Towards more drug like inhibitors of trypanosome alternative oxidase

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New tools are required to ensure the adequate control of the neglected tropical disease human African trypanosomiasis. Annual reports of infection have recently fallen to fewer than 5000 cases per year; however, current therapies are hard to administer and have safety concerns, hence are far from ideal. Trypanosome alternative oxidase is an exciting target for controlling the infection; it is unique to the parasite and inhibition of this enzyme with the natural product ascofuranone has shown to clear in vivo infections. We report the synthesis and associated structure activity relationships of inhibitors based upon this natural product with correlation to T. b. brucei growth inhibition in an attempt to generate molecules that possess improved physicochemical properties and potential for use as new treatments for human African trypanosomiasis.

Keywords: Medicinal chemistry, drug design, multiparameter optimisation, human African trypanosomiasis.

Human African trypanosomiasis (HAT), otherwise known as African sleeping sickness, is a neglected tropical disease caused by human infection by the protozoan parasites T. b. gambiense or T. b. rhodesiense, transmitted by tsetse flies in sub-Saharan Africa. Without treatment, HAT is invariably fatal; the drugs that are currently approved for treatment of both the haemolymphatic (stage 1) and encephalitic (stage 2) phases of the disease have either toxic side effects or require complex administration procedures. Resistant strains of the parasites have been observed in the clinic and there is a requirement for new treatment options to guarantee the successful control of the disease. In 2009 it was estimated that occurrences of the disease had fallen below 20 000 incidences a year and that 65 million people are at risk of transmission.

The trypanosome, first discovered by Sir David Bruce in 1894, undergoes diverse lifecycle adaptations conferring viability in both the tsetse fly vector and mammalian host. A predominant feature of Trypanosoma brucei is that it is capable of successfully evading host immune responses, predominantly due to its capacity to diversify the surface antigens that it presents to its host; this ability has been a major factor for preventing the development of an efficacious vaccine against the pathogen. T. b. gambiense is responsible for >95% of cases of HAT and is a zoonotic disease, residing in a variety of domestic animals and livestock. Trypanosome infection in cattle and other livestock also has a large burden on the health and associated economic output of animals; the cost of delivering trypanocidal agents to control ‘Nagana’ or African animal trypanosomiasis (AAT) is estimated to be 140 million USD per year.

Current treatments for HAT are far from ideal. Stage 1 of the disease is treated with suramin for T. b. rhodesiense or pentamidine for T. b. gambiense, both compounds display undesired toxic side effects in the clinic. Resistance against pentamidine has also been observed and linked to mutations in the P2 amino purine uptake transporters. Both suramin and pentamidine are unable to permeate into the CNS to sufficient levels, and thus are ineffective against stage 2 infections. The organoarsenide melarsoprol and the more recent combination therapy of nifurtimox and eflornithine (NECT) are currently used to treat infections of T. b. gambiense and T. b. rhodesiense respectively. Both treatments have their limitations. Melarsoprol shows significant toxic effects in those treated, causing encephalopathy in 5-10% of people treated with the drug. Incidences of resistance to melarsoprol are becoming more commonplace, again linked to mutations in the P2 amino purine uptake transporters; associated failure rates in the clinic have been reported to be between 20%-30% in the early 2000’s. NECT requires the administration of eflornithine via slow intravenous infusion of 200 mg/kg every 12 hours for 7 days as it is only trypanostatic, thus requiring trypanocidal action by the co-administered nifurtimox and innate host clearance mechanisms.

With the limitations of the currently approved medicines for treating HAT and the increasing reports of resistance to these therapies, it is vital to discover new and improved treatments against the pathogen. An ideal target for drug discovery for HAT is the trypanosome alternative oxidase (TAO). TAO is the sole terminal oxidase enzyme in the
aerobic respiratory pathway for the long slender blood stage form of *Trypanosoma brucei* subspecies. The enzyme utilises oxygen and ubiquinol as substrates for the efficient generation of cellular ATP as the parasite does not express regular cytochrome respiratory complexes. TAO is a di-iron (non-heme) oxidoreductase that is located in the inner-mitochondrial membrane of the trypanosome. As TAO is not found in mammalian systems, there is a unique opportunity to attain compounds with enhanced selectivity and circumvent on-target toxicity. It has recently been reported that the pentamidine and melarsoprol drug resistant parasites have an increased sensitivity to inhibition of TAO, as these parasites have a reduced capacity to efflux cellular glycerol that is produced as the result of anaerobic respiration. The crystal structure of TAO has been reported, allowing a structure based design approach to new molecules. Nanomolar inhibition of TAO has been reported with the natural product ascofuranone (AF) and its analogues in validated biochemical assays, demonstrating correlation in *ex vivo* growth inhibition and efficacy in *in vivo* clearance models.

**Results and Discussion**

The biochemical assessment of the bicyclic 1,2-isoxazole intermediate prepared to access the aldehyde replacement with the nitrile analogue of CCB (2), had shown unexpectedly high potency for inhibiting TAO. Previous reports had postulated that a hydrogen bond donor was needed at this position for potent inhibition of TAO.

![Figure 1. Summary of previous work](image)

Our previous work identified robust synthetic routes to close analogues of AF (1) and structure activity relationships of the synthesized molecules, highlighting the requirement of both the chloro and methyl substituents on the aromatic ring for high potency (figure 1). With these routes in place we set out to further explore structurally similar analogues with the aim to reduce lipophilicity, and improve the drug like properties of these molecules. Our aim of reducing lipophilicity was to specifically improve aqueous solubility, decrease metabolism and improve the potential to achieve efficacious CNS concentrations, as new compounds for HAT must be effective against both stages of the disease.

There are many hurdles preventing permeability of organic drug molecules to the brain. The blood brain barrier provides significant protection to xenobiotics; tight junctions around capillaries prevent permeation and the expression of ABC-transporters actively transport solubilised organic molecules out and away from the barrier and the brain. Compounds that traverse readily into the CNS tend to have high passive permeability, have low efflux from ABC-transporters like P-glyco-protein (P-gp) and have low metabolic turnover in human liver microsomes, providing the basis for the generation of a predictive scoring tool for CNS penetration.

![Figure 2. Per atom contribution to lipophilicity of ascofuranone (AF) (1) and colletochlorin B (CCB) (2)](image)

A major contribution to the poor multi-parameter optimisation (MPO) predicted CNS-penetration score for AF (1) and colletochlorin B (CCB (2)) is attributed to its high lipophilicity. Clearly the major contributor to the high lipophilicity of CCB (2) is from the geranyl ‘lipophilic tail’ (figure 2). Compounds with a cLogP above 5 are commonly observed to be P-gp substrates, and tend to have a high metabolic liability. We herein report the synthesis and SAR investigation of analogues focused on reducing the lipophilicity of the tail region of structural analogues of AF (1) and CCB (2), with an aim to improve physicochemical properties and increase the predicted CNS penetration MPO score.

![Figure 3. Isoxazole Kemp elimination](image)
Scheme 1. Mono-phenol analogue synthesis

Reaction conditions: a) trimethylphenylammonium tribromide, CH₂Cl₂ / MeOH rt, 3 h, 77%, b) NH₂OH·HCl, EtOH, reflux, 16 h, 90%, c) NCS, Et₃N, MeCN, 0°C – rt, 16 h, 70%, d) dimethyl sulfate, acetone, rt, 16 h, 98%, e) i) BH₃·THF, 1-octene, 0°C, 20 min, ii) toluene, H₂O, K₃PO₄, RuPhos, Pd(OAc)₂, 50°C, 16 h, 90% f) TMS-Cl, NaI, MeCN, 80°C, 16 h, 65%.

Selective bromination of the phenolic aldehyde was achieved by treatment with trimethylbenzylammonium tribromide at 0°C; non-selective and over bromination was observed using N-bromosuccinimide. The aldehyde (6) could be successfully converted to the nitrile (7) by oxime formation with hydroxylamine hydrochloride and subsequent in situ dehydration. Protection of the phenol (8) to the anisole (9) with dimethylsulfate was required to facilitate a high yielding Suzuki-Miyaura cross-coupling using tri-n-octylborane as the coupling partner that was formed in situ by reacting 1-octene with a solution of tM borane in THF. Final deprotection of the anisol (10) to the free phenol using trimethylsilyl chloride and sodium iodide provided the monophenol analogue (11) for biochemical and ex vivo assessment (table 1).

Table 1. Biological assay assessment of isoxazole and mono-phenol analogues

<table>
<thead>
<tr>
<th>#</th>
<th>Structure</th>
<th>TAO pIC₅₀ †</th>
<th>T. b. b. pEC₅₀ *</th>
<th>HepG2 pCC₅₀ #</th>
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<tr>
<td>2</td>
<td><img src="image1" alt="Structure" /></td>
<td>8.5 ± 0.3</td>
<td>8.4 ± 0.1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td><img src="image2" alt="Structure" /></td>
<td>8.4 ± 0.2</td>
<td>6.4 ± 0.1</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Structure" /></td>
<td>8.3 ± 0.4</td>
<td>7.0 ± 0.2</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>11</td>
<td><img src="image4" alt="Structure" /></td>
<td>8.9 ± 0.3</td>
<td>7.6 ± 0.1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>46</td>
<td><img src="image5" alt="Structure" /></td>
<td>7.0 ± 0.1</td>
<td>5.1 ± 0.2</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>10</td>
<td><img src="image6" alt="Structure" /></td>
<td>4.5 ± 0.1</td>
<td>&lt; 4.3</td>
<td>&lt; 4.3</td>
</tr>
</tbody>
</table>

†: Negative log concentration and standard deviation of compounds required for 50% inhibition of trypanosome alternative oxidase; *: Negative log concentration and standard deviation of compounds required for 50% growth inhibition of T. b. brucei Lister427; #: Negative log concentration and standard deviation of compounds required for 50% cytotoxicity of HepG2 cell line. (n ≥ 2 in all assays)

Interestingly, the monophenol analogue (11) retained high potency against TAO that corresponded to the most potent inhibition of T. b. brucei growth for a compound not containing the benzaldehyde functionality present in AF and...
CCB. The removal of one of the flanking substituents ortho to the brominated coupling position facilitated its reactivity and thus exploration of this tail. This synthetic route was adopted for further diversification of this tail region. The des-methyl starting material was accessible from commercially available sources and was used to initially investigate tail modifications.

The introduction of polarity into the lipophilic tail was devised to reduce cLogP and improve upon the predicted CNS penetration MPO score (>4 required for high predicted CNS exposure). The introduction of amide functionality in alternate positions in the chain were planned to mimic the n-octyl (C₈H₁₇) or geranyl chain. The inclusion of the amide functionality had the largest predicted improvement to both cLogP and MPO score (Row C – table 2). 1-Alkene esters were converted to trialkylboranes using the methodology previously employed to access the n-octyl analogues (scheme 2). These were then coupled with the brominated intermediate (14). The esters were converted to the desired amides by either in situ hydrolysis under the demethylation conditions, with subsequent HATU amide coupling with the required alkylamine, or a direct aminolysis of the ester by using 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) and the desired alkylamine with subsequent anisole deprotection with TMSCl / NaI to provide the amide chain compounds for biological assessment (table 3).

Table 2. cLogP and predicted MPO scores for planned analogues.

<table>
<thead>
<tr>
<th>Row</th>
<th>Structure</th>
<th>cLogP</th>
<th>MPO Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>(4)</td>
<td>6.2</td>
<td>3.5</td>
</tr>
<tr>
<td>B</td>
<td>(11)</td>
<td>6.3</td>
<td>3.8</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>3.5</td>
<td>5.5</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>4.6</td>
<td>4.8</td>
</tr>
<tr>
<td>E</td>
<td>(43)</td>
<td>4.9</td>
<td>4.2</td>
</tr>
</tbody>
</table>

cLogP calculated from ChemAxon Marvin Sketch, calculated MPO as described by Wager et al.²⁶

Scheme 2. Amide tail linker synthesis

Reaction conditions: a) NBS, MeCN, rt, 16 h, 85%, b) dimethyl sulfate, acetone, reflux, 3 h, 90%, c) i) BH₃·THF, 1-alkene ester, 0 °C, 20 min, ii) toluene, H₂O, K₃PO₄, RuPhos, Pd(OAc)₂, 16 h, 50°C, 90%, d) TMSCl, NaI, MeCN, 80 °C, 16 h, 35-85%. e) HATU, Et₃N, R¹R²NH, MeCN, rt, 3 h, 60-90%, f) TBD, R⁵R⁶NH, MeCN, rt, 1 – 16 h 70-85%.

The acrylic ester could not be successfully coupled under these conditions; we postulate that the required trialkylborane may not form as the alkene is too electron deficient. The desired ester was synthesized by a Heck-Mizoroki cross-coupling and subsequent reduction of the double-bond, before chlorination and amide formation with TBD (scheme 3).

Scheme 3. Heck cross-coupled amide tail linker synthesis

Reaction conditions: a) ethyl acrylate, SingaCycle™, K₂CO₃, NMP, 100 °C, 16 h, 30%, b) triethylsilane, Pd/C, EtOH, rt, 16 h, 78%, c) SO₂Cl₂, Et₂O, rt, 96 h, 57%, d) TBD, n-butylamine, MeCN, 16h, 39%.

A selection of ether chain analogues were also planned and synthesised (D – table 2), these were expected to be more chemically similar to that of the aliphatic chain with a smaller amount of polarity introduced in comparison to the amide. These compounds were less chemically tractable; in our hands the anisole de-protection to the phenol proved challenging. The deprotection conditions that we explored led to the cleavage of the desired ether functionality present in the chain to the terminal alcohol. To mitigate this synthetic hurdle, alternative protecting groups were investigated. Bulkier silyl protecting groups hindered the cross-coupling and failed to provide the desired ether tail analogues. Reaction with the phenolic ester (27) provided some cross-coupled product, the stability of this protecting group perhaps unsurprisingly, proved to be labile under the reaction coupling conditions. The de-acetylated products (29 to 31) were observed and isolated from the reactions however significant proportions of the starting material ester (28) saponified before cross-coupling providing
a major by-product of the deacetylated phenol (14) thus resulting in lower isolated yields (scheme 4).

**Scheme 4. Ether tail linker synthesis**

Reaction conditions: a) Ac₂O, pyridine, DCM, rt, 16 h, 74%, b) i) BH₃·THF, 1-alkene ether, 0°C, 20 min, ii) toluene, H₂O, K₃PO₄, RuPhos, Pd(OAc)₂, 16 h, 50 °C, 7-24%.

**Table 3. Biological assessment of polar tail analogues**

<table>
<thead>
<tr>
<th>#</th>
<th>R³</th>
<th>cLogP</th>
<th>TAO pIC₅₀</th>
<th>T. b. b. pEC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>CH₃</td>
<td>6.3</td>
<td>8.9 ± 0.3</td>
<td>7.6 ± 0.1</td>
</tr>
<tr>
<td>46</td>
<td>H</td>
<td>5.8</td>
<td>7.0 ± 0.1</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>27</td>
<td>CH₃</td>
<td>3.5</td>
<td>5.5 ± 0.2</td>
<td>&lt;4.3</td>
</tr>
<tr>
<td>19</td>
<td>H</td>
<td>3.0</td>
<td>&lt;4.5</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>H</td>
<td>2.9</td>
<td>4.6 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>H</td>
<td>3.0</td>
<td>4.6 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>H</td>
<td>3.4</td>
<td>5.1 ± 0.1</td>
<td>&lt;4.3</td>
</tr>
<tr>
<td>23</td>
<td>H</td>
<td>3.6</td>
<td>5.4 ± 0.2</td>
<td>&lt;4.3</td>
</tr>
<tr>
<td>29</td>
<td>H</td>
<td>4.1</td>
<td>4.8 ± 0.1</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>30</td>
<td>H</td>
<td>4.1</td>
<td>5.6 ± 0.1</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>31</td>
<td>H</td>
<td>4.1</td>
<td>5.2 ± 0.1</td>
<td>&lt;4.3</td>
</tr>
</tbody>
</table>

*¹: Negative log concentration and standard deviation of compounds required for 50% inhibition of trypanosome alternative oxidase; *#: Negative log concentration and standard deviation of compounds required for 50% growth inhibition of *T. b. brucei* Lister 427. (n ≥ 2 in all assays performed).

Assessment of these less lipophilic analogues showed a large decrease in potency compared to the *n*-octyl analogues, highlighting that even minor increases in polarity in this part of the lipophilic tail was not tolerated. The most promising analogues included polarity at the terminal of the chain (22 and 23); however, disappointingly, these compounds resulted in no measurable inhibition of *T. b. brucei* growth (table 3).

This data suggested that a lipophilic tail was required for the potent inhibition of TAO. To further probe this observation; analogues were prepared to assess the length of tail needed for potent inhibition of TAO and whether total lipophilicity could be decreased by reduction of the carbon chain length (E - table 2). Previous reports had shown large decreases in potency against TAO at carbon chain length below 3. The C₁-7 analogues (39 to 45) were prepared in a similar fashion to that of the *n*-octyl analogue (11); C₁-4 analogues were however prepared directly using the alkyl boronic acid or trimeric alkyl boroxines as the volatile alkene hydrocarbons were not accessible (scheme 5).

**Scheme 5. Carbon chain length analogue synthesis**
Reaction conditions: a) i) BH$_3$·THF, 1-alkene, 0 °C, 20 min, ii) toluene, H$_2$O, K$_3$PO$_4$, RuPhos, Pd(OAc)$_2$, 16 h, 50 °C, 65-90%, or 1-boronic acid/boroxine, K$_3$PO$_4$, RuPhos, Pd(OAc)$_2$, 16 h, 80 °C b) TMSCl, NaI, MeCN, 80 °C, 16 h, 35-85%.

Table 4. Biological assessment of carbon chain length analogues

<table>
<thead>
<tr>
<th>#</th>
<th>R$^3$</th>
<th>cLogP</th>
<th>TAO pIC$_{50}$$^\dagger$</th>
<th>T. b. b. pEC$_{50}$$^*$</th>
</tr>
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<tbody>
<tr>
<td>11</td>
<td>n-C$<em>8$H$</em>{17}$</td>
<td>6.3</td>
<td>8.9 ± 0.3</td>
<td>7.6 ± 0.1</td>
</tr>
<tr>
<td>45</td>
<td>n-C$<em>7$H$</em>{15}$</td>
<td>5.8</td>
<td>8.5 ± 0.1</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td>44</td>
<td>n-C$<em>6$H$</em>{13}$</td>
<td>5.4</td>
<td>8.2 ± 0.1</td>
<td>6.1 ± 0.1</td>
</tr>
<tr>
<td>43</td>
<td>n-C$<em>5$H$</em>{11}$</td>
<td>4.9</td>
<td>7.8 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>42</td>
<td>n-C$_4$H$_9$</td>
<td>4.5</td>
<td>7.7 ± 0.1</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td>41</td>
<td>n-C$_3$H$_7$</td>
<td>4.1</td>
<td>7.1 ± 0.1</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>39</td>
<td>n-CH$_3$</td>
<td>3.2</td>
<td>6.2 ± 0.1</td>
<td>4.7 ± 0.1</td>
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</tbody>
</table>

$^\dagger$: Negative log concentration and standard deviation of compounds required for 50% inhibition of trypanosome alternative oxidase; $^*$: Negative log concentration and standard deviation of compounds required for 50% growth inhibition of *T. b. brucei* Lister 427. ($n \geq 2$ for all assays performed).

Interestingly, the reduced carbon chain length analogues showed good potency against TAO (table 4), until a notable decrease in enzymatic inhibition was observed at compounds with C3 or below, corroborating previous reports. What was more evident was the decrease observed in efficacy against *T. b. brucei* growth inhibition as chain length and cLogP decreased. Compounds showed a distinct drop off in potency against *T. b. brucei* for each carbon reduction. To interrogate this data further correlations of cLogP and biochemical and cellular potency were carried out (figure 4).
Biochemical potency for these compounds track to a lipophilic ligand efficiency of 3. Cellular potency, however correlates to a lipophilic ligand efficiency of 1, relating to a 100-fold decrease in potency from the biochemical activity to the cellular assay. The 2 log decrease in cellular potency observed here could be due a combination of reasons that we are currently investigating, it is of interest that CCB (2) shows a smaller decrease in potency from the biochemical to cellular assay. The permeability of the compounds though the cell and mitochondrial membranes of the trypanosome may reduce as the lipophilicity decreases. The compounds could be substrates for efflux transport, that may reduce the intracellular concentration. The TAO inhibition kinetics of the compounds may be altered with the reduction in carbon chain length that could result in faster dissociation, resulting in reduced efficacy on target. The compounds may not locate to the desired cell compartment as lipophilicity may be required for the inhibitors to be retained in the inner mitochondrial membrane where the enzyme is located. Colletochlorin B could be acting on an unknown complimentary anti-parasitic target. Ongoing studies to better understand these data will aid future analogue design and synthesis to identify more drug like inhibitors of TAO.

We also noticed positive correlation between cLogP and both biochemical and cellular activity (figure 4). Examples of potency correlating to high lipophilicity have previously been observed with other membrane proteins and enzymes requiring lipophilic substrates.\textsuperscript{33-34}

**Conclusions**

HAT continues to provide a challenge for the development of new therapies; cases continue to be reported with resistance more frequently observed. Discoveries of new brain penetrant \textit{T. b. gambiense} and \textit{T. b. rhodesiense} trypanocides are vital for ensuring the adequate control of HAT in the future. Our work in this area has been to identify potent inhibitors of TAO that possess improved physicochemical properties than that of the natural products from which they are derived. Our exploration in this area has previously shown the requirement for the methyl and chloro substituents on the aromatic ring for high potency inhibition of TAO. Our efforts reported here for modulating the lipophilicity of this molecule by the introduction of polar functionality in the tail region of this chemotype has proved challenging. Polar functionality in this region of the molecule was shown to not be tolerated with large decreases in potency against TAO observed. The reduction in the carbon chain length of the tail resulted in identifying analogues that retained high inhibitory activity of TAO, however \textit{ex vivo} efficacy against \textit{T. b. brucei} growth was diminished. We will continue to try to understand this disconnect and use this generated data for future compound design towards more drug like inhibitors of TAO.

**Materials and Methods**

**Experimental Details.** ChemAxon Calculator Plugins were used for structure property prediction and calculation (cLogP), Marvin 15.5.4, 2015, ChemAxon (http://www.chemaxon.com). All commercial reagents were purchased from Sigma-Aldrich, Alfa Aesar, Apollo Scientific, Fluorochem or Tokyo Chemical Industry and of the highest available purity. Unless otherwise stated, chemicals were used as supplied without further purification. Anhydrous solvents were purchased from Acros (AcroSeal\textsuperscript{TM}) or Sigma-Aldrich (SureSeal\textsuperscript{TM}) and were stored under nitrogen. 40-60 petrol ether refers to the fraction with a boiling point between 40 °C and 60 °C. Anhydrous solvents and reagents were used as purchased. Thin layer chromatography (TLC) was carried out using glass plates pre-coated with Merck silica gel 60 F254. Melting points were determined using an OptiMelt apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded at 500 MHz on a Varian VNMR 500 MHz spectrometer (at 30 °C), using residual isotopic solvent (CHCl\textsubscript{3}, δ = 7.27 ppm, DMSO δ = 2.50 ppm, MeOH δ = 3.31 ppm) as an internal reference. Chemical shifts are quoted in parts per million (ppm). Coupling constants (J) are recorded in Hertz (Hz). Carbon nuclear magnetic resonance spectra were recorded at 125 MHz on a Varian 500 MHz spectrometer and are proton decoupled, using residual isotopic solvent (CHCl\textsubscript{3}, δ = 77.00 ppm, DMSO δ = 39.52 ppm, MeOH δ = 49.00 ppm) as an internal reference. Proton and carbon spectra assignments are supported by DEPT editing. High resolution mass spectrometry (HRMS) data (ESI) was recorded on Bruker Daltonics, Apex III, ESI source: Apollo ESI with methanol as spray solvent. Only molecular ions, fractions from molecular ions and other major peaks are reported as mass/charge (m/z) ratios. LCMS data was recorded on a Waters 2695 HPLC using a Waters 2487 UV detector and a Thermo LCQ ESI-MS. Samples were eluted through a Phenomenex Lunar 3 μm C8 50 mm × 4.6 mm column, using water and acetonitrile acidified by 0.1% formic acid at 1 ml/min and detected at 254 nm. The gradient employed was a 7 min method 30-90% MeCN over 5 min gradient, held at 90% MeCN for 1 min, then re-equilibrated to 30% MeCN over 1 min. All experiments were carried out under an inert atmosphere of N\textsubscript{2} unless otherwise stated.

**TAO Absorbance Assay.** 1-Ubiquinol turnover was measured by recording the increase in absorbance at 278 nm (Greiner 96-well UV star flat bottom plates with BMG PHERAstar FS photo spectrometer) to monitor the increase of 1-ubiquinone concentration kinetically over 6 min with purified rTAO (3 nM). A final concentration of 15 μM 1-ubiquinol was used under the following conditions: 50 mM Tris-HCl; 0.05% (w/v) CoE8; pH 7.4; 25 °C. The sigmoidal curve of the inhibition was observed by 10-point 3-fold serial dilution of test compounds to generate IC\textsubscript{50} values.

**Growth inhibition assays.** Bloodstream form \textit{Trypanosoma brucei brucei} Lister 427 parasites were continuously
passaged in HMI-9 medium formulated from IMDM medium (Invitrogen), 10% heat-inactivated fetal bovine serum, 10% Serum Plus medium supplement (SAFC Biosciences), 1 mM hypoxanthine (Sigma-Aldrich), 50 µM bathocuproine disulfonic acid (Sigma-Aldrich), 1.5 mM cysteine (Sigma-Aldrich), 1 mM pyruvic acid (Sigma-Aldrich), 39 µg/mL thymidine (Sigma-Aldrich), and 14 µL/L beta-mercaptoethanol (Sigma-Aldrich); all concentrations of added components refer to that in complete HMI-9 medium. The parasites were cultured in 10 mL of HMI-9 medium in T75 CELL-STAR tissue culture flasks at 37 °C / 5% CO₂.

To determine growth inhibitory potency of compounds against *T. b. brucei* bloodstream form parasites, 200 nL of 10-point, 3-fold serially diluted compounds in DMSO were transferred to the wells of white, solid bottom 384-well plates (Greiner Bio-One) by either Eppendorf 555 acoustic liquid handling system or Mosquito. Then, 104 of *T. b. brucei* parasites in 40 µL of HMI-9 medium were added to each well, and the plates were incubated for 48 hours at 37 °C in 5% CO₂ incubators. Parasite numbers in individual plate wells were determined through quantification of intracellular ATP amount. The CellTiter-Glo luminescent cell viability reagent (Promega) was added to plate wells, and ATP-dependent luminescence signal was measured on Tecan M1000 plate reader after 30 min incubation. Suramin an anti-trypanosomal drug was used positive control and DMSO was used as negative control. pIC₅₀ values were calculated using Graph Pad Prism software by plotting the luminescence values in sigmoidal dose response curves. Suramin was used as a positive control in screening. (pIC₅₀ 6.7 ± 0.1)

**Hep-G2 Cytotoxicity Assay.** Human hepatocellular carcinoma (HepG2) cells were obtained from ATCC and grown in RPMI media. 25 µL of 1.6 x 10⁴ cells / mL were dispensed into sterile 384 well Greiner Bio-One plates and incubated at 37 °C in 5% CO₂ incubator for 24 h. Once the cells adhered, 125 nL of 10-point, 3-fold serially diluted compounds in DMSO were transferred on to cells. After incubating for additional 96 h at 37 °C in 5% CO₂ incubator, cells were washed with CCK-8 reagent to each well. Plates were further incubated for 3 h followed by absorbance reading at 450 nM using Envision reader. Absorbance values were used for determination of cytotoxic concentration (pCC₅₀) required to inhibit 50% growth. Purvynic was used as positive control in screening (pCC₅₀ 6.3 ± 0.1).

**Synthesis**

5-Chloro-7-[(2E)-3,7-dimethylocta-2,6-dien-1-yl]-1,2-benzoaxazol-6-ol (3)

To a solution of triphenylphosphine (42 mg, 0.16 mmol) in dichloromethane (2 mL) was added 2,3-dichloro-5,6-dicyano-p-benzoquinone (37 mg, 0.16 mmol). The dark reaction mixture was stirred at room temperature for 1 minute during which time the colour faded. 4-Chloro-2,2E)-3,7-dimethylocta-2,6-dien-1-yl]-6-[(hydroxyimino)methyl]benzene-1,3-diol (35 mg, 0.10 mmol) in dichloromethane (0.5 mL) was added to the reaction mixture in 1 portion and this was stirred for 1 minute before being concentrated under reduced pressure. Purification by flash column chromatography (10 g silica), eluting with a gradient of petrol ether : ethyl acetate (100 : 0) to (90 : 10), gave the title compound as a white solid (24 mg, 72%). ¹H NMR (500 MHz, Chloroform-d) δ 8.60 (s, 1H), 6.10 (s, 1H), 5.42 – 5.32 (m, 1H), 5.10 – 5.01 (m, 1H), 3.66 (d, J = 7.3 Hz, 2H), 2.58 (s, 3H), 2.11 – 2.05 (m, 2H), 2.05 – 1.98 (m, 2H), 1.84 (d, J = 1.2 Hz, 3H), 1.64 (d, J = 1.4 Hz, 3H), 1.57 (d, J = 1.2 Hz, 3H).
Dimethyl sulfate (0.85 mL, 8.95 mmol) was added to a suspension of 5-bromo-3-chloro-4-hydroxy-benzonitrile (2.10 g, 8.52 mmol), and potassium carbonate (1.41 g, 10.22 mmol) in water (80 mL). The reaction mixture was heated to reflux for 16 hours. The reaction mixture was partitioned between water (10 mL) and EtOAc (20 mL). The organic layer was separated, washed with water (2 × 10 mL), washed with brine (1 × 10 mL), and dried over MgSO₄. The resulting precipitate was collected by filtration and concentrated in vacuo, to give the title compound as a granular white solid (462 mg, 74%).

<table>
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<th>Compound</th>
<th>Formula</th>
<th>Molar Mass</th>
<th>Found</th>
<th>Calculated</th>
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<td>C₁₅H₁₅BrClNO</td>
<td>283.1126</td>
<td>283.1126</td>
<td></td>
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<tr>
<td>3-Chloro-4-methoxy-2-methyl-5-octyl-benzonitrile (10)</td>
<td>C₂₈H₂₈BrClNO</td>
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<td>524.1159</td>
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<tr>
<td>Methyl 4-(3-chloro-5-cyano-2-methoxy-phenyl)butanoate (28)</td>
<td>C₁₅H₁₅BrClNO₂</td>
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<tr>
<td>5-(3-chloro-5-cyano-2-methoxy-phenyl)pentanoate (16)</td>
<td>C₁₅H₁₅BrClNO₂</td>
<td>275.1125</td>
<td>275.1125</td>
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</tr>
</tbody>
</table>
3-Chloro-5-cyano-4-methoxy-2-methyl-pentyl benzonitrile (37)
1H NMR - (500 MHz, CDCl3) δ 7.35 (s, 1H), 3.85 (s, 3H), 2.60 (t, J = 7.6 Hz, 2H), 2.54 (s, 3H), 1.61-1.48 (m, 2H), 1.41-1.17 (m, 6H), 0.87 (t, J = 6.8 Hz, 3H). 13C NMR - (126 MHz, CDCl3) δ 158.1 (C), 139.3 (C), 136.3 (C), 132.0 (CH), 129.6 (C), 117.7 (C), 109.3 (C), 60.9 (CH), 31.6 (CH), 30.1 (CH), 29.7 (CH2), 29.1 (CH2) 22.5 (CH3), 18.8 (CH3), 14.0 (CH). 1H NMR - (500 MHz, CDCl3) δ 7.35 (s, 1H), 3.86 (s, 3H), 2.62 (t, J = 7.7 Hz, 2H), 2.55 (s, 3H), 1.59-1.51 (m, 2H), 1.41-1.31 (m,

General protocol for alkylboronic acid Suzuki-Miyaura cross-coupling.

Dicyclohexyl-[2-(2,6-diisopropoxyphenyl)phenyl]phosphine (9 mg, 0.02 mmol) and palladium(II) acetate (2 mg, 0.01 mmol) was added to a degassed suspension of 5-bromomethyl-4-methoxy-2-methylbenzonitrile (100 mg, 0.38 mmol), boronic acid or trialkylboroxine (0.58 mmol) and tripotassium phosphate (244 mg, 1.15 mmol) in toluene (2 mL). The reaction mixture was sealed, degassed, put under a nitrogen atmosphere and heated to 100°C for 16-72 h. The reaction mixture was filtered through a pad of celite, the filtrate was partitioned between ethyl acetate and water, the organics were separated, washed with brine, dried over MgSO4, filtered and concentrated under vacuum. The residue was purified by flash chromatography eluting with petroleum ether to 10%-25% ethylacetate in petroleum ether. Material was further purified by reverse phase flash chromatography, eluting with water to MeOH if required to provide the cross-coupled analogues described (36-67%).

3-Chloro-5-methoxy-2-methylbenzonitrile (32)
1H NMR - (500 MHz, CDCl3) δ 7.35 (s, 1H), 3.84 (s, 3H), 2.55 (s, 3H), 2.29 (s, 3H). 13C NMR - (126 MHz, CDCl3) δ 158.3 (C), 139.5 (C), 132.9 (C), 131.5 (C), 129.6 (C), 117.6 (C), 109.2 (C), 60.2 (CH3), 18.8 (CH3), 16.0 (CH). 1H NMR - (500 MHz, CDCl3) δ 7.37 (s, 1H), 3.86 (s, 3H), 2.75-2.60 (m, 2H), 2.56 (s, 3H), 1.22 (t, J = 7.5 Hz, 3H). 13C NMR - (126 MHz, CDCl3) δ 158.0 (C), 139.4 (C), 137.5 (C), 134.1 (C), 129.6 (C), 117.8 (C), 109.5 (C), 60.9 (CH3), 22.3 (CH3), 18.8 (CH3), 14.4 (CH). 1H NMR - (500 MHz, CDCl3) δ 7.35 (s, 1H), 3.86 (s, 3H), 2.60 (t, J = 7.6 Hz, 2H), 2.55 (s, 3H), 1.66-1.56 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H). 13C NMR - (126 MHz, CDCl3) δ 158.2 (C), 139.4 (C), 136.0 (C), 132.1 (CH), 129.7 (C), 117.8 (C), 109.3 (C), 60.9 (CH3), 31.7 (CH3), 23.3 (CH3), 18.9 (CH3), 13.9 (CH). 5-Butyl-3-chloro-4-methoxy-2-methylbenzonitrile (35)
1H NMR - (500 MHz, CDCl3) δ 7.35 (s, 1H), 3.86 (s, 3H), 2.62 (t, J = 7.7 Hz, 2H), 2.55 (s, 3H), 1.59-1.51 (m, 2H), 1.41-1.31 (m,
1H NMR - (500 MHz, CDCl₃) δ 7.31 (s, 1H), 13.8 (CH₃), 2.54 (s, 3H), 2.25 (s, 3H).
3-Chloro-4-hydroxy-2-methyl-benzonitrile (39)
1H NMR - (500 MHz, CDCl₃) δ 7.29 (s, 1H), 6.21 (br s, 1H), 2.53 (3H, 2H), 2.25 (3H, 3H). 13C NMR - (126 MHz, CDCl₃) δ 153.4 (C), 138.3 (C), 124.2 (C), 120.8 (C), 110.4 (C), 104.9 (C), 187.1 (CH₃). LRMS, (ESI+) [M-H]+ 179.9 m/z.
3-Chloro-5-ethyl-4-hydroxy-2-methyl-benzonitrile (40)
1H NMR - (500 MHz, CDCl₃) δ 7.29 (s, 1H), 6.14 (s, 1H), 2.66 (q, J = 7.6 Hz, 2H), 2.54 (3H, 3H), 1.21 (t, J = 7.6 Hz, 3H). 13C NMR - (126 MHz, CDCl₃) δ 153.0 (C), 138.3 (C), 134.1 (CH), 130.1 (C), 121.0 (C), 118.1 (C), 105.2 (C), 23.1 (CH₃), 18.8 (CH₃), 13.4 (CH₃). LRMS, (ESI+) [M-H]+ 194.0 m/z.
3-Chloro-4-hydroxy-2-methyl-propyl-benzonitrile (41)
1H NMR - (500 MHz, CDCl₃) δ 7.29 (s, 1H), 6.21 (br s, 1H), 2.66 (t, J = 7.7 Hz, 2H), 2.53 (s, 3H), 1.62-1.56 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H). 13C NMR - (126 MHz, CDCl₃) δ 153.2 (C), 138.3 (C), 132.2 (CH), 128.7 (C), 121.1 (C), 118.1 (C), 105.0 (C), 31.9 (CH₃), 22.2 (CH₃), 18.8 (CH₃), 13.7 (CH₃). LRMS (ESI+) [M-H]+ 207.9 m/z.
5-Butyl-3-chloro-4-hydroxy-2-methyl-benzonitrile (42)
1H NMR - (500 MHz, CDCl₃) δ 7.29 (s, 1H), 6.22 (br s, 1H), 2.61 (t, J = 7.8 Hz, 2H), 2.53 (s, 3H), 1.60-1.50 (m, 2H), 1.40-1.29 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H). 13C NMR - (126 MHz, CDCl₃) δ 153.2 (C), 138.2 (C), 132.1 (CH), 128.9 (C), 121.1 (C), 118.1 (C), 105.0 (C), 31.2 (CH₃), 29.6 (CH₃), 22.3 (CH₃), 18.7 (CH₃), 13.8 (CH₃). LRMS (ESI+) [M-H]+ 221.9 m/z.
3-Chloro-4-hydroxy-2-methyl-5-pentyl-benzonitrile (43)
1H NMR - (500 MHz, CDCl₃) δ 7.30 (s, 1H), 6.10 (br s, 1H), 2.62 (t, J = 7.8 Hz, 2H), 2.54 (s, 3H), 1.65-1.52 (m, 2H), 1.37-1.27 (m, 4H), 0.89 (t, J = 6.8 Hz, 3H). 13C NMR - (126 MHz, CDCl₃) δ 153.1 (C), 138.2 (C), 132.2 (CH), 128.9 (C), 121.0 (C), 118.1 (C), 105.1 (C), 31.4 (CH₃), 29.9 (CH₃), 28.8 (CH₃), 22.4 (CH₃), 18.8 (CH₃), 14.0 (CH₃). LRMS (ESI+) [M-H]+ 235.9 m/z.
3-Chloro-5-hexyl-4-hydroxy-2-methyl-benzonitrile (44)
1H NMR - (500 MHz, CDCl₃) δ 7.30 (s, 1H), 6.10 (br s, 1H), 2.62 (t, J = 7.8 Hz, 2H), 2.54 (s, 3H), 1.65-1.52 (m, 2H), 1.37-1.27 (m, 4H), 0.89 (t, J = 6.8 Hz, 3H). 13C NMR - (126 MHz, CDCl₃) δ 153.1 (C), 138.2 (C), 132.2 (CH), 128.9 (C), 121.0 (C), 118.1 (C), 105.1 (C), 31.4 (CH₃), 29.9 (CH₃), 28.8 (CH₃), 22.4 (CH₃), 18.8 (CH₃), 14.0 (CH₃). LRMS (ESI+) [M-H]+ 235.9 m/z.
\[ ^{1}H\text{ NMR} - (500 \text{MHz, CDCl}_3) \delta 7.29 (s, 1H), 6.18 (br s, 1H), 2.61 (t, J = 7.8 Hz, 2H), 2.53 (s, 3H), 1.61-1.50 (m, 2H), 1.38-1.23 (m, 6H), 0.92-0.84 (m, 3H). \]  
\[ ^{13}C\text{ NMR} - (126 \text{ MHz, CDCl}_3) \delta 153.1 (C), 138.2 (C), 132.2 (CH), 128.9 (C), 121.0 (C), 118.1 (C), 105.0 (C), 31.6 (CH), 30.0 (CH), 29.0 (CH), 28.9 (CH), 22.6 (CH), 18.8 (CH), 14.1 (CH). \]  
LRMS, (ESI) [M-H] \[ \text{249.9 m/z.} \]

**3-Chloro-5-heptyl-4-hydroxy-2-methyl-benzonitrile (45)**

\[ ^{1}H\text{ NMR} - (500 \text{MHz, CDCl}_3) \delta 7.30 (s, 1H), 6.11 (br s, 1H), 2.61 (t, J = 7.5, 2H), 2.54 (s, 3H), 1.61-1.53 (m, 2H), 1.35-1.22 (m, 8H), 0.88 (t, J = 6.2 Hz, 3H). \]  
\[ ^{13}C\text{ NMR} - (126 \text{ MHz, CDCl}_3) \delta 153.1 (C), 138.2 (C), 132.2 (CH), 128.9 (C), 121.0 (C), 118.1 (C), 105.1 (C), 31.7 (CH), 30.0 (CH), 29.2 (CH), 29.0 (CH), 22.6 (CH), 18.8 (CH), 14.1 (CH). \]  
LRMS, (ESI) [M-H] \[ \text{264.0 m/z.} \]

**5-Chloro-4-hydroxy-5-octylbenzonitrile (46)**

m.p. 60.1 – 61.8 °C. IR (neat, \( v_{\text{max}} \)) cm\(^{-1}\) 3362, 2927, 2849, 2230 (CN), 1595, 1471, 1316, 1241, 1168. \[ ^{1}H\text{ NMR} (500 \text{MHz, CDCl}_3) \delta 7.49 (s, 1H), 7.34 (s, 1H), 6.07 (s, 1H), 2.66 (m, 2H), 1.59 (m, 2H), 1.49-1.17 (m, 10H), 0.88 (m, 3H). \]  
\[ ^{13}C\text{ NMR} (126 \text{ MHz, CDCl}_3) \delta 153.4 (C), 133.9 (C), 132.2 (CH), 130.4 (CH), 120.5 (C), 118.2 (CH), 104.6 (C), 32.0 (CH), 30.3 (CH), 29.5 (CH), 29.5 (CH), 29.4 (CH), 29.2 (CH), 22.8 (CH), 14.3 (CH). \]  
HRMS (ESI) m/z [M-H] calc for \( C_9H_8ClNO \) \[ \text{264.1161, found 264.1151.} \]

**General protocol for HATU amide coupling.**

Triethylamine (0.07 mL, 0.49 mmol) was added to a solution of carboxylic acid (0.2 mmol), HATU (0.08 g, 0.22 mmol), and amine (0.3 mmol) in acetonitrile (0.5 mL). The reaction was stirred at RT for 16 h. The reaction mixture was partitioned between ethyl acetate (5 mL) and water (5 mL). The organic layer was separated, washed with brine, dried over MgSO\(_4\), filtered and concentrated under reduced pressure. The residue was purified by flash silica chromatography eluting with petroleum ether to ethyl acetate in petroleum ether, then further purified by reverse phase chromatography eluting with water to MeOH if necessary to provide the amides described (29-67%).

**4-(3-Chloro-5-cyano-2-hydroxy-phenyl)-N-propyl-butanamide (19 b)**

\[ ^{1}H\text{ NMR} - (500 \text{MHz, CDCl}_3) \delta 10.73 (br s, 1H), 7.95 (br t, 1H), 7.73 (s, 1H), 7.45 (s, 1H), 2.97, (m, 2H), 2.56 (t, J = 7.4 Hz, 2H), 2.04 (t, J = 7.4 Hz, 2H), 1.71 (m, 2H), 1.37 (m, 2H), 0.81 (t, J = 7.4 Hz, 3H). \]  
\[ ^{13}C\text{ NMR} - (126 \text{ MHz, CDCl}_3) \delta 172.0 (C), 134.4 (CH), 120.3 (C), 119.4 (CH), 118.5 (C), 118.1 (CH), 104.6 (C), 32.0 (CH), 30.3 (CH), 29.5 (CH), 29.5 (CH), 29.4 (CH), 29.2 (CH), 22.8 (CH), 14.3 (CH). \]  
HRMS (ESI) m/z [M-H] calc for \( C_{19}H_{19}ClNO \) \[ \text{281.0 m/z.} \]

**5-(3-Chloro-5-cyano-2-hydroxy-phenyl)-N-ethyl-pentanamide (20 b)**

IR (neat, \( v_{\text{max}} \)) cm\(^{-1}\) 3362, 3246, 2927, 2225 (CN), 1716, 1199, 1250. \[ ^{1}H\text{ NMR} (500 \text{MHz, CDCl}_3) \delta 8.25 (s, 1H), 7.32 (s, 1H), 6.80 (s, 1H), 4.16 (q, J = 7.3 Hz, 2H), 2.84 (m, 2H), 2.70 (m, 2H), 2.44 (s, 3H), 1.25 (t, J = 7.3 Hz). \]  
\[ ^{13}C\text{ NMR} (126 \text{ MHz, CDCl}_3) \delta 175.9 (C), 158.4 (C), 142.5 (C), 135.1 (CH), 125.8 (C), 119.0 (CH), 118.6 (C), 104.3 (C), 61.8 (CH), 35.0 (CH), 24.0 (CH), 20.1 (CH), 14.0 (CH). \]  
HRMS (ESI) m/z [M-H] calc for \( C_{20}H_{20}ClNO_2 \) \[ \text{256.0944, found 256.0941.} \]

Ethyl 3-(5-cyano-2-hydroxy-4-methyl-phenyl)propanoate (26)

Sulfuryl chloride (0.02 mL, 0.26 mmol) was added to a solution of ethyl 3-(5-cyano-2-hydroxy-4-methyl-phenyl)propanoate (0.05 g, 0.21 mmol) in diethyl ether (1 mL). The reaction mixture was stirred at RT for 96 h. Further sulfuryl
chloride (0.02 mL, 0.26 mmol) was added and the reaction mixture was stirred for a further 16 h. The reaction mixture was partitioned between 1 M HCl (aq) (10 mL) and ethyl acetate (2 x 20 mL). The combined organics were separated, dried over MgSO₄, filtered and concentrated under vacuum. The residue was purified by flash silica chromatography, eluting with petroleum ether to provide the compound as a white solid (0.043 g, 75%). IR (neat, v_max), cm⁻¹ 3369, 2928, 2220 (CN), 1632, 1562, 1157. ¹H NMR (500 MHz, CDCl₃) 6 7.52 (d, J = 2.0 Hz, 1H), 7.37 (d, J = 2.0 Hz, 1H), 5.44 (br s, 1H), 3.88 (s, 3H), 2.80 (d, J = 5.0 Hz, 2H), 2.17 (t, J = 7.6 Hz, 2H), 1.68 (m, 2H), 1.59 (m, 2H), 1.37 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) 6 173.5 (C), 158.2 (C), 139.4 (C), 132.4 (C), 131.8 (C), 128.9 (C), 117.7 (C), 108.4 (C), 61.1 (CH₃), 37.3 (CH₃), 35.4 (CH₂), 33.2 (CH₂), 30.1 (CH₃), 29.9 (CH₃), 29.2 (CH₃), 29.2 (CH₃), 25.0 (CH₃). LRMS (ESI⁺) [M+H⁺] 263.1 m/z.

N-Butyl-3-(3-chloro-5-cyano-2-hydroxy-4-methyl-phenyl)propanamide (27)

IR (neat, v_max), cm⁻¹ 3369, 2928, 2220 (CN), 1632, 1562, 1157. ¹H NMR (500 MHz, CDCl₃) 6 10.35 (br s, 1H), 7.24 (s, 1H), 5.55 (br s, 1H), 3.26 (m, 2H), 2.91 (m, 2H), 2.59 (m, 2H), 2.54 (s, 3H), 1.45 (m, 2H), 1.28 (m, 2H), 0.90 (t, J = 7.5 Hz, 3H). ¹³C NMR - (126 MHz, CDCl₃) 6 173.6 (C), 155.6 (C), 140.0 (C), 132.6 (CH), 127.4 (C), 123.9 (C), 118.3 (C), 104.0 (C), 39.9 (CH₃), 36.3 (CH₃), 31.4 (CH₃), 25.0 (CH₃), 19.9 (CH₃), 18.9 (CH₃), 13.6 (CH₃). HRMS (ESI⁺) m/z [M+Na⁺] calcd for C₉H₁₇ClN₂O₂ 317.1027, found 317.1019.

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All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS
AAT, African animal trypanosomiasis; AF, ascorfuranone; CCB, colletchochlorin B; CNS, central nervous system; HAT, human African trypanosomiasis; LipE, lipophilic efficiency; MPO, multi-parameter-optimisation; NECT, Nifurtimox and Eflornithine combination therapy; TAO, trypanosome alternative oxidase; T. b. brucei, trypanosoma brucei brucei; TBD, 1,5,7-triazacyclo[4.4.0]dec-5-ene

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Colletochlorin B (CCB) (2)

Tail cLogP 3.54

Aromatic cLogP 2.68

Decrease cLogP

TAO pIC50

\[ \text{cLogP} \]