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Optimization of the electrophile of chloronitrobenzamide leads active against *Trypanosoma brucei*

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ABSTRACT

We previously reported the phenylchloronitrobenzamides (PCNBs), a novel class of compounds active against the species of trypanosomes that cause Human African Trypanosomiasis (HAT). Herein, we explored the potential to adjust the reactivity of the electrophilic chloronitrobenzamide core. These studies identified compound **7d** that potently inhibited the growth of trypanosomes (EC_{50} = 120 nM for *Trypanosoma b. brucei*, 18 nM for *Trypanosoma b. rhodesiense*, and 38 nM for *Trypanosoma b. gambiense*) without significant cytotoxicity against mammalian cell lines (EC_{50} > 25 μM for HepG2, HEK293, Raji, and BJ cell lines) and also had good stability in microsomal models ($t_{1/2}$ > 4 h in both human and mouse). Overall these properties indicate the compound **7d** and its analogs are worth further exploration as potential leads for HAT.

Human African trypanosomiasis (HAT), a lethal disease widespread in sub-Saharan Africa, is caused by two sub-species of the eukaryotic protozoan parasite *Trypanosoma brucei* (*rhodesiense* and *gambiense*).[1] *T. brucei* is vectored between mammalian hosts (cattle and humans) by the tsetse fly. Few chemotherapeutics are available for HAT. The classic drugs are suramin and pentamidine, for treatment of early-stage disease, and melarsoprol and eflornithine, for treatment of late-stage disease.[1] Suramin and melarsoprol have serious side effects and all of the drugs have poor clinical effect in late stage disease, and emerging drug resistance.[2,3] Recently a drug combination, nifurtimox–eflornithine, has entered late stage development for *T. b. gambiense* HAT.[4] However, there is still an urgent need for new drugs against HAT with better efficacy and lower toxicity.[5,6]

We have recently reported a new class of lead compounds inhibiting proliferation of *T. brucei spp.*, the chloronitrobenzamides (PCNBs) that were discovered by phenotypic screening (Fig. 1, **1**).[7] The initial examination of structure–activity relationships (SARs) revealed that the 2-chloro-4-nitro group of the PCNBs was essential for potency with any change of the substitution pattern on this phenyl ring causing great reduction of potency. In subsequent intensive SAR studies it was discovered that incorporation of benzothiazolyl substitution on the B-ring of PCNB **2** significantly increased anti-trypanosomal potency.[2] Additionally, substitution with piperazine or methyl piperazine on A-ring of PCNBs improved the activity and pharmacological properties.

In an effort to reduce complexity and molecular weight and improve pharmacological properties of the PCNBs, we examined whether or not the chloronitrobenzamide electrophile could be replaced. These studies are reported herein.

All compounds used in this study were prepared using the synthetic scheme as outlined in Scheme 1. A common intermediate **4** was prepared by the published method.[8–10] All variant electrophile compounds **5** were synthesized by amide formation with either acid chlorides or acids, using standard conditions. All compounds were purified to >95% as verified by HPLC/MS/ELSD. The identity of all compounds was confirmed by MS and NMR.

All compounds were subjected to a standardized testing scheme measuring: potency for inhibition of trypanosome growth, potency for inhibition of mammalian cell growth, solubility, and permeability. Compounds were serially diluted in 10 threefold steps from a 10,000 μM DMSO stock. The resulting concentration series of each PCNB were transferred to the assay wells using hydrodynamic pins (V&P Scientific, San Diego, CA), giving a final DMSO concentration of 0.2% and a concentration range downward from 33 μM to 0.5 nM. Trypanosome growth inhibition was measured after 48 h as previously described in 384-well plates.[11,12] The cytotoxicity of all compounds was measured after 72 h for four human cell lines; Raji, a Burkett's lymphoma derived line; BJ, an immortalized foreskin fibroblast derived line; HEK 293, an embryonic kidney fibroblast derived line; and HepG2, a hepatocellular carcinoma derived line, using the CellTiter Glo method (Promega). Each growth

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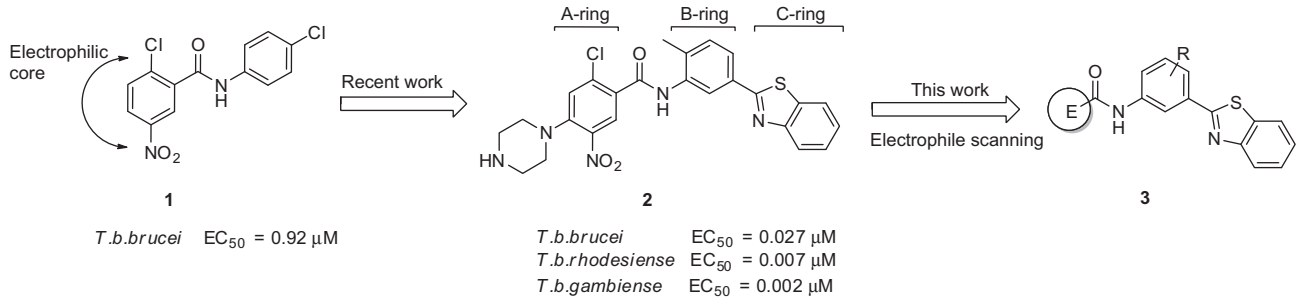
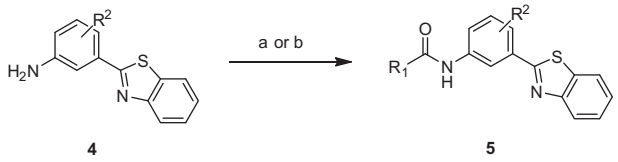


Figure 1. *Para*chloronitrobenzamide (*P*CNB) compounds investigated.

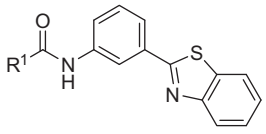


Scheme 1. Synthesis of candidate electrophiles. Reagents and conditions: (a) acid chlorides, diisopropylethylamine, dichloromethane, rt; (b) acids, EDC·HCl, diisopropylethylamine, dichloromethane, rt.

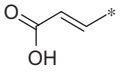
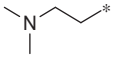
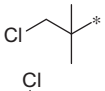
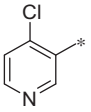
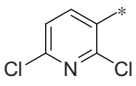
proliferation experiment was carried out in triplicate. The kinetic solubility was evaluated in PBS buffer (pH 7.4) to aid in interpretation of cellular data and provide preliminary evaluation of physiochemical characteristics. The passive permeability was measured against PBS buffer (pH 7.4) sinks using the Parallel Artificial Membrane Permeability Assay (PAMPA) as implemented by pIon. The data are summarized in Table 1.

Several groups of related electrophiles were tested. The first group contained α,β-unsaturated amides (**6a–d**). All compounds in this set failed to inhibit *T. brucei* proliferation. In addition

Table 1
Summary of pharmacological properties of compound **6**



6

No	R_1	EC_{50} (μM)					Solubility[b] (μM)	PAMPA[b] (Pe, $\times 10^{-6}$ cm/s)
		T. b. brucei[a]	HEK293[b]	HepG2[b]	Raji[b]	BJ[b]		
1	na	0.92 ± 0.2	>25	>25	>25	>25	3	536
6a		>33	>25	>25	>25	>25	0.18	797
6b		>33	>25	>25	>25	>25	0.11	1641
6c		>33	>25	>25	>25	>25	0.42	987
6d		>33	>25	>25	>25	>25	56	4
6e		>33	>25	21	>25	>25	56	2081
6f		0.12 ± 0.06	10 ± 1	16 ± 1	1.7 ± 0.2	8.5 ± 1.6	1.0	660
6g		1.2 ± 0.2	>25	>25	>25	>25	0.83	769
6h		>33	>25	>25	>25	>25	<DL[c]	891
6i		0.30 ± 0.18	>25	>25	12	>25	0.32	732
6j		25 ± 6	>25	>25	>25	>25	0.52	677
6k		11 ± 6	>25	>25	>25	>25	0.35	104