCH or HetCH), 122.1 (CH, ArCH or HetCH), 115.2 (CH, ArCH), 113.6 (CH, ArCH), 55.2 (CH_3 , OMe), 52.2 (CH_3 , OMe).

General procedure for the basic hydrolysis of benzothiophene methyl esters to carboxylic acids

Typically carried out on a 100 mg scale. A solution of the methyl ester in dioxane (typically approximately 0.2 M) was stirred at room temperature with aqueous NaOH (2.0 equiv.) in a capped vial overnight. If the methyl ester could still be detected *via* TLC analysis after this time, a further portion of aqueous NaOH was added and the reaction continued until the starting material could no longer be detected *via* TLC analysis. The reaction was quenched with aqueous HCl (3.0 equiv.), then water. The product was extracted with ethyl acetate and the combined organic extracts washed with brine, before being dried with $MgSO_4$. The solvents were removed *in vacuo* to leave the pure carboxylic acid. A representative product was 3-(3-methoxyphenyl)benzo[*b*]thiophene-2-carboxylic acid: white crystals, 97%. Mp 206–208 °C. **HRMS-ESI** (m/z): $[M + Na]^+$ calcd for $C_{16}H_{12}O_3SNa$, 307.0399; found, 307.0406. **FTIR** $\bar{\nu}_{max}$ (cm^{-1}): 2972 (br., w, OH), 1684 (s, CO), 1242 (s), 1034 (s), 728 (vs). **1H NMR** (500 MHz, $DMSO-d_6$): δ 8.07 (d, J = 8.1 Hz, 1H, HetCH), 7.54 (app. t, J = 7.4 Hz, 1H, ArCH or HetCH), 7.48–7.36 (m, 3H, ArCH and HetCH), 7.06–7.00 (m, 1H, ArCH or HetCH), 6.98–6.91 (m, 2H, ArCH), 3.78 (s, 3H, OMe). **^{13}C NMR** (125 MHz, $DMSO-d_6$): δ 163.2 (QC, CO), 158.8 (QC, C-OMe), 142.0 (QC, HetC), 139.7 (QC, HetC), 139.3 (QC, HetC), 135.6 (CH, ArCH or HetCH), 129.8 (QC, ArC), 129.0 (CH, ArCH or HetCH), 127.2 (QC, HetC), 125.1

(CH, ArCH or HetCH), 124.6 (CH, ArCH or HetCH), 122.8 (CH, ArCH or HetCH), 121.9 (CH, ArCH), 115.3 (CH, ArCH), 113.4 (CH, ArCH), 55.07.

General procedure for the synthesis of WEHI-0086109 (1) derivatives *via* Schotten–Baumann amidation

Typically carried out on a 100 mg scale. Where required, the appropriate acyl chloride was freshly prepared from the corresponding carboxylic acid: to a solution of the carboxylic acid in dioxane (typically approximately 0.04 M) was added thionyl chloride (5.0 equiv.) and the reaction mixture heated at reflux overnight, before the solvents and excess thionyl chloride were removed *in vacuo* to leave the acyl chloride, which was used immediately. A solution of the acyl chloride (1.0 equiv.), the appropriate benzothiazole amine fragment (1.0 equiv.) and pyridine (1.5 equiv.) in toluene (typically approximately 0.05 M with respect to the acyl chloride) was heated at reflux overnight, or until the starting materials could no longer be detected *via* TLC analysis. The solvent was removed *in vacuo* before the residue was washed with aqueous HCl (2.0 M) and extracted with CH_2Cl_2 , before the organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. If required, the crude material was subjected to either column chromatography or recrystallisation to give the pure carboxamide. A representative product was *N*-(5,5-dimethyl-7-oxo-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)-3-(3-methoxyphenyl)benzo[*b*]thiophene-2-carboxamide (**12**): purified *via* column chromatography (EtOAc/hexane, 30:70), off-white solid, 54%. An analytical sample was obtained *via* recrystallisation in CH_2Cl_2 layered with hexane and used for biological testing. Mp 182–184 °C. **HRMS-ESI** (m/z): $[M - H]^-$ calcd for $C_{25}H_{21}N_2O_3S_2$, 461.0999; found, 461.0963. **FTIR** $\bar{\nu}_{max}$ (cm^{-1}): 3316 (w, NH), 1653 (s, CO), 1508 (vs), 1369 (s), 1287 (s), 1225 (s), 1157 (s), 1032 (s), 698 (s). **1H NMR** (400 MHz, $CDCl_3$): δ 9.18 (br. s, 1H, NH), 7.95–7.93 (m, 1H, HetCH), 7.59 (dd, J = 8.5, 7.4 Hz, 1H, ArCH or HetCH), 7.54–7.50 (m, 2H, ArCH or HetCH), 7.41–7.37 (m, 1H, ArCH or HetCH), 7.19 (ddd, J = 8.4, 2.6, 1.0 Hz, 1H, ArCH or HetCH), 7.09 (ddd, J = 7.5, 1.6, 1.0 Hz, 1H, ArCH or HetCH), 7.01 (dd, J = 2.6, 1.6 Hz, 1H, ArCH), 3.88 (s, 3H, OMe), 2.65 (s, 2H, CH_2 -CO), 2.40 (s, 2H, CH_2 -C-N), 1.06 (s, 6H, CH_3). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 192.1 (QC, CO), 162.33 (QC, C-N or C-NH), 162.30 (QC, C-N or C-NH), 160.6 (QC, CO-NH or C-OMe), 159.9 (QC, CO-NH or C-OMe), 140.7 (QC, HetC), 140.1 (QC, HetC), 139.8 (QC, HetC), 134.1 (QC, ArC or HetC), 132.2 (QC, ArC or HetC), 131.2 (CH, ArCH or HetCH), 127.6 (CH, ArCH or HetCH), 125.3 (CH, ArCH or HetCH), 125.2 (CH, ArCH or HetCH), 124.5 (QC, HetC), 122.7 (CH, ArCH or HetCH), 121.7 (CH, ArCH or HetCH), 115.9 (CH, ArCH or HetCH), 115.1 (CH, ArCH or HetCH), 55.5 (CH_3 , OMe), 51.8 (CH_2 , CH_2 -C-N), 40.9 (CH_2 , CH_2 -CO), 34.9 (QC, $C(CH_3)_2$), 28.3 (CH_3 , 2 $\times$ CH_3). **HPLC**: t_R 9.57 min, 97% purity at 254 nm.

General procedure for the synthesis of WEHI-0086109 (1) derivatives *via* EDC coupling

Typically carried out on a 200 mg scale. A suspension of the benzothiophenyl carboxylic acid (1.00 equiv.), the appropriate

thiazole amine fragment (1.05 equiv.) and DMAP (20 mol%) in CH_2Cl_2 (0.05 M with respect to the carboxylic acid) was stirred at room temperature. To this was added EDC-HCl (1.2 equiv.) and the reaction mixture stirred at room temperature under a nitrogen atmosphere. After 24 hours, the reaction mixture was cooled to 0 °C and quenched with aqueous HCl (1.0 M). The precipitated product was collected by filtration and washed with cold water, followed by cold CH_2Cl_2 , and dried under vacuum. A representative product was *N*-(5,5-dimethyl-7-oxo-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)benzo[*b*]thiophene-2-carboxamide (**16**): synthesised using commercially available benzothiophene-2-carboxylic acid. White solid, 55%. Mp 292–293 °C. HRMS-ESI (*m/z*): $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_2\text{S}_2\text{Na}$, 379.0545; found, 379.0551. FTIR $\bar{\nu}_{\text{max}}$ (cm^{-1}): 3237 (w, NH), 1664 (s), 1635 (vs, CO), 1536 (s), 1289 (s), 1207 (s), 879 (m), 719 (s). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 13.51 (br. s, 1H, NH), 8.60 (br. s, 1H, HetCH), 8.09 (d, $J = 7.9$ Hz, 1H, HetCH), 8.02 (d, $J = 7.6$ Hz, 1H, HetCH), 7.56–7.47 (m, 2H, HetCH), 2.83 (s, 2H, $\text{CH}_2\text{-CO}$), 2.44 (s, 2H, $\text{CH}_2\text{-C-N}$), 1.08 (s, 6H, CH_3). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ 191.6 (QC, CO), 163.4 (br, C-N or C-NH), 162.6 (br, C-N or C-NH), 160.8 (br, CO-NH), 141.2 (QC, HetC), 138.9 (QC, HetC), 136.5 (br, QC, HetC), 128.6 (CH, HetCH), 127.3 (CH, HetCH), 126.0 (CH, HetCH), 125.3 (CH, HetCH), 123.0 (CH, HetCH), 122.4 (br, QC, HetC), 51.1 (CH_2 , $\text{CH}_2\text{-C-N}$), 34.7 (QC, $\text{C}(\text{CH}_3)_2$), 27.8 (CH_3 , $2 \times \text{CH}_3$). The signal arising from the $\text{CH}_2\text{-CO}$ carbon is obscured by the residual solvent peak, but was determined via a gHSQC experiment to appear at approximately 40.2 ppm. HPLC: t_{R} 7.48 min, >99% purity at 254 nm.

Assay protocol for *T. b. brucei* and HEK293 screening^{11b}

Compound activity against *T. b. brucei* Strain 427 was assessed in a resazurin-based viability assay, modified from an assay described by Sykes and Avery.^{11b} Briefly, 1200 parasites per mL of logarithmic phase *T. b. brucei* parasites were added to a 384-well microtiter plate (BD biosciences) in 55 μL of HMI-9 media containing 10% FCS and incubated for 24 hours at 37 °C/5% CO_2 . Serial compound concentrations were prepared in DMEM media and 5 μL added to assay plates to give final concentrations ranging from 83.34 to 0.04 μM . Plates were incubated for 48 hours at 37 °C/5% CO_2 . After this time, 10 μL of 0.49 mM resazurin (Sigma Aldrich) prepared in HMI-9 media containing 10% FCS was added to assay plates and incubated for a further 2 hours at 37 °C/5% CO_2 , followed by 22 hours at room temperature. Assay plates were read at 535 nm excitation/590 nm emission on an Envision® multiplate reader (PerkinElmer, Massachusetts, USA). Data was analysed and IC_{50} values calculated using the software GraphPad Prism 5. pentamidine and diminazene were used as controls. For the HEK293 cytotoxicity assay, 55 μL of DMEM media with 10% FCS (Gibco) containing 7.27×10^4 cells per mL of confluent HEK293 cells was added to each well of a 384-well microtiter plate (BD Biosciences) and incubated for 24 hours at 37 °C/5% CO_2 . Serial compound concentrations were prepared in DMEM and 5 μL of this dilution subsequently added to assay plates to give final compound concentrations ranging from 83.34 to 0.04

μM . Plates were incubated for 48 hours at 37 °C/5% CO_2 . After this time, 10 μL of 0.49 mM resazurin (Sigma Aldrich) prepared in DMEM media containing 10% FCS was added to assay plates and incubated for a further 2 hours at 37 °C/5% CO_2 , followed by 22 hours at room temperature. Assay plates were read at 535 nm excitation/590 nm emission on an Envision® multiplate reader (PerkinElmer, Massachusetts, USA). Data was analysed and IC_{50} values calculated using the software GraphPad Prism 5. Puromycin was used as a control.

Assay protocol for *T. b. rhodesiense* and L6 screening^{19a}

The stock of *T. b. rhodesiense* STIB900 was isolated in 1982 from a human patient in Tanzania and, after several mouse passages, cloned and adapted to axenic culture conditions.^{17b} Minimum essential medium (MEM, 50 μL) supplemented with 25 mM HEPES, 1 g L^{-1} additional glucose, 1% MEM non-essential amino acids (100 $\times$), 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 $\mu\text{g mL}^{-1}$ were prepared. Then 4×10 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 μL was added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 70 hours. After this time, 10 μL Alamar Blue (resazurin, 12.5 mg in 100 mL double-distilled water) was then added to each well and incubation continued for a further 2–4 hours.^{19c} The plates were then read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analysed with the graphic program Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA), which calculated IC_{50} values by linear regression^{19d} and 4-parameter logistic regression from the sigmoidal dose inhibition curves. Melarsoprol (Arsobal Sanofi-Aventis, received from WHO) was used as control. The *in vitro* L6 cytotoxicity assays were performed in 96-well microtiter plates, each well containing 100 μL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4000 L6 cells (a primary cell line derived from rat skeletal myoblasts)^{19e,f} Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 $\mu\text{g mL}^{-1}$ were prepared. After 70 hours of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. Alamar Blue (10 μL) was then added to each well and the plates incubated for another 2 hours. The plates were then read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values were calculated by linear regression^{19d} and 4-parameter logistic regression from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA). Podophyllotoxin (Sigma P4405) was used as control.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research was supported by Research Training Program scholarships awarded to Ronald W. Brown and Ashraf M. Abdel-Megeed from the University of Wollongong. The authors would like to thank Elouise Gaylard for assistance with the *T. b. brucei* and HEK293 assays.

References

- 1 WHO Fact Sheet: Trypanosomiasis, human African (sleeping sickness), [https://www.who.int/en/news-room/fact-sheets/detail/trypanosomiasis-human-african-\(sleeping-sickness\)](https://www.who.int/en/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness)) (accessed April 7, 2020).
- 2 For relevant reviews on African Sleeping Sickness and drug discovery in this area, see: (a) R. Brun, J. Blum, F. Chappuis and C. Burri, *Lancet*, 2010, **375**, 148; (b) D. Steverding, *Parasites Vectors*, 2008, **1**, 3; (c) D. Steverding, *Parasites Vectors*, 2010, **3**, 15; (d) J. R. Franco, P. P. Simarro, A. Diarra and J. G. Jannin, *Clin. Epidemiol.*, 2014, **6**, 257; (e) M. C. Field, D. Horn, A. H. Fairlamb, M. A. J. Ferguson, D. W. Gray, K. D. Read, M. De Rycker, L. S. Torrie, P. G. Wyatt, S. Wyllie and I. H. Gilbert, *Nat. Rev. Microbiol.*, 2017, **15**, 217–231; (f) M. De Rycker, B. Baragaña, S. L. Duce and I. H. Gilbert, *Nature*, 2018, **559**, 498.
- 3 P. Babokhov, A. O. Sanyaolu, W. A. Oyibo, A. F. Fagbenro-Beyioku and N. C. Iriemenam, *Pathog. Global Health*, 2013, **107**, 242.
- 4 G. Priotto, S. Kasparian, W. Mutombo, D. Ngouama, S. Ghorashian, U. Arnold, S. Ghabri, E. Baudin, V. Buard, S. Kazadi-Kyanza, M. Ilunga, W. Mutangala, G. Pohlig, C. Schmid, U. Karunakara, E. Torreele and V. Kande, *Lancet*, 2009, **374**, 56.
- 5 (a) N. Baker, H. P. de Koning, P. Mäser and D. Horn, *Trends Parasitol.*, 2013, **29**, 110; (b) A. H. Fairlamb and D. Horn, *Trends Parasitol.*, 2018, **34**, 481.
- 6 R. W. Brown and C. J. T. Hyland, *Med. Chem. Commun.*, 2015, **6**, 1230.
- 7 (a) F. W. Jennings and G. M. Urquhart, *Z. Parasitenkd.*, 1983, **69**, 577; (b) W. Raether and H. Seidenath, *Ann. Trop. Med. Parasitol.*, 1983, **77**, 13.
- 8 For the preclinical studies of fexinidazole, see: (a) E. Torreele, B. Bourdin Trunz, D. Tweats, M. Kaiser, R. Brun, G. Mazué, M. A. Bray and B. Pécoul, *PLoS Neglected Trop. Dis.*, 2010, **4**, e923; for a succinct review on the importance of fexinidazole, see: (b) M. P. Pollastri, *Trends Parasitol.*, 2018, **34**, 178; for a review on fexinidazole and other drugs in preclinical development for the treatment of African sleeping sickness, see: (c) M. Berninger, I. Schmidt, A. Ponte-Sucre and U. Holzgrabe, *Med. Chem. Commun.*, 2017, **8**, 1872.
- 9 R. T. Jacobs, B. Nare, S. A. Wring, M. D. Orr, D. Chen, J. M. Sligar, M. X. Jenks, R. A. Noe, T. S. Bowling, L. T. Mercer, C. Rewerts, E. Gaukel, J. Owens, R. Parham, R. Randolph, B. Beaudet, C. J. Bacchi, N. Yarlett, J. J. Plattner, Y. Freund, C. Ding, T. Akama, Y. K. Zhang, R. Brun, M. Kaiser, I. Scandale and R. Don, *PLoS Neglected Trop. Dis.*, 2011, **5**, e1151.
- 10 Acoziborole, <https://www.dndi.org/diseases-projects/portfolio/acoziborole/> (accessed April 7, 2020).
- 11 (a) M. L. Sykes, J. B. Baell, M. Kaiser, E. Chatelain, S. R. Moawad, D. Ganame, J.-R. Ioset and V. M. Avery, *PLoS Neglected Trop. Dis.*, 2012, **6**, e1896; (b) M. L. Sykes and V. M. Avery, *Am. J. Trop. Med. Hyg.*, 2009, **81**, 665.
- 12 For examples of SAR investigations of other hit compounds arising from the same HTS, see: (a) S. Russell, R. Rahmani, A. J. Jones, H. L. Newson, K. Neilde, I. Cotillo, M. Rahmani Khajouei, L. Ferrins, S. Qureishi, N. Nguyen, M. S. Martinez-Martinez, D. S. Weaver, M. Kaiser, J. Riley, J. Thomas, M. De Rycker, K. D. Read, G. R. Flematti, E. Ryan, S. Tanghe, A. Rodriguez, S. A. Charman, A. Kessler, V. M. Avery, J. B. Baell and M. Piggott, *J. Med. Chem.*, 2016, **59**, 9686; (b) A. Manos-Turvey, E. E. Watson, M. L. Sykes, A. J. Jones, J. B. Baell, M. Kaiser, V. M. Avery and R. J. Payne, *Med. Chem. Commun.*, 2015, **6**, 403; (c) R. Rahmani, K. Ban, A. J. Jones, L. Ferrins, D. Ganame, M. L. Sykes, V. M. Avery, K. L. White, E. Ryan, M. Kaiser, S. A. Charman and J. B. Baell, *J. Med. Chem.*, 2015, **58**, 6753; (d) A. A. Rashad, A. J. Jones, V. M. Avery, J. Baell and P. A. Keller, *ACS Med. Chem. Lett.*, 2014, **5**, 496; (e) L. Ferrins, M. Gazdik, R. Rahmani, S. Varghese, M. L. Sykes, A. J. Jones, V. M. Avery, K. L. White, E. Ryan, S. A. Charman, M. Kaiser, C. A. S. Bergström and J. B. Baell, *J. Med. Chem.*, 2014, **57**, 6393; (f) L. Ferrins, R. Rahmani, M. L. Sykes, A. J. Jones, V. M. Avery, E. Teston, B. Almohaywi, J. Yin, J. Smith, C. Hyland, K. L. White, E. Ryan, M. Campbell, S. A. Charman, M. Kaiser and J. B. Baell, *Eur. J. Med. Chem.*, 2013, **66**, 450.
- 13 W. C. Van Voorhis, J. H. Adams, R. Adelfio, V. Ah Yong, M. H. Akabas, P. Alano, A. Alday, Y. A. Resto, A. Alsibae, A. Alzualde, K. T. Andrews, S. V. Avery, V. M. Avery, L. Ayong, M. Baker, S. Baker, C. B. Mamoun, S. Bhatia, Q. Bickle, L. Bounaadja, T. Bowling, J. Bosch, L. E. Boucher, F. F. Boyom, J. Brea, M. Brennan, A. Burton, C. R. Caffrey, G. Camarda, M. Carrasquilla, D. Carter, M. B. Cassera, K. C.-C. Cheng, W. Chindaoudomsate, A. Chubb, B. L. Colon, D. D. Colón-López, Y. Corbett, G. J. Crowther, N. Cowan, S. D'Alessandro, N. Le Dang, M. Delves, J. L. DeRisi, A. Y. Du, S. Duffy, S. A. E.-S. El-Sayed, M. T. Ferdig, J. A. F. Robledo, D. A. Fidock, I. Florent, P. V. T. Fokou, A. Galstian, F. J. Gamo, S. Gokool, B. Gold, T. Golub, G. M. Goldgof, R. Guha, W. A. Guiguemde, N. Gural, R. K. Guy, M. A. E. Hansen, K. K. Hanson, A. Hemphill, R. H. van Huijsduijnen, T. Horii, P. Horrocks, T. B. Hughes, C. Huston, I. Igarashi, K. Ingram-Sieber, M. A. Itoe, A. Jadhav, A. N. Jensen, L. T. Jensen, R. H. Y. Jiang, A. Kaiser, J. Keiser, T. Ketas, S. Kicka, S. Kim, K. Kirk, V. P. Kumar, D. E. Kyle, M. J. Lafuente, S. Landfear, N. Lee, S. Lee, A. M. Lehan, F. Li, D. Little, L. Liu, M. Llinás, M. I. Loza, A. Lubar, L. Lucantoni, I. Lucet, L. Maes, D. Mancama, N. R. Mansour, S. March, S. McGowan, I. M. Vera, S. Meister, L. Mercer, J. Mestres, A. N. Mfopa, R. N. Misra, S. Moon, J. P. Moore, F. M. R. da Costa, J. Müller, A. Muriana, S. N. Hewitt, B. Nare, C. Nathan, N. Narraidoo, S. Nawaratna, K. K. Ojo,

- D. Ortiz, G. Panic, G. Papadatos, S. Parapini, K. Patra, N. Pham, S. Prats, D. M. Plouffe, S.-A. Poulsen, A. Pradhan, C. Quevedo, R. J. Quinn, C. A. Rice, M. A. Rizk, A. Ruecker, R. St. Onge, R. S. Ferreira, J. Samra, N. G. Robinett, U. Schlecht, M. Schmitt, F. S. Villela, F. Silvestrini, R. Sinden, D. A. Smith, T. Soldati, A. Spitzmüller, S. M. Stamm, D. J. Sullivan, W. Sullivan, S. Suresh, B. M. Suzuki, Y. Suzuki, S. J. Swamidass, D. Taramelli, L. R. Y. Tchokouaha, A. Theron, D. Thomas, K. F. Tonissen, S. Townson, A. K. Tripathi, V. Trofimov, K. O. Udenze, I. Ullah, C. Vallieres, E. Vigil, J. M. Vinetz, P. V. Vinh, H. Vu, N.-A. Watanabe, K. Weatherby, P. M. White, A. F. Wilks, E. A. Winzeler, E. Wojcik, M. Wree, W. Wu, N. Yokoyama, P. H. A. Zollo, N. Abla, B. Blasco, J. Burrows, B. Laleu, D. Leroy, T. Spangenberg, T. Wells and P. A. Willis, *PLoS Pathog.*, 2016, **12**, e1005763.
- 14 Benzo[b]thiophene-2-carboxamides have been shown to have anti-malarial activity: (a) M. Pieroni, E. Azzali, N. Basilico, S. Parapini, M. Zolkiewski, C. Beato, G. Annunziato, A. Bruno, F. Vacondio and G. Costantino, *J. Med. Chem.*, 2017, **60**, 1959.
- 15 C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Delivery Rev.*, 1997, **23**, 3.
- 16 T. T. Talele, *J. Med. Chem.*, 2018, **61**, 2166.
- 17 The low yields obtained for benzothiophenes 3 (7–46%, Scheme 2) reflect those observed in the literature. (a) P. Čapek, Y. Zhang, D. J. Barlow, K. L. Houseknecht, G. R. Smith and T. J. Dickerson, *ACS Chem. Neurosci.*, 2011, **2**, 288; (b) M. Sarker, T. Shahrin and M. G. Steinmetz, *Org. Lett.*, 2011, **13**, 872.
- 18 M. Miyasaka, K. Hirano, T. Satoh and M. Miura, *Adv. Synth. Catal.*, 2009, **351**, 2683.
- 19 T. b. rhodesiense/L6 assays were carried out by Dr Marcel Kaiser via the previously reported method. (a) I. Orhan, B. Sener, M. Kaiser, R. Brun and D. Tasdemir, *Mar. Drugs*, 2010, **8**, 47; (b) T. Baltz, D. Baltz, C. Giroud and J. Crockett, *EMBO J.*, 1985, **4**, 1273; (c) B. Rätz, M. Iten, Y. Grether-Bühler, R. Kaminsky and R. Brun, *Acta Trop.*, 1997, **68**, 139; (d) J. Huber and J. C. Koella, *Acta Trop.*, 1993, **55**, 257; (e) C. Page, M. Page and C. Noel, *Int. J. Oncol.*, 1993, **3**, 473; (f) S. A. Ahmed, R. M. Gogal and J. E. Walsh, *J. Immunol. Methods*, 1994, **170**, 211.
- 20 M. Gupta, H. J. Lee, C. J. Barden and D. F. Weaver, *J. Med. Chem.*, 2019, **62**, 9824.
- 21 *The Drugs for Neglected Diseases initiative: target product profile for sleeping sickness*, <https://dndi.org/diseases/sleeping-sickness/target-product-profile/> (accessed September 2, 2020).