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# Evaluation of pyrrolidine and pyrazolone derivatives as inhibitors of trypanosomal phosphodiesterase B1 (TbrPDEB1)

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ABSTRACT

Human African trypanosomiasis (HAT) is a parasitic disease, caused by the protozoan pathogen *Trypanosoma brucei*, which affects thousands every year and which is in need of new therapeutics. Herein we report the synthesis and assessment of a series of pyrrolidine and pyrazolone derivatives of human phosphodiesterase 4 (hPDE4) inhibitors for the assessment of their activity against the trypanosomal phosphodiesterase TbrPDEB1. The synthesized compounds showed weak potency against TbrPDEB1.


Human African trypanosomiasis (HAT), or African sleeping sickness, is a parasitic disease caused by the protozoan pathogen *Trypanosoma brucei*. HAT is predominantly found in poor, remote rural regions of sub-Saharan Africa. Currently, over 60 million people in 36 countries in sub-Saharan Africa are at risk, with approximately 10,000 infections annually.[1] HAT is a neglected tropical disease (NTD), in that relatively little attention has been paid to finding new treatments.

Four drugs are approved for the treatment of HAT: pentamidine, suramin, eflornithine, and melarsoprol. The nifurtimox–eflornithine combination therapy (NECT) has also been approved for HAT, which provides safety and dosing advantages over eflornithine monotherapy.[2,3] Though effective, these drugs are limited by serious and sometimes lethal side effects, inconvenient route of administration, and increasing incidence of drug resistance.[3,4] Considering the economic and social burden that HAT produces, further work is needed in finding safe and effective drugs that are safer, simpler to administer, and cheaper than those currently available. With this in mind, our research efforts have been directed toward repurposing established classes of inhibitors of druggable human targets to be inhibitors of essential parasite targets.[5] Phosphodiesterases (PDEs) are a family of enzymes that regulate signal transduction mediated by cAMP and cGMP in the cell, and inhibition of these enzymes reduces cAMP or cGMP PDE degradation, thus affecting the physiological processes controlled by these second messengers. Numerous PDE inhibitors are used as therapeutics.[6] *Trypanosoma brucei* expresses five PDEs, two of which, TbrPDEB1 and TbrPDEB2, have together been shown by RNAi to be essential for parasite proliferation.[7] The catalytic domains of human PDEs share 30–35% sequence identity to those of the parasite enzymes TbrPDEB1 and TbrPDEB2,[8] thus we reasoned that human PDE inhibitors repurposing could be a useful approach. We tested a range of human PDE (hPDE) inhibitors against TbrPDEB1 and B2, and we observed that the hPDE4 inhibitor piclamilast (**1**, Fig. 1) inhibits both TbrPDEB1 and B2 and *T. brucei brucei* (Tbb) cell growth in vitro.[9] Cilomilast (**2**), another commercialized hPDE4 inhibitor, and some of its analogs, have been found to inhibit TbrPDEB1.[10] We observed the prototypical hPDE4 inhibitor rolipram (**3**) to be inactive against TbrPDEB1.

Another class of human PDE4 phthalazinones has also been pursued, leading to the discovery of compound **4**, which is the most active TbrPDEB1/B2 inhibitor to date ($IC_{50}$ 3.98 nM and 6.0 nM, respectively, for TbrPDEB1 and B2).[11] The related phthalazinone compound **5** shows an $IC_{50}$ of 278 nM against TbrPDEB1.[12] In addition, pyrazolone **6** was identified through a scaffold merging approach.[13] Despite the obvious structural similarity between compounds **1**–**3**, **5**, and **6**, their reported TbrPDEB1 $IC_{50}$ values fall in a wide range. These molecules share the cyclopentyl-substituted catechol functionality, and differ in the region of the molecule (highlighted in red) pointing toward the catalytic metals in the binding site region. This suggested to us that this 'headgroup' region must be a major driver of potency against TbrPDEB1. With this in mind, in this Letter we report our attempt to find novel

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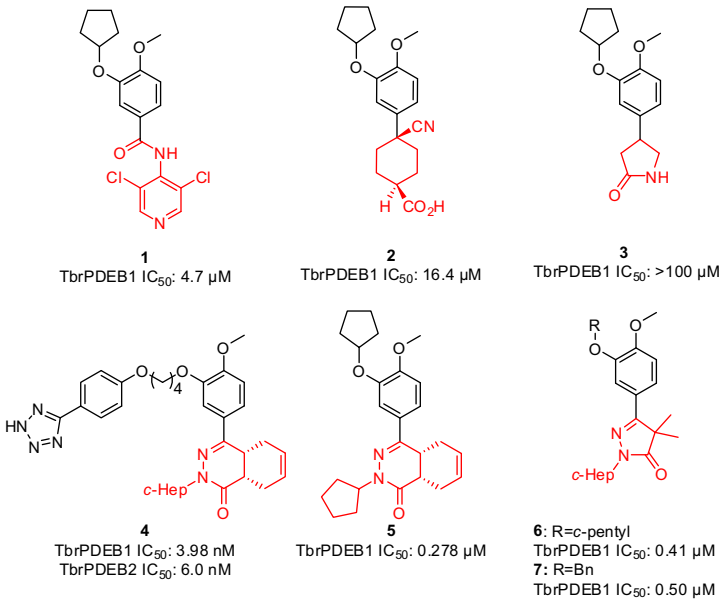
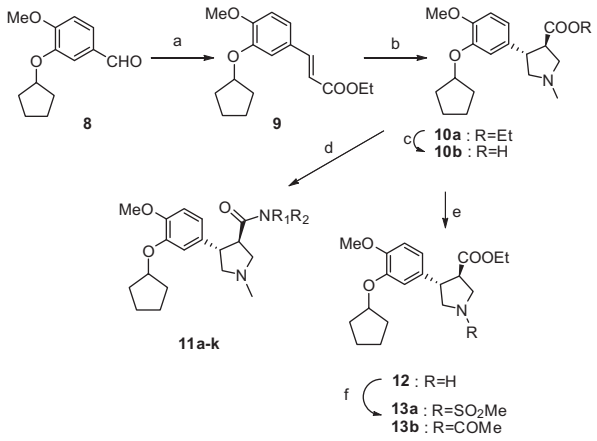



**Figure 1.** Compounds previously studied as TbrPDEB1 inhibitors.

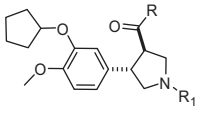


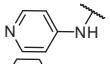
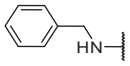
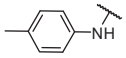
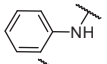
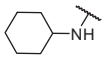
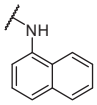

**Scheme 1.** Synthesis of pyrrolidine derivatives. Reagents and conditions: (a) (carbethoxymethylene)triphenylphosphorane, $CH_3CN$, MW, 150 °C, 20 min; (b) formaldehyde, sarcosine, $MgSO_4$, toluene, 170 °C, 24 h; (c) LiOH, $H_2O$, MeOH, THF, rt, 2 h; (d) appropriate amine, $Me_3Al$, toluene, 80 °C, 12 h; (e) (i) 1-chloroethyl chloroformate, DMAP, 1,2-dichloroethane, reflux, overnight; (ii) MeOH, reflux 4 h; (f) acetyl chloride or methanesulfonyl chloride, DMAP, DMF, rt, overnight.

TbrPDEB1 inhibitors driven by the replacement of the pyrrolidinone moiety of compound **3** with different five-membered rings.

As a starting point, we prepared racemic *trans*-3,4-disubstituted pyrrolidine analogs as an intended mimic of the pyrazolinone headgroup of **4** (Scheme 1). The sequence was initiated with Wittig olefination of aldehyde **8** to obtain the phenylacrylic acid ester **9**. Iminium ylide cycloaddition of compound **9** with sarcosine and formaldehyde in refluxing toluene gave the corresponding *trans*-3,4-disubstituted *N*-methyl pyrrolidine ethyl ester **10a**.[14] A small library of amides (compounds **11a–k**) has been prepared starting from the ester **10a** by reaction with the corresponding aluminum amide, while LiOH hydrolysis of ester **10a** gave the corresponding acid **10b**. Demethylation of **10a** with 1-chloroethyl chloroformate produced the pyrrolidine **12**,[15] which was converted to compounds **13a** and **13b** with methanesulfonyl chloride or acetyl chloride, respectively. Compounds **10**, **11**, and **13** were all found to be weak inhibitors of TbrPDEB1 (Table 1).

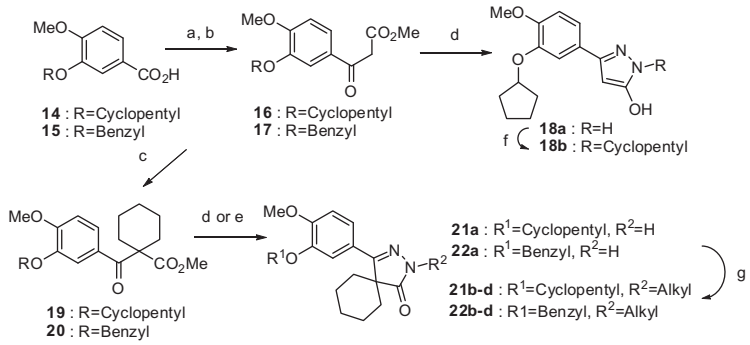
**Table 1**
*rac*-(*trans*-3,4)-Disubstituted pyrrolidine analogs tested against TbrPDEB1



| Compd | R | $R_1$ | TbrPDEB1 (% inh)[a] |
|---|---|---|---|
| **10a** | $OCH_2CH_3$ | $CH_3$ | 8 ± 11 |
| **10b** | OH | $CH_3$ | 17 ± 9 |
| **11a** |  | $CH_3$ | 5 ± 5[b] |
| **11b** |  | $CH_3$ | 7 ± 5[b] |
| **11c** |  | $CH_3$ | 8 ± 0 |
| **11d** |  | $CH_3$ | 6 ± 6 |
| **11e** |  | $CH_3$ | 16 ± 11 |
| **11f** |  | $CH_3$ | 10 ± 5 |
| **11g** |  | $CH_3$ | 16 ± 6 |
| **11h** | $NHCH_3$ | $CH_3$ | 8 ± 0 |
| **11i** | $NHCH_2CH_3$ | $CH_3$ | 5 ± 4 |
| **11j** |  | $CH_3$ | 1 ± 2 |
| **11k** |  | $CH_3$ | 10 ± 3 |
| **13a** | $OCH_2CH_3$ | $SO_2CH_3$ | 3 ± 4 |
| **13b** | $OCH_2CH_3$ | $COCH_3$ | 15 ± 0 |

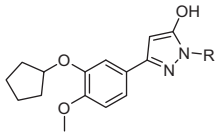
[a] Data shown are average of 2 replicate independent experiments. Compounds were tested at 10 μM concentrations.
[b] Replicate of 3 independent experiments.

We then prepared two pyrazolone-based inhibitors (compounds **18a** and **18b**), as shown in Scheme 2. Acid **14** was converted to the corresponding acyl chloride with thionyl chloride




**Scheme 2.** Synthesis of pyrazolone derivatives. Reagents and conditions: (a) $SOCl_2$, DMF, 90 °C, 3 h; (b) MeOAc, LDA, THF, −78 °C then rt, 1 h; (c) 1,5-diiodopentane, $K_2CO_3$, DMF, 90 °C, overnight; (d) hydrazine hydrochloride, acetic acid, 120 °C, 2 h; (e) hydrazine hydrochloride, DIPEA, BuOH, 120 °C, 3 h; (f) alkyl bromide, $K_2CO_3$, DMF, 70 °C, 2 h; (g) NaH, alkyl bromide, DMF, 60 °C, 3 h.

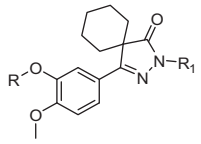
**Table 2**
Pyrazolone analogs tested against TbrPDEB1




| Compd | R | $R_1$ | TbrPDEB1 (% inh)[a] |
|---|---|---|---|
| **18a** |  | H | 0 |
| **18b** |  |  | 5 ± 3 |

[a] Data shown are average of 2 replicate independent experiments. Compounds were tested at 10 μM concentrations.

**Table 3**
Spiro pyrazolone analogs tested against TbrPDEB1




| Compd | R | $R_1$ | TbrPDEB1 (% inh)[a] |
|---|---|---|---|
| **21a** |  | H | 11 ± 10 |
| **21b** |  |  | 18 ± 2 |
| **21c** |  |  | 9 ± 4[b] |
| **21d** |  |  | 9 ± 6[b] |
| **22a** |  | H | 14 ± 20 |
| **22b** |  |  | 11 ± 10[b] |
| **22c** |  |  | 7 ± 5 |
| **22d** |  |  | 10 ± 3 |

[a] Data shown are average of 2 replicate independent experiments. Compounds were tested at 10 μM concentrations.
[b] Replicate of 3 independent experiments.

and reacted with the lithium enolate of methyl acetate to provide **16**. The β-keto-ester intermediate **16** was cyclized to the desired pyrazolol derivative **18a** using hydrazine hydrochloride in refluxing acetic acid. Compound **18a** was alkylated with bromocyclopentane to obtain compound **18b**. Compounds **18a–b** were also found to have little activity against TbrPDEB1 (Table 2).

We looked toward increasing the size of the headgroup region, looking to mimic the size and shape of compounds **4–7** more closely, and therefore prepared the spirocyclic compounds **21a–d** (Scheme 2). The β-keto-ester **16** was first alkylated with 1,5-diiodopentane to give compound **19** which cyclized to **21a** when treated with hydrazine. This could be N-alkylated with various alkyl bromides to give compounds **21b–d**.

Besides preparing the cyclopentyl-substituted catechol analogs, we noted that improved *T brucei* cellular potency had been reported for **7** (Tbb $EC_{50}$: 6.3 μM) when compared to **6** (>64 μM).[13] Thus, we prepared the benzyl-substituted analogs **22a–d**. Compounds **21** and **22** were tested against TbrPDEB1 (Table 3), and, despite the structural similarity of these compounds and the known actives **6** and **7**, we found that these analogs had very little ability to inhibit TbrPDEB1. We can conclude, based on this and on our previous attempts to explore structural variations around compound **1** that the SAR is extraordinarily tight for this class of compounds.[9,12] With this in mind, our efforts are focused on obtaining a better understanding of the subtle structural features needed for an optimal enzyme inhibition.

## Acknowledgment

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## Supplementary data

The synthetic preparation and characterization of new compounds, along with biological assay conditions, and a recapitulation of the tables from the manuscript that include compound registry numbers may be found in the Supplementary data. All screening data reported in this Letter is freely available on the Collaborative Drug Discovery database (www.collaborativedrug.com). Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.04.061.

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