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# Discovery of halo-nitrobenzamides with potential application against human African trypanosomiasis

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ABSTRACT

A series of halo-nitrobenzamide were synthesized and evaluated for their ability to block proliferation of *Trypanosoma brucei brucei*. A number of these compounds had significant activity against the parasite, particularly 2-chloro-*N*-(4-chlorophenyl)-5-nitrobenzamide **17** which exhibited low micromolar inhibitory potency against *T. brucei* and selectivity towards both malaria and mammalian cells.



The protozoan parasite *Trypanosoma brucei* causes Human African trypanosomiasis (HAT), a major health concern in sub-Saharan Africa with an estimated 50,000 cases and 60 million at risk of infection.[1] Several drugs such as Melarsoprol and Eflornithine (DFMO) have been developed to treat HAT (Fig. 1).[2,3] However these drugs used have serious side effects, poor clinical effect, and emerging drug resistance (Fig. 1).[4–7] Therefore, there is urgent need for new drugs against HAT with low toxicity.[8,9]

As part of our ongoing search for novel chemical entities with antitrypanosomal or antimalarial effects, all compounds synthesized in our laboratory or purchased by our laboratory are routinely assayed to detect such activity. Compound **3** emerged as a hit compound inhibiting *T. brucei* proliferation in this campaign. It has two distinct features: a potentially electrophilic center and a hydrophobic side chain. The halo-nitro substituted benzene structure is well known as an electrophile. Thus, compound **3** might be forming a covalent bond with nucleophiles such as cysteine and lysine in the binding pocket of its target.[10] Compound **3** inhibited *T. brucei* proliferation with fairly good potency ($EC_{50}$ = 1.5 μM) but had poor selectivity against malaria (3D7 strain, $EC_{50}$ = 7.3 μM) and mammalian cells (HepG2, $EC_{50}$ = 2.5 μM). In addition compound **3** had poor solubility (<0.1 μM) and permeability (<1 × $10^{-6}$ cm/s). Therefore we began optimizing this chemical structure to improve both pharmacological properties and selectivity. Here we report this new class of *T. brucei* inhibitors (Fig. 2).

All of compounds investigated in this report were synthesized from commercially available anilines or phenols and benzoic acid by amide or ester coupling reactions induced with polymer bound EDC (Scheme 1). All compounds were evaluated for their ability to inhibit proliferation of cultured *T. brucei brucei* and *Plasmodium falciparum* (3D7 strain). Additionally, the growth inhibitory activity against HepG2 cells was determined to set a cellular therapeutic index. The solubility and passive permeability of the small molecules were also investigated to support the cell based assay results and to guide the earliest stages of discovery towards tractable compounds (Fig. 3).

First we explored putative nucleophilic ring group of hit compound **3** to determine if the 2-fluoro-5-nitrobenzamide moiety

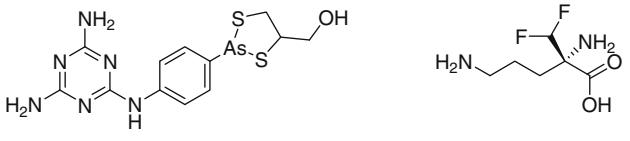


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**Figure 1.** Melarsoprol **1** and eflornithine **2**.

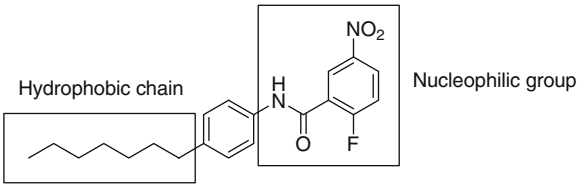


**Figure 2.** Chemical structure of hit compound **3**.

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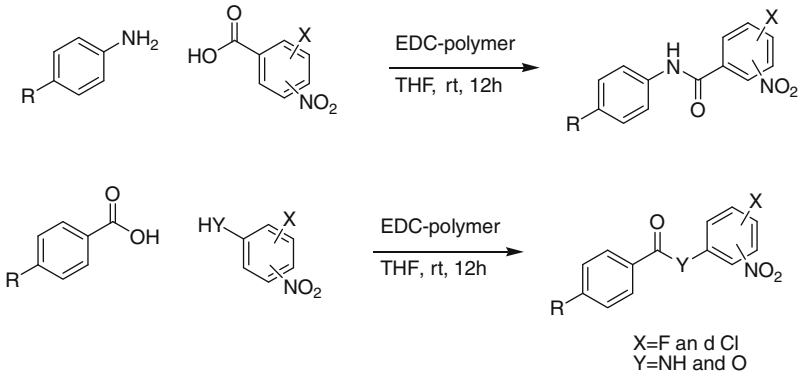


**Scheme 1.** General synthetic route for halo-nitrobenzene esters and amides.

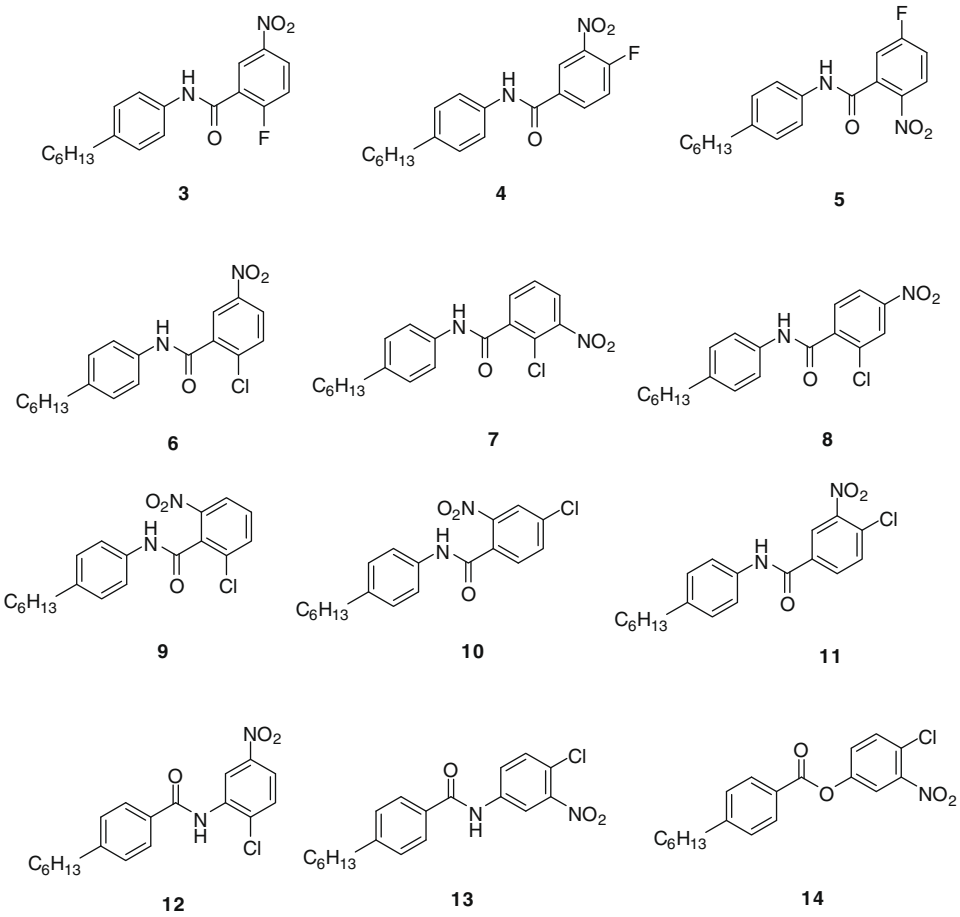


**Figure 3.** Halo-nitrobenezene compounds investigated.

was essential for inhibition *T. brucei* proliferation (Table 1). 4-Fluoro-3-nitrobenzamide compound **4** showed weak *T. brucei* inhibitory activity ($EC_{50}$ = 24 μM) and relatively low cytotoxicity ($EC_{50}$ = 10.3 μM) in HepG2 cells compared to original hit compound **3**. 3-Fluoro-5-nitrobenzamide compound **5** also showed weak *T. brucei* inhibitory activity ($EC_{50}$ = 22.5 μM) but no toxicity in HepG2 cells ($EC_{50}$ >50 μM). All of fluoro-nitrobenzamide compounds **3–5** lacked growth inhibitory activity against malaria. However, all of these compounds had poor solubility (<0.1 μM) and permeability ($<1 \times 10^{-6}$ m/s). Next we investigated whether fluorine is replaceable with chlorine. 2-Chloro-5-nitro benzamide compound **6** showed not only inhibitory activity similar to initial hit **3** against *T. brucei* ($EC_{50}$ = 1.0 μM), but also high selectivity against HepG2 cells ($EC_{50}$ >50 μM). However, compound **6** also showed poor solubility (<0.1 μM) and modest permeability ($7 \pm 13 \times 10^{-6}$ cm/s). Other chloro-nitrobenzamide compounds **8-11** showed weak *T. brucei* inhibitory activity (10–30 μM). Reverse halo-nitrophenyl amides **12** and **13** and ester **14** were also investigated. 4-Chloro-5-nitrophenyl amide **12** showed moderate activity ($EC_{50}$ = 6.0 μM) but compounds **13** and **14** showed no inhibitory activity and weak activity ($EC_{50}$ >50 and 24.0 μM), respectively. All of this series showed good selectivity against malaria ($EC_{50}$ >15 μM) and HepG2 cells ($EC_{50}$ >50 μM) and poor solubility and permeability (<0.1 μM and $<1 \times 10^{-6}$ cm/s, respectively). All compounds in this series showed relatively high *A* log *P*, regardless of structure.[11]

**Table 1**
Activities and pharmacological properties of halo-nitrobenzamides

| Compound | Yield (%) | $IC_{50}$ versus *T. brucei* | $IC_{50}$ versus *P. falcip* | $IC_{50}$ versus HepG2 | Solubility (μM) | Permeability $10^{-6}$ cm/s | *A* log *P* |
|---|---|---|---|---|---|---|---|
| **3** | | 1.5 ± 0.4 | 7.3 ± 1.0 | 2.5 ± 0.5 | <0.1 | <1 | 5.94 |
| **4** | 86 | 24 ± 1.2 | >15 | 10.3 ± 7.7 | <0.1 | <1 | 5.48 |
| **5** | 68 | 22.5 ± 2.5 | >15 | >50 | <0.1 | <1 | 5.48 |
| **6** | 75 | 1.0 ± 0.1 | >15 | >50 | <0.1 | 7 ± 13 | 5.48 |
| **7** | 40 | >50 | >15 | >50 | <0.1 | <1 | 5.94 |
| **8** | 66 | 19.0 ± 3.6 | >15 | >50 | <0.1 | 82 ± 142 | 5.94 |
| **9** | 23 | 29.8 ± 1.4 | >15 | >50 | <0.1 | 3 ± 5 | 5.94 |
| **10** | 63 | 20.8 ± 4.8 | >15 | >50 | <0.1 | <1 | 5.94 |
| **11** | 64 | 11.9 ± 0.7 | >15 | >50 | <0.1 | <1 | 5.94 |
| **12** | 55 | >50 | >15 | >50 | <0.1 | <1 | 5.94 |
| **13** | 64 | 6.0 ± 1.3 | >15 | >50 | <0.1 | <1 | 5.94 |
| **14** | 99 | 24.0 ± 1.9 | >15 | >50 | <0.1 | <1 | 5.94 |

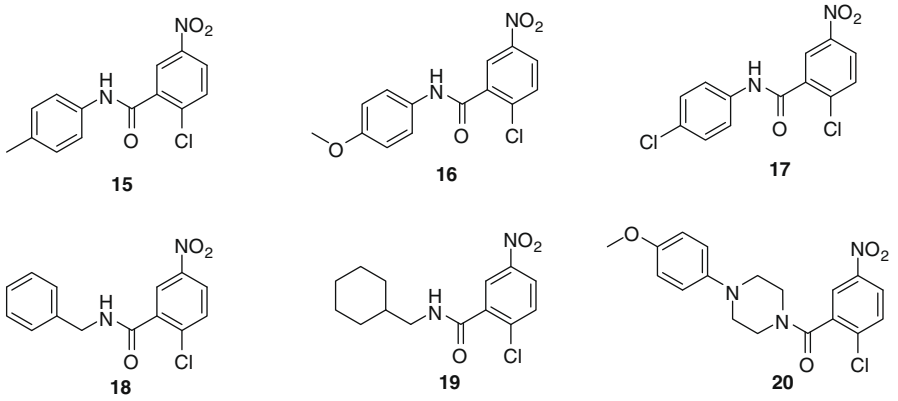


**Figure 4.** 2-Chloro-5-nitrobenezamide compounds.

**Table 2**
Activities and pharmacological properties of 2-chloro-5-nitrobenzamides

| Compound | Yield (%) | $IC_{50}$ versus *T. brucei* | $IC_{50}$ versus *P. falcip.* | $IC_{50}$ versus HepG2 | Solubility (μM) | Permeability Pe, $10^{-6}$ cm/s | *A* log *P* |
|---|---|---|---|---|---|---|---|
| **15** | 94 | 7.7 ± 0.6 | >15 | >50 | 5.7 ± 0.1 | 363 ± 56 | 3.66 |
| **16** | 65 | 19.2 ± 1.0 | >15 | >50 | 9.9 ± 0.5 | 248 ± 38 | 3.16 |
| **17** | 51 | 1.5 ± 1.0 | >15 | >50 | 2.9 ± 0.1 | 536 ± 87 | 3.84 |
| **18** | 49 | >50 | >15 | >50 | 3.9 ± 0.2 | 186.5 ± 50 | 3.18 |
| **19** | 51 | 27.0 ± 3.1 | >15 | >50 | 7.5 ± 0.4 | 418 ± 27 | 3.79 |
| **20** | 56 | 24.5 ± 13.7 | >15 | >50 | 5.9 ± 0.2 | 386 ± 21 | 3.43 |

Next we explored variation of the hydrophobic side chain to determine if it was possible to improve the poor physicochemical properties of the original series. The results are summarized in Figure 4 and Table 2. 4-Methylphenylamide **15** and 4-methoxyphenylamide **16** exhibited moderate activity against *T. brucei* proliferation ($EC_{50}$ = 7.7 μM and 19.2 μM, respectively), and showed improvement of solubility (5.7 μM and 9.9 μM, respectively) and permeability ($363 \times 10^{-6}$ cm/s and $248 \times 10^{-6}$ cm/s, respectively). 4-chloroaniline **17** showed not only good inhibitory activity against *T. brucei* ($EC_{50}$ = 1.5 μM) but also reasonable solubility (2.9 μM) and permeability ($536 \times 10^{-6}$ cm/s) without change of selectivity against HepG2 ($EC_{50}$ >50 μM). Benzylamine **18** was inactive against *T. brucei* and cyclohexyemthylamine **19** and 4-methoxyphenylpiperazine **20** showed moderate activity ($EC_{50}$ = 27.0 μM and 24.5 μM, respectively). This entire series also showed good selectivity against malaria and mammalian cells. The replacement of the hexyl chain resulted in reduced *A* log *P* (3.16–3.84) and improved solubility (30–90-fold). In contrast to the earlier series, all compounds showed reasonable permeability. In addition, the electron withdrawing chloro group improved antitrypanosomal activity. Conclusively chloro-substituted compound **17** showed the most balance of potency, selectivity, and physicochemical properties.

In summary we have reported the synthesis and evaluation of a series of halo-nitrobenzamides made to explore the SAR of a hit selected from screening for inhibitors of *T. brucei* proliferation. The optimized inhibitor exhibited potent activity against *T. brucei* proliferation and selectivity against malaria and mammalian cells. Although the mechanism of action of these series remains unclear the halo-nitrobenzamides warrant further examination as leads for the therapy of human African trypanosomiasis.

## Acknowledgements

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## Supplementary data

Experimental procedures and characterization data for all final compounds are available. Supplementary data associated with this

article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.022.

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