

Synthesis and assessment of catechol diether compounds as inhibitors of trypanosomal phosphodiesterase B1 (TbrPDEB1).

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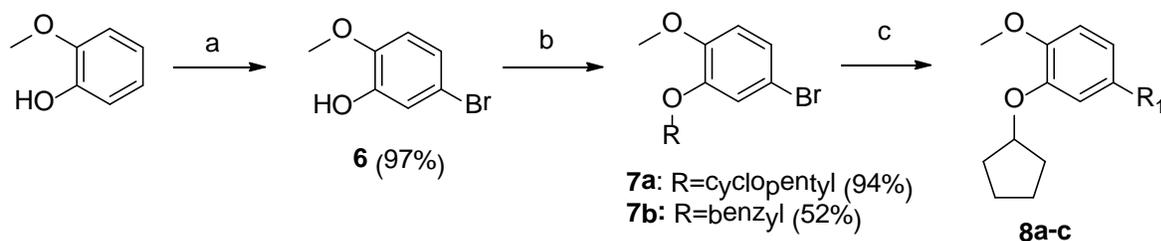
Abstract. Human African trypanosomiasis (HAT) is a parasitic neglected tropical disease that affects 10,000 patients each year. Current treatments are sub-optimal, and the disease is fatal if not treated. Herein, we report our continuing efforts to repurpose the human phosphodiesterase 4 (hPDE4) inhibitor piclamilast to target trypanosomal phosphodiesterase TbrPDEB1. We prepared a range of substituted heterocyclic replacements for the 4-amino-3,5-dichloro-pyridine head group of piclamilast, and found that these compounds exhibited weak inhibitory activity of TbrPDEB1.

Human African trypanosomiasis (HAT) is a neglected tropical disease caused by the parasites *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. Together, over 60 million people in 36 countries in sub-Saharan Africa are at risk, with approximately 10,000 infections annually.¹ HAT is fatal unless treated and the four drugs approved for this indication: pentamidine, suramin, eflornithine, and melarsoprol, are inadequate for a variety of reasons, including cost, toxicity, and lack of oral bioavailability. For instance, melarsoprol is especially toxic as it induces reactive encephalopathy in 5-10% of patients, killing approximately half of them.² As such, new medicines are desperately needed but pharmaceutical companies tend to deprioritize diseases such as HAT due to an inability to recover research costs from the extremely poor who are the most affected by the disease.

In order to speed up the drug discovery process, a drug repurposing approach³ has been taken against two *T. brucei* phosphodiesterases (PDEs), TbrPDEB1 and TbrPDEB2.^{4,6} Simultaneous RNAi knockdown of both is fatal,⁷ suggesting that small molecule inhibitors of these enzymes could be useful interventions.^{4,8} Humans have 11 PDEs that have been well explored, producing numerous clinical drug candidates.⁹ The catalytic domains of human PDEs are 30-35% homologous to those of the parasite enzymes TbrPDEB1 and TbrPDEB2. Recent crystallographic evidence confirms that there are key regions of the trypanosomal protein that may allow for selective inhibition over human PDEs.¹⁰ We previously reported that human PDE4 inhibitor piclamilast (**1**) represents a promising lead series for optimization towards selective TbrPDEB inhibitors,⁴ and others have shown analogous catechol-derived inhibitors to have high potency against the trypanosomal enzyme.^{8, 11} In this report, we describe our efforts to explore replacements for the 2,6-dichloro-4-pyridylamide headgroup of **1** by assessing their potency against TbrPDEB1.

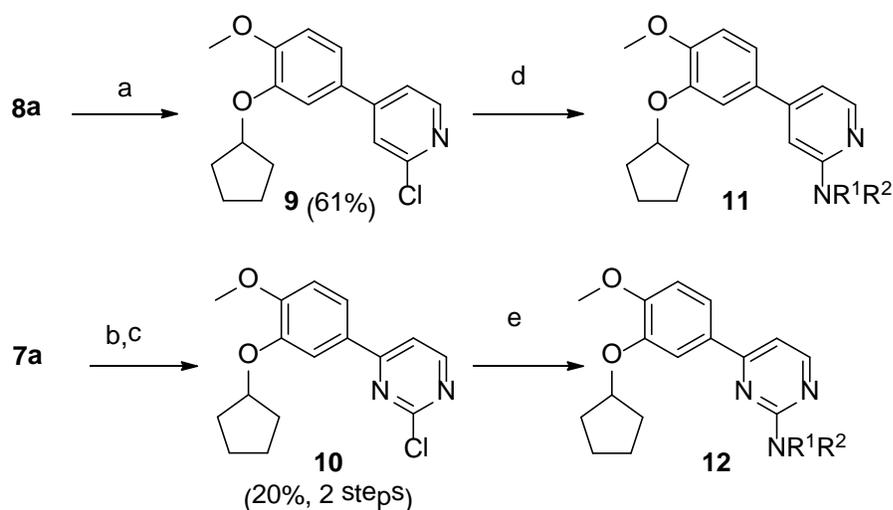
Our rationale for focusing first on the headgroup region of **1** is shown in **Figure 1**. The optimization of phthalazinones as human PDE4 (hPDE4) inhibitors has been described, leading to the discovery of **2** with an IC₅₀ of 0.6 nM against hPDE4.¹² This compound was later discovered in a screening campaign to be a potent inhibitor of TbrPDEB1 (3.98 nM),⁸ and we found that this compound comparably inhibits

We prepared putative heterocyclic aromatic headgroup replacements for the phthalizinone moiety as shown in **Scheme 1**. Electrophilic bromination of guaiacol by a previously published method afforded 5-bromo-2-methoxyphenol, intermediate **6**.¹⁶ This intermediate reacted with cyclopentanol under Mitsunobu conditions to produce the dialkoxy catechol intermediate **7a**. A small library of boronic acids was used in Suzuki reactions with intermediate **7a** to yield analogs **8a-c**, which were found to be weak inhibitors of TbrPDEB1 (**Table 1**).



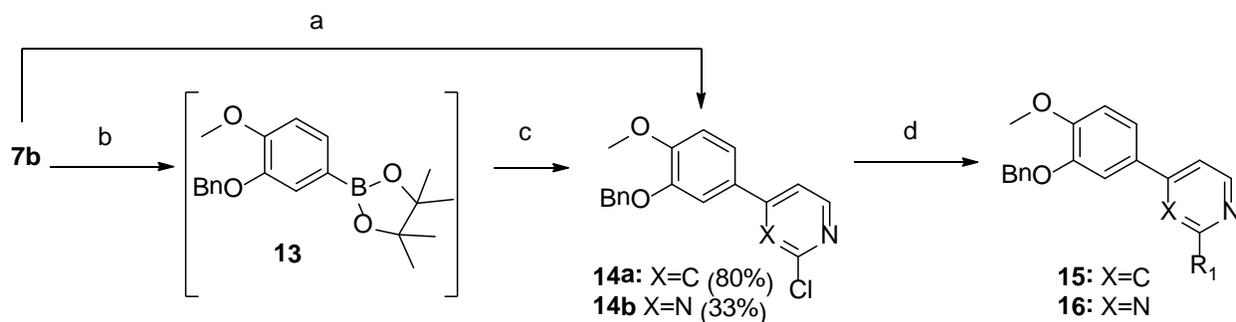
Scheme 1. Synthesis of **8a-c**. Reagents and conditions: (a) (i) TFAA, *t*-BuOK, MeCN, rt, 45 min; (ii) NBS, MeCN, rt, *o/n*; (b) ROH, PPh₃, DEAD, toluene, rt, 2h; (c) R-B(OH)₂, Na₂CO₃, Pd(PPh₃)₄, toluene/EtOH/H₂O (4:1:1), 105°C, *o/n*.

We also prepared a series of pyrimidine and pyridine-derived inhibitors, as shown in **Scheme 2**. Compound **8a** could be converted to the chloropyridine intermediate **9** by a sequence of N-oxide formation and chlorination with POCl₃. The analogous pyrimidine intermediate **10** was made via borylation of intermediate **7a**, followed by a Suzuki reaction with 2,4-dichloropyrimidine. Chloride displacement with small aliphatic and benzylic amines was achieved to provide the pyrimidine and pyridine-derived analogs **11** and **12** (**Table 2**).



Scheme 2. Synthesis of **11** and **12**. Reagents and conditions: (a) (i) *m*-CPBA, CHCl_3 , 0°C to RT, *o/n*; (ii) POCl_3 , TEA, CHCl_3 , MW, 100°C , 1 h; (b) *n*-BuLi, then bis-(pinacolato)diborane, -78°C to rt, *o/n*; (c) 2,4-dichloropyrimidine, Na_2CO_3 , PPh_3 , $\text{Pd}_2(\text{dba})_3$, *tol*/*EtOH*/ H_2O (4:1:1), 105°C , *o/n*; (d) $\text{R}^1\text{R}^2\text{NH}$, DIEA, NMP or DMF, MW, 250°C , 1 h; (e) $\text{R}^1\text{R}^2\text{NH}$, DIEA, NMP or DMF, 80°C , *o/n*.

Figure 1 shows a related TbrPDEB1 inhibitor chemotype (**4**) that showed equal enzymatic potency (though improved cellular potency) where the cyclopentyl catechol ether was replaced with a benzyl ether.¹¹ In light of this, we opted to also make benzyl-substituted analogs of the compounds in **Scheme 2**, which was achieved by the route shown in **Scheme 3**. The brominated intermediate **7b** was converted to the 2-chloropyridyl intermediate **14a** under Suzuki conditions in modest yields. We prepared the pyrimidine template **16** via the boronate; we elected to utilize palladium catalysis for the preparation of **13**, as the lithium-halogen exchange route applied in **Scheme 2** led to undesirable side reactions. The crude boronate **13** was reacted with 2,4-dichloropyrimidine under Suzuki conditions to obtain intermediate **14b**. As before, the pyrimidine and pyridine libraries were produced from halide displacement with various amines. We note that the dimethyl-substituted analogs (**11a**, **12a**, **15a**, **16a**) were obtained as side-products when performing the displacement reactions in DMF, the chloride having reacted with dimethyl amine generated by thermal decomposition of the solvent.



Scheme 3. Synthesis of **15** and **16**. Reagents and conditions: (a) 2-chloro-4-pyridylboronic acid, Na_2CO_3 , PPh_3 , $\text{Pd}_2(\text{dba})_3$, $\text{tol}/\text{EtOH}/\text{H}_2\text{O}$ (4:1:1), 105°C , *o/n*; (b) bis-(pinacolato)diborane, KOAc , $\text{PdCl}_2(\text{dppf})$, dioxane, MW, 45 min, 145°C ; (c) 2,4-dichloropyrimidine, Na_2CO_3 , $\text{Pd}_2(\text{dba})_3$, $\text{tol}/\text{EtOH}/\text{H}_2\text{O}$ (4:1:1), 105°C , *o/n*; (d) $\text{R}^1\text{R}^2\text{NH}$, DMF or NMP, heat.

All analogs generated were tested for inhibition of TbrPDEB1 at a concentration of $10\ \mu\text{M}$ (Table 2). Regrettably, we observed that all analogs showed weak (< 50%) inhibition of TbrPDEB1. We conclude that, though the headgroup of **5** is responsible for significant improvement of potency over **1**, the substituted aromatic headgroup replacements we prepared do not appropriately occupy the space that the headgroup of **5** does. We hypothesize that larger, nonplanar headgroups may be needed.

Figure 2 shows an overlay of **5** (green) against the cyclopentyl substituted analogs in **8c** (black) and **11a** (purple). This figure was generated by creating a conformer database of the analogs (using OMEGA version 2.4.3 OpenEye Scientific Software, Santa Fe, NM.),¹⁷ and generating optimal shape and electrostatic overlays against a minimized conformation of **5** (using ROCS version 3.0.0 and EON version 2.1.0). It is clear from this analysis that there is poor overlap between these new analogs and the fused ring of the N-alkylphthalizone core, which most likely attributes to the observed differences in potency. Further, though the pyridyl, pyrimidinyl, or quinolinyl nitrogen is positioned close to the carbonyl of **5**, these lone pairs may not reach far enough into the metal binding pocket to create good binding interactions suggested by previous crystallography results.^{10, 18-21} With this in mind, our optimization efforts continue in this class of compounds, with an eye towards a better recapitulation of the headgroup-metal interactions.

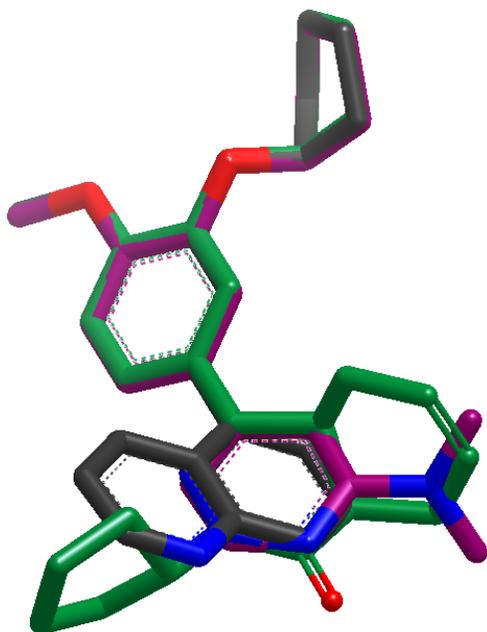


Figure 2 – Overlay of 5 (green) with compounds 8c (black) and 11a (purple).

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Supporting information. Supporting information includes synthetic preparations, compound characterization, biological assay conditions, and a recapitulation of the tables from the manuscript that include compound registry numbers. All data is freely available on the Collaborative Drug Discovery database (www.collaborativedrug.com).

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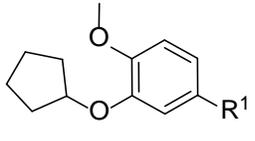
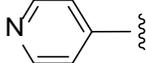
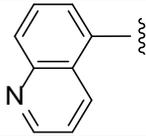
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Data Tables

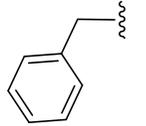
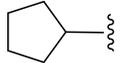
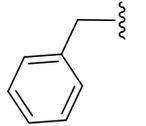
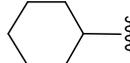
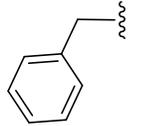
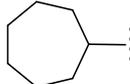
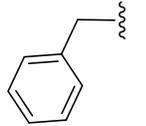
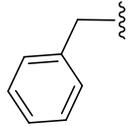
Table 1. Aryl analogs tested against TbrPDEB1

		
Compound	R ¹	TbrPDEB1 (% inh) ^a
8a		18.7 ± 10.7 ^b
8b		7.6 ± 2.6
8c		69.1 ± 13.9

^a Data shown are average of 3 replicate independent experiments. ^bReplicate of 5 independent experiments.

Table 2. Pyridine and Pyrimidine analogs tested against TbrPDEB1

Compound	R ¹	R ²	X	TbrPDEB1 (% inh) ^a
11a			C	34.0 ± 22.8
11b			C	27.1 ± 6.1
12a			N	15.8 ± 21.2
12b			N	29.8 ± 0.3
12c			N	12.7 ± 17.9
12d			N	20.6 ± 8.8
12e			N	32.9 ± 6.0
12f			N	3.9 ± 0.5
15a			C	48.0 ± 3.1
15b			C	28.4 ± 2.8
15c			C	7.2 ± 33
16a			N	14.5 ± 20.5
16b			N	16.1 ± 22.8

16c			N	21.8 ± 4.6^b
16d			N	23.2 ± 10.9
16e			N	22.3 ± 11.2
16f			N	18.5 ± 5.14

^a Data shown are average of 2 replicate independent experiments. ^bReplicate of 4 independent experiments.