



Synthesis and evaluation of human phosphodiesterases (PDE) 5 inhibitor analogs as trypanosomal PDE inhibitors. Part 1. Sildenafil analogs

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ABSTRACT

Parasitic diseases, such as African sleeping sickness, have a significant impact on the health and well-being in the poorest regions of the world. Pragmatic drug discovery efforts are needed to find new therapeutic agents. In this Letter we describe target repurposing efforts focused on trypanosomal phosphodiesterases. We outline the synthesis and biological evaluation of analogs of sildenafil (**1**), a human PDE5 inhibitor, for activities against trypanosomal PDEB1 (TbrPDEB1). We find that, while low potency analogs can be prepared, this chemical class is a sub-optimal starting point for further development of TbrPDE inhibitors.

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Tropical diseases such as human African trypanosomiasis (HAT), leishmaniasis, and Chagas' disease are often referred to as 'neglected' diseases, as the low level of funding of drug discovery research is disproportionate to the total disease burden. For example, collectively these three diseases endanger over 73 million people globally¹ and create a burden of 4.5 million disability-adjusted life-years.^{2,3} Yet, few compounds have advanced to clinical studies for these diseases.⁴ The primary reason for this disparity is the high cost of drug discovery and unlikelihood that these research costs will be recouped by a research organization via drug sales to highly impoverished populations.

As a result, cost-effective methods for identification of lead matter and expediting compound optimization efforts are acutely needed. We apply the method of 'target repurposing', wherein essential parasite enzymes are matched with proven, druggable human homologs.⁴ By doing so, the research outputs of large and expensive programs of historical drug discovery projects can be used to initiate and drive optimization projects against parasitic targets.

Phosphodiesterases (PDEs) are a family of enzymes that maintain the balance of cAMP and cGMP in the cell, opposed to

adenylate and guanylate cyclase, respectively. Humans possess eleven PDEs, several of which have been fruitfully pursued for drug discovery. The most well-known of these is PDE5, an enzyme that is inhibited by erectile dysfunction drugs such as ViagraTM (sildenafil, **1**), CialisTM (tadalafil, **2**), and LevitraTM (vardenafil, **3**), Figure 1. Other PDEs are of demonstrated relevance to inflammatory conditions and CNS indications, such as schizophrenia.^{5–7} *Trypanosoma brucei*, the causative agent of HAT, expresses five cAMP-specific PDEs,⁸ two of which (TbrPDEB1 and TbrPDEB2) have together been shown by both RNAi^{9,10} and small molecules^{10,11} to be essential for parasite proliferation. While initial benchmarking and early optimization experiments have uncovered a susceptibility of TbrPDEB1 and B2 to human PDE4 inhibitors,¹¹ the two trypanosomal enzymes are also 31% identical to PDE5 within their respective catalytic domains, and previous reports cited low potency of **1** and **2** against the trypanosomal enzymes; thus, we describe here early structure–activity relationships (SAR) of (**1**), and report such work for **2** in a subsequent article.¹²

We initially tested **1** for its inhibitory activity against the catalytic domain of TbrPDEB1, and observed low potency (51% at 100 μM). However, we deemed this chemotype worth further exploration given the extensive knowledge of SAR and structural biology around it. We performed activity scoping experiments by making strategic changes to the structure of **1**, to explore the impact of variation of the *N*-methyl group and propyl chain on

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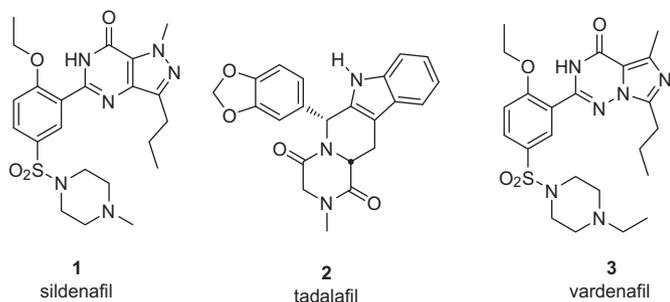


Figure 1. Human PDE5 inhibitors marketed for erectile dysfunction.

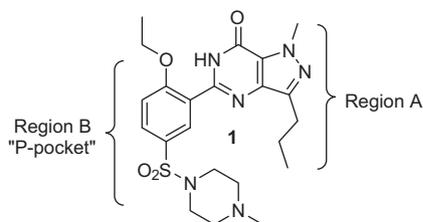
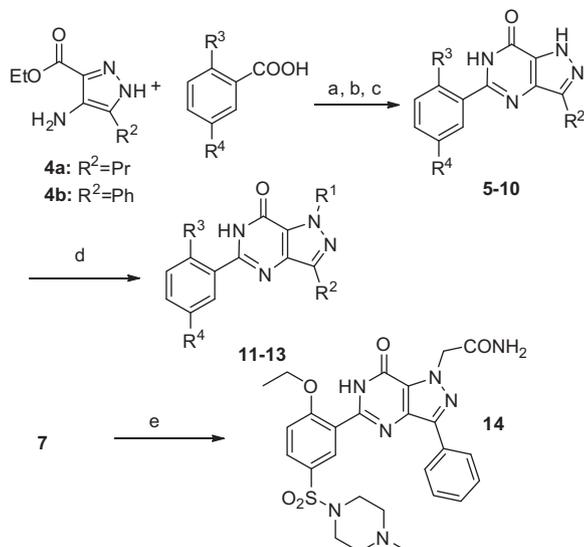


Figure 2. Strategy for exploration of the sildenafil core.

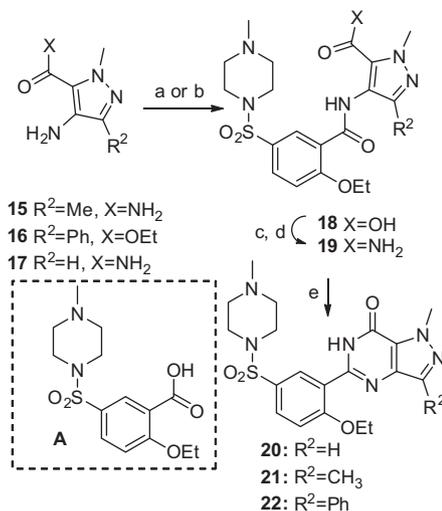
the pyrazolopyrimidinone core (Region A, Fig. 2), and of the specific functionality on Region B. We were particularly interested in exploring the western portion of **1**, as this region of the molecule was proposed to project into a parasite-specific pocket (the P-pocket). This pocket was first observed in the *Leishmania major* phosphodiesterase LmjPDEB1¹³ and is predicted to also exist in TbrPDEB1,¹¹ but, importantly, is absent from all human PDEs.

Compounds that explore Regions A and B were synthesized using the routes outlined in Schemes 1 and 2. The known aminopyrazole **4a**¹⁴ or **4b**¹⁵ was acylated with the appropriate benzoic acid and cyclized under basic conditions to give **5–10**. Pyrazole N-arylation was achieved using copper catalysis¹⁶ to prepare **11–13**. Alkylation of **7** with bromoacetamide provided **14**.

The preparation of Strategy B analogs of compound **1** is illustrated in Scheme 2. The appropriate aminopyrazole **15**,¹⁷ **16**,¹⁸ or



Scheme 1. Synthesis of **5–14**. Reagents and conditions: (a) PyBroP, TEA, DCE, 120 °C, MW, 10 min; (b) NH₄OH, dioxane, rt; (c) NaOEt/EtOH, 120 °C, MW, 10 min; (d) R¹-I, CuI, *trans*-cyclohexane-1,2-diamine, Cs₂CO₃, DMF, 110 °C; (e) NaH, 2-bromoacetamide, 0 °C to rt. Refer to Table 1 for the identity of R-groups.



Scheme 2. Synthesis of **20–22**. Reagents and conditions: (a) CDI, **A**, 70 °C, EtOAc, o/n; (b) PyBroP, **A**, Et₃N, DCE, MW 120 °C 20 min; (c) SOCl₂; (d) NH₃, *i*PrOH; (e) NaOEt, EtOH, MW 120 °C 10 min.

17¹⁹ is acylated with **A** using either CDI or PyBroP; these reaction conditions surprisingly resulted in the partial-to-complete hydrolysis of the primary amide (of **14** and **15**) or ester (of **16**). Thus, the resulting carboxylic acid **18** was converted to the primary amide **19** via treatment with thionyl chloride, followed by ammonia in isopropanol. Cyclization to the desired products was effected under basic conditions.

Following synthesis, the analogs were tested in biochemical assays¹¹ at a single concentration. Notably, with one exception (**7**), none of the analogs that varied the pyrazole N1 substituent (H, Me, 3-pyridyl, or acetamide) nor the C3 position (H, Me, Pr, Ph) showed improved potency over **1**.

The removal of the *N*-methylpiperazinyl sulfonamide head group resulted in compounds with significant loss in solubility, and as such biochemical screening data was not possible with some analogs (Table 1, entries 8, 12–14).

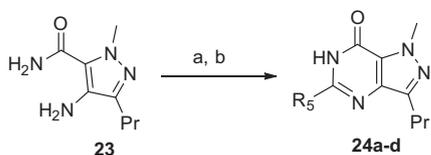
A broad exploration of heterocyclic substitutions in Region B was undertaken by application of parallel synthesis (Scheme 3).

Table 1
Results of biochemical screening of analogs of **1**

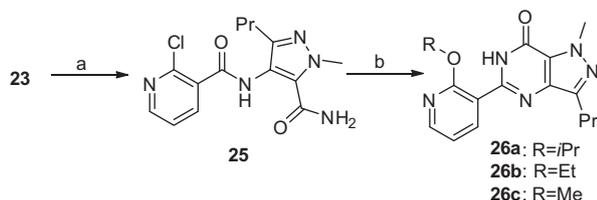
Entry	Compd	R ₁	R ₂	R ₃	R ₄	TbrPDEB1 ^a (%inh)
1	1	CH ₃	Pr	OEt		51.5
2	7	H	Ph	OEt		70.9
3	13	3-Pyr	Pr	OEt		32.4
4	22	CH ₃	Ph	OEt		17.5
5	10	H	Pr	OEt		16.0
6	20	CH ₃	H	OEt		13.6
7	8	CH ₃	Pr	H		8.4
8	21	CH ₃	CH ₃	OEt		ND ^b
9	6	H	Ph	OEt	H	22.9
10	9	CH ₃	Pr	OEt	H	21.8
11	14	CH ₂ CONH ₂	Ph	OEt	H	7
12	11	Ph	Ph	OEt	H	ND ^b
13	12	3-Pyr	Ph	OEt	H	ND ^b
14	5	H	Pr	OEt	H	ND ^b

^a Standard assay conditions: 100 μM, 10% DMSO.

^b Compound showed lack of solubility, which precluded testing.



Scheme 3. Synthesis of **24a–d**. Reagents and conditions: (a) R_5CO_2H , imidazole-HCl, CDI, then **23**, 50 °C, o/n; (b) EtOH, NaOEt, 120 °C, 2 h. Refer to Table 2 for the identity of R-groups.



Scheme 4. Synthesis of **26a–c**. Reagents and conditions: (a) 2-Chloronicotinic acid, PyBroP, Et₃N; (b) R-OH, KOtBu.

Table 2
Analogues of sildenafil (Strategy A)

#	Compound	R ₅	TbrPDEB1 ^a (% inh)
1	26c	2-Methoxy-3-pyridyl	77.2
2	24a	3-Methyl-2-pyridyl	72.1
3	26a	2-Isopropoxy-3-pyridyl	53.8
4	26b	2-Ethoxy-3-pyridyl	41.5
5	24b	2-Methyl-5-pyrazinyl	30.0
6	24c	2,4-Dimethyloxazol-5-yl	29.0
7	24d	Thiazol-4-yl	25.4

^a Standard assay conditions: 100 μM, 10% DMSO.

This was achieved by condensing the commercially available pyrazole amino amide **23** with various monocyclic heteroaromatic carboxylic acids that were available in pre-weighed quantities from a commercial vendor (ASDI, Inc). Following this amidation reaction, cyclization was achieved by treatment with sodium ethoxide in ethanol.

A series of 2-alkoxy-3-pyridyl variations were prepared as shown in Scheme 4. The acylated aminopyrazole **25** was cyclized under basic conditions, using an appropriate alcoholic solvent, which yielded the 2-alkoxy-pyridyl derivatives **26**.

Table 2 summarizes the biochemical results for analogs prepared using this parallel methodology that display >25% inhibition at 100 μM (characterization and biochemical data for all compounds are listed in the Supplementary data). In brief, heterocycles show improved potency at TbrPDEB1, specifically, 2-methoxy-3-pyridyl and 3-methyl-2-pyridyl show the best potency (entries 1–2); larger 2-alkoxy groups (entries 3 and 4) lead to reduced activity. Unfortunately, dose–response experiments with **26c** were precluded by poor solubility at higher concentrations.

While we appreciate that the potencies of the compounds shown in Tables 1 and 2 are modest, it is clear that **7**, **26c** and **24a** represent qualitative potency improvement over **1**. Nonetheless, the current exploration of the various regions of the sildenafil template is not suggestive of promising structure–activity relationship trends.

In conclusion, with the broad interest in repurposing PDE inhibitors for neglected diseases^{11,20,21} and the extensive precedent in medicinal chemistry for human PDE5 inhibitors, we note our finding that these initial sildenafil analogs do not represent as strong starting points for further optimization for TbrPDEB inhibition as the PDE4 chemotypes previously reported.¹¹ This information may be deemed important for re-prioritizing the focus of these efforts.

Acknowledgments

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Supplementary data

Full tabulation of all inhibitors prepared and screened in the context of this project is included in the Supplementary data, along with spectroscopic characterization of new compounds. Screening data will be made publically available as a public data set in the Collaborative Drug Discovery database (www.collaborativedrug.com).

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bmcl.2012.01.119](https://doi.org/10.1016/j.bmcl.2012.01.119).

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